Extraction of Proteins from Natural Plants: Overview of Novel and Physical Extraction Methods

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Abstract

Proteins typically consist of carbon, nitrogen, hydrogen, oxygen, and sulfur, leading to a complex arrangement of amino acid molecules interconnected by peptide bonds. An in-depth examination of alternative protein-rich options is essential due to the decreasing availability of traditional protein sources and the necessity to satisfy the dietary needs of the growing global population. The primary objective of developing concentrates and isolates from a protein source is to enhance protein concentration by eliminating non-protein components. This process enables the utilization of a reduced quantity of protein in food formulation, thereby delivering targeted nutritional and functional attributes. Protein concentrates represent a viable alternative derived from various sources, such as dairy products, plants, and insects. The extraction strategies employed, spanning from traditional solvent extraction to innovative methods like membrane separation and enzyme-assisted techniques, offer avenues to enhance yield, preserve nutritional quality, and minimize environmental effects. These concentrates serve a crucial role in the food industry by enhancing dietary profiles, formulating functional foods, and addressing the increasing demand for plant-based and alternative protein sources. This review enhances our comprehension of this vital component in the quest for nutrient-rich and sustainable protein sources by elucidating their origins, extraction methods, and applications.

Keywords: Protein Extraction, Novel Extraction Techniques, Conventional Extraction Methods, Protein Concentrate.

1. Introduction

It is projected that the population of the world will increase by more than 9 billion by the year 2050. In addition to this, it has been calculated that the global food output has to expand by seventy percent from its current level (Singh et al., 2022). Since conventional agricultural intensification techniques are no longer seen as environmentally benign, reaching this food production target is becoming more difficult. Protein is a macronutrient that is expected to see a significant rise in demand in the future. The worldwide market for protein ingredients was valued at USD 38.02 billion in 2019, and it was projected to increase from 2020 to 2027 at a CAGR (compound annual growth rate) of 9.1% (Protein ingredients market study, 2019) (Okolie et al., 2019; Özyurt et al., 2021; Sharma et al., 2023). Furthermore, by 2054, the world's protein demand is predicted to reach 943.5 million metric tons, which means there have to be markets for alternative proteins, such as plant-based proteins. Protein is a vital macronutrient important to bolstering the growth and development of the body. The situation will worsen if the supply and distribution of protein-rich foods and supplements are not sustained, if climate change mitigation plans are not put into action, and if renewable plant-based foods are not used as sources of energy and nutrition (Mathews et al., 2022). The increasing demand in the plant protein market, along with the pressure on traditional crop sources, is the primary factor motivating the extraction of proteins from alternative plant sources. This demand, when limited to the food industry, arises from an increasing consumer consciousness and awareness regarding the food they consume. The extraction process also aids in freeing proteins from their associated compounds, including sugars, fatty acids, polyphenols, pigments, and antinutrients, resulting in proteins that possess a neutral colour and flavour (Mathews et al., 2023).

Proteins are now used as functional constituents to change the texture and stability of the food, as opposed to earlier times when they were mainly used for bodybuilding. They have also been crucial in the development of new processed foods by: a) providing customized structures (e.g., 3D food printing); b) creating meat and dairy substitutes, increasing consumer options; c) reducing allergenic qualities; and d) isolating enzymes and bioactive peptides for shelf life extension and other specific uses (Prandi et al., 2022; Segatto et al., 2022). The intrinsic protein allergenicity and commercial production stress from the current animal proteins and traditional plant proteins, including soybean and pea, will likely be lessened by the new sources, trends, and alterations in alternative plant protein extraction. However, because they lack some essential amino acids, plant proteins are generally regarded as being less nutritious than animal proteins. Additionally, the nutritional value of these protein sources is heavily influenced by their processing, bioavailability, amino acid profile, purity, digestibility, and anti-nutritional factors (Pattnaik et al., 2021).

Research is ongoing on plant-based proteins as a cost-effective way to improve the nutritional content of foods. Affordable protein sources for value-added food products are becoming more important. Protein may be found in several dietary sources, such as plants, animals, and nutritional supplements. Most of the protein isolates and concentrates used in food production come from dairy, wheat, and soy (Fatima, Singh, Pandey, et al., 2024; Parandi et al., 2023). However, other protein sources, like oilseeds and lentils, are being studied because of dietary choices and limits like halal, vegetarianism, and food allergies. Proteins may execute distinct technical functions depending on their classification as a formulation, isolate, or concentrate, as well as their specific source and concentration level. These attributes include digestibility score, emulsification, heat stability, solubility, and flavor-binding capacity. The separation and extraction of proteins are crucial phases in the process (Cao et al., 2023; Chatterjee et al., 2015). Dry fractionation and wet extraction are the two main procedures for protein extraction and separation. The majority of plant protein constituents are generated using wet extraction methods, including standard procedures like salt extraction dialysis (SED), alkaline extraction-isoelectric precipitation (AE-IP), and micellar precipitation (MP). They also include recently developed methodologies such as enzyme-assisted extraction (EAE), microwave-assisted extraction (MAE), membrane technology (MT), and ultrasound-assisted extraction (UAE). Various factors, including the oilseed, pulse, and cereal composition (polysaccharides, fibre content, and fat), the used part, and the required protein quantity in the components, among others, affect the selection of the optimal plant protein extraction or separation method. Nonetheless, these methods have not been completely used in the extraction of plant-based protein. This study addresses the need to compile recent data on the status of innovative and environmentally sustainable protein extraction technologies. The shortcomings and future possibilities of these unique approaches are thoroughly examined.

1.1 Significance of Protein Extraction

Protein extraction is an essential procedure in most biological and biochemical investigations. It entails the isolation and purification of proteins from many biological sources, including cells, tissues, or fluids. Isolated proteins may be examined for their structure, function, and interactions with other molecules(Bouloumpasi et al., 2024). Methods such as X-ray crystallography, mass spectrometry, and enzyme tests depend on natural proteins. Protein extraction facilitates the quantification of protein concentrations in various samples, which is crucial for elucidating biological mechanisms, diagnosing conditions, and assessing therapeutic responses. Protein extraction is essential for finding and isolating prospective drug targets, creating protein-based therapies (such as antibodies and enzymes), and examining drug-protein interactions (Bhagwat & Padalia, 2020). It is used in the synthesis of recombinant proteins for diverse purposes, including industrial enzymes, biofuels, and diagnostic reagents. Protein extraction is crucial for isolating proteins from plant and animal sources for incorporation into food items such as meat replacements, protein supplements, and dairy alternatives. Protein extraction is a basic technology with extensive applications across several industries. Figure 1 shows the types of protein extraction methods It establishes the basis for comprehending protein function, advancing novel technologies, and enhancing human health and well-being (Bhagwat & Padalia, 2020). Figure 2 illustrates the extraction of protein from leaf.

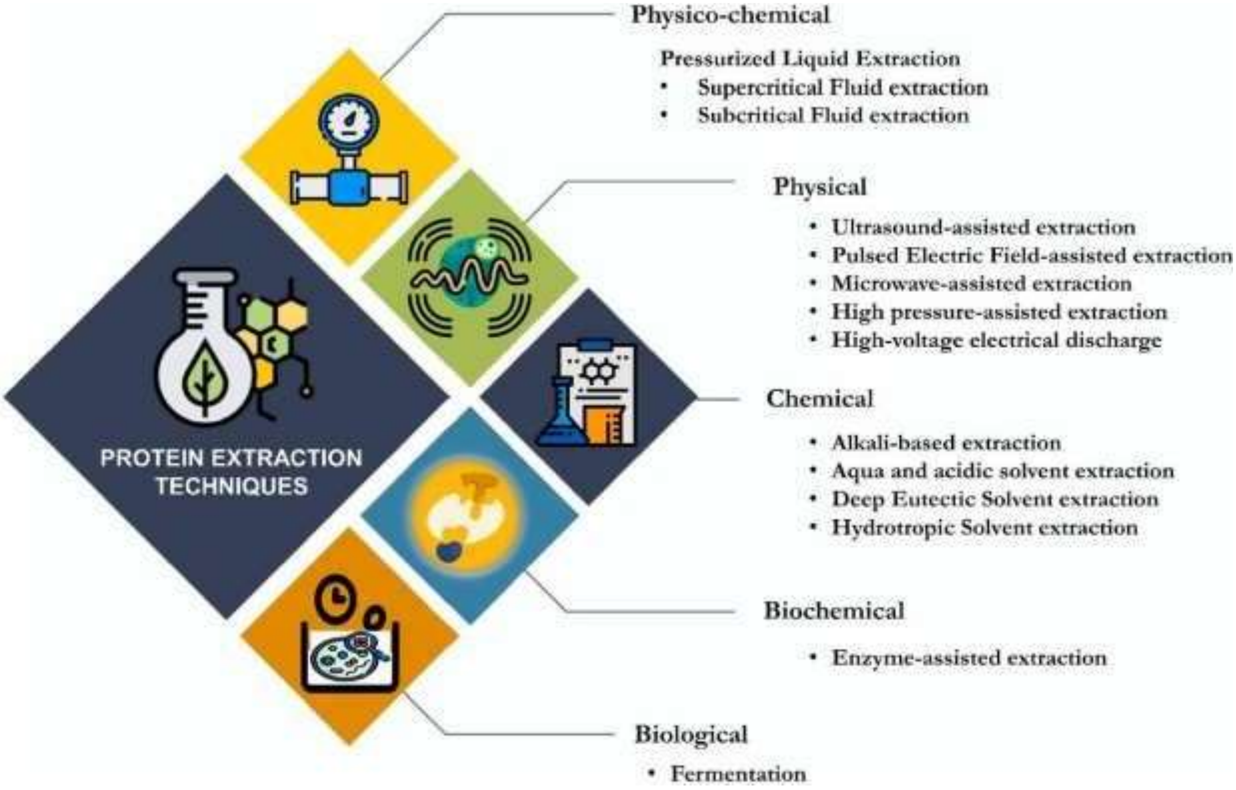

Figure 1 Types of protein extraction methods (Ravindran et al., 2024) (Reproduced with permission from Elsevier)

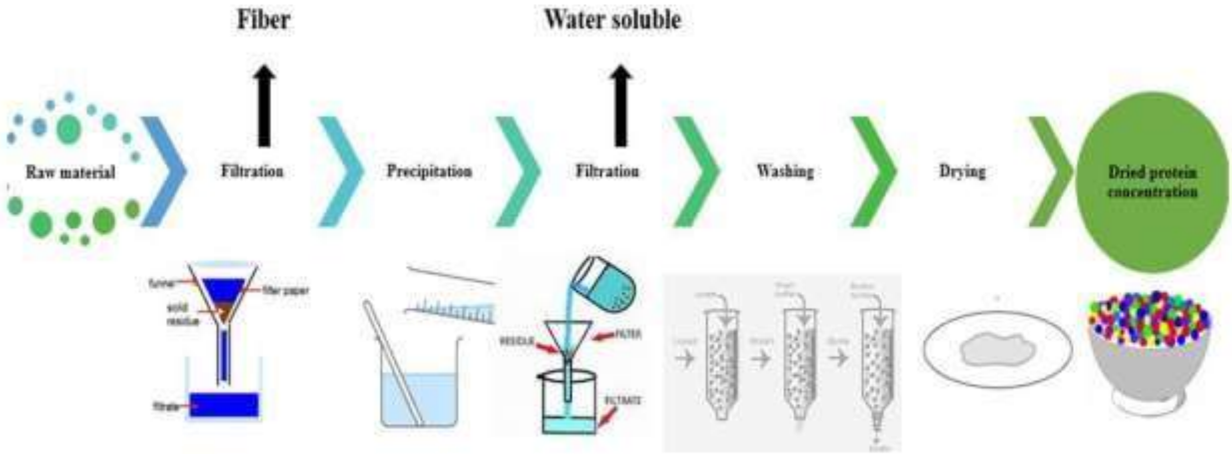

Figure 2 Extraction of protein from leaf (Fatima et al., 2024) (Reproduced with permission from Elsevier)

2. Novel Extraction Methods

Conventional extraction techniques often need substantial quantities of organic solvents, elevated temperatures, and prolonged processing durations. These variables may result in environmental issues and the deterioration of the target chemicals. Researchers have devised innovative extraction methods that provide several benefits compared to traditional procedures.

2.1 Enzyme assisted protein extraction

Enzyme-assisted extraction (EAE) is an effective technique for obtaining high-quality plant proteins. The extraction of cellular proteins is hindered by rigid cell walls. The primary objective of EAE is to break down the integrity of cell walls by the enzymatic degradation of its major components: hemicellulose, cellulose, and pectin (Fatima, Singh, Pandey, et al., 2024). Figure 3 shows the schematic of protein extraction using Enzyme assisted method Pectinases and carbohydrates, which facilitate specialized functions in cell wall degradation, assist in the release of cellular proteins from cereal, legume, and oilseed seeds. Proteases operate optimally at a certain pH to prevent protein denaturation. A typical concentration of protease for various extraction procedures is 1–5% g or mL enzyme per gram of substrate. Under certain physiological conditions, these enzymes may also inhibit the formation of complexes between the released proteins and other cellular components, including carbohydrates and phytates (Zhou et al., 2022). Enzymes need certain acidic and alkaline environments to attain optimal catalytic efficiency. Carbohydrases often function well in moderately acidic circumstances, whereas several proteases like mildly alkaline environments. The optimal pH and temperature ranges for proteases functioning in an alkaline environment are 8–10 and 45–60 °C, respectively (Gul et al., 2023). Protein extraction from rice bran has been investigated using an enzyme complex including several proteases (papain) and carbohydrases (cellulase, arabinose, pectinase, xylanase, glucanase, and hemicellulase). Proteases had the highest protein production, whilst carbs showed no significant effect. In conjunction with EAE, mechanical processing techniques, including sonication, ultrasound, and microwave treatment, enhance both the yield and quality of protein extracts. Dehulled rapeseed press cakes had elevated levels of protein (36–40%) and carbs (35%). They were subjected to treatment with pectinolytic, cellulolytic, and xylanolytic enzyme preparations to extract proteins. Proteolytic enzymes significantly increase protein extraction yield by 1.7-fold by the hydrolysis of pectic and glucan polysaccharides, facilitating the breakdown of cell walls. 74%

and 56% of the proteins were effectively eliminated from dehulled and whole-pressed rapeseed cakes, respectively (Mathews et al., 2022; Nuchdang et al., 2022). Table 1 presents the summary of protein extraction using Enzyme assisted technique

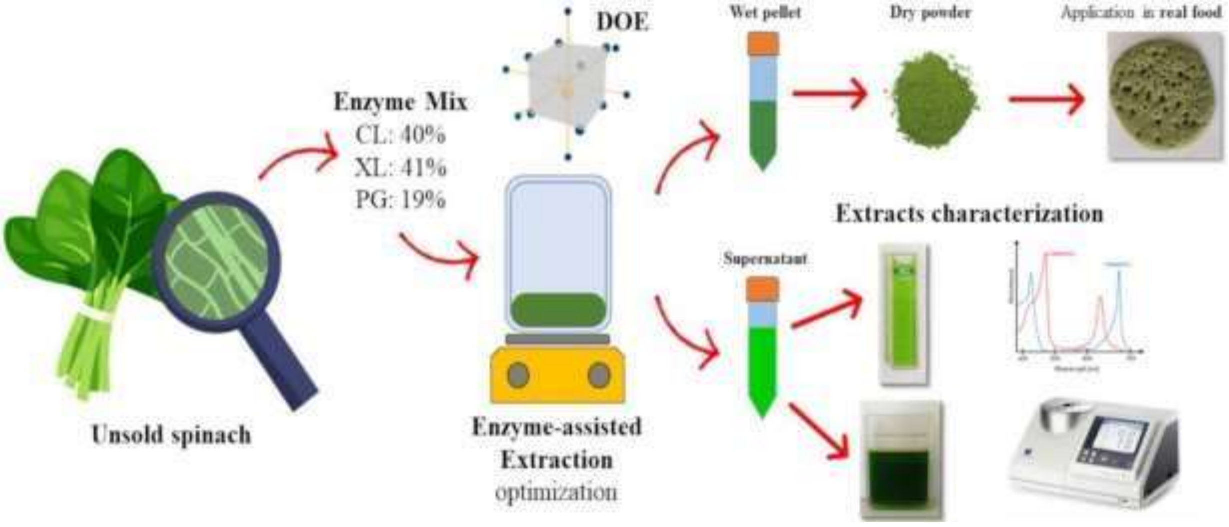

Figure 3 Schematic of protein extraction using Enzyme assisted method.

Table 1 Summary of protein extraction using Enzyme assisted technique.

Sr.No.	**Natural Material**	**Extraction Conditions**	**Yield of Proteins recovered**	**Technological functional properties (%)**	**Reference**
1	Soybean flakes	Phytase and acid protease, pH- 4.5, T- 50 °C and Time- 30 min	74.83	Good thermal stability	(Wei et al., 2018)
2	Grits of soybean	β-glucanase, pH- 5.5, T-50 °C and Time- 180 min	45.93	Enhanced solubility	(Perović et al., 2020)
3	Okara	Concentration- 4%, Cellulase, xylanase, pH- 6.2, T- 53 °C	58.00	Good emulsion activity	(de Figueiredo et al., 2018)
4	Red Seawood	Concentration- 0.2-0.4%, Alcalase, Shearzyme pH- 7, T-60 °C, Time- 14 h	85.50	Good water holding capacity	(Kumar et al., 2021)
5	Akebia trifoliata	Cellulose 0.1 g, pH-10, T-60 °C, Time- 2 h	52.78	Superior emulsion properties	(Jiang et al., 2021)

6	Red bean	Protex 6L activity-3.5%, pH- 8.4, T-56 °C, Time- 3.5 h	73.34	Good stability	(LIU et al., 2008)

7	Rapeseed Meal	Cellulase activity- 18 FPU/g pH- 4.8, T-50 °C, Time- 6 h	77.00	Enhanced antioxidant activity	(Li et al., 2023)
8	Sesame bran	Alcalase activity- 0.12-2.40 AU/100g, pH- 8, T-55 °C, Time- 10-120 min	91.70	Highest protein recovery	(Görgüç et al., 2019)
9	Sesame seeds	Neutrase, Pectinex, T-50 °C, Time- 6 h	89.70	Max. Protein Recovery	(Tirgarian et al., 2019)
10	Peanut	Alcalase, Nutrase, pH- 9.5, T-55 °C, Time- 90 min	71.38	Enhanced protein recovery	(Jiang et al., 2021)

2.3 Deet Eutectic solvents, Aqua, and acidic solvents based Extraction

Deep eutectic solvents (DES) are known as a category of ionic liquid (IL) equivalents due to their comparable features and qualities to ILs. The distinct chemical features of DES make it appropriate for use in food and metal processing applications (Fatima, Singh, Pandey, et al., 2024). Typically, deep eutectic solvents (DES) are derived from the complexation of quaternary ammonium salts, including N, N-diethyl-2-hydroxy ethanamide chloride, tetramethylammonium chloride, choline chloride, tetra butyl phosphonium bromide, methyl triphenyl phosphonium bromide, and tetra propylammonium bromide, which serve as hydrogen bond acceptors (HBA), in conjunction with hydrogen bond donors (HBD) such as glycerol, sorbitol, thiourea, acetamide, benzamide, imidazole, malonic acid, and urea. Figure 4 shows the deep eutectic solvent-based ultrasound-assisted extraction and quantitative analysis by HPLC for proteins. The growing demand for environmentally sustainable processes in green chemistry, along with the accepted benefits of deep eutectic solvents (DES), have proven DES as an attractive substitute to traditional organic solvents across multiple fields (Kumar et al., 2021). Traditional organic solvents, including methanol, acetonitrile, ethanol, hexane, and acetone, have been used in food processing technologies such as extraction, separation, and pre-concentration. Despite the affordability and ease of processing of these typical organic solvents, the risk of environmental contamination remains a significant problem (Özyurt et al., 2021). DES has been investigated as a sustainable, eco-friendly processing technique for the extraction of various food components to overcome these restrictions. Deep Eutectic Solvents (DESs), recognized as eco-friendly or green solvents, have garnered heightened attention among the scientific community. Due to its affordability, biodegradability, and low toxicity, the proliferation of novel DESs has significantly risen (Cao et al., 2023). Li et al., (2023) Studied the extraction of pumpkin seed protein using poly(ethylene glycol)-based deep eutectic solvents under synergistic ultrasound-microwave extraction conditions. The maximum protein yield (93.95%) was observed for aqueous poly(ethylene glycol) (PEG 200)-based deep eutectic solvent under these optimized conditions. The concentration of PEG 200-based DES: 28% w/w; solid-to-liquid ratio: 28 g/ml; microwave power: 140 W; extraction temperature: 43 °C.

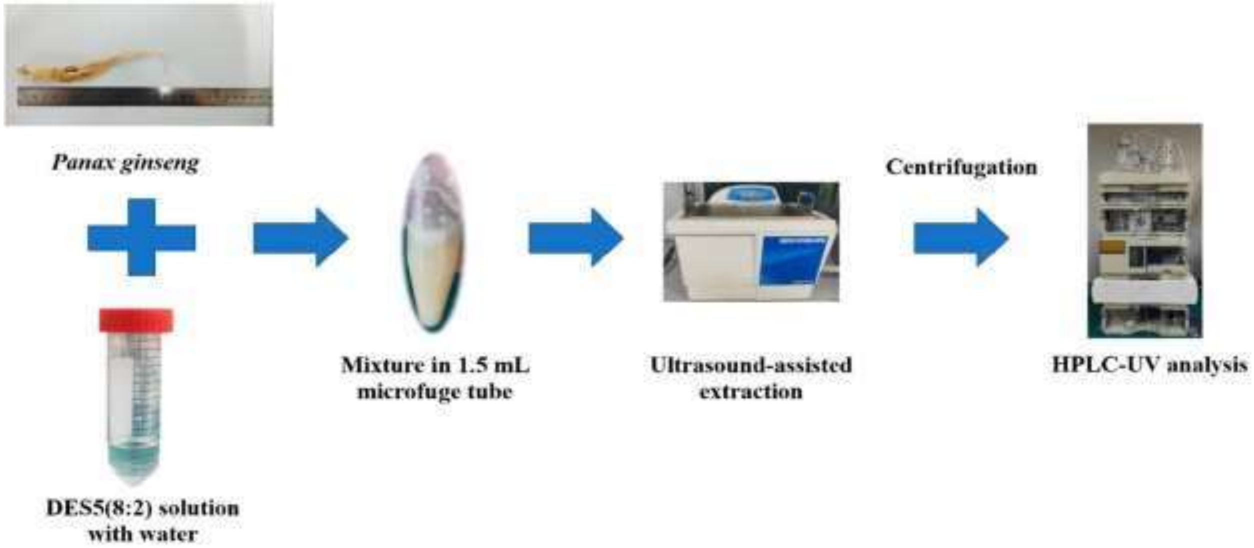

Figure 4 Deep eutectic solvent-based ultrasound-assisted extraction and quantitative analysis by HPLC for proteins (Tu et al., 2022) (Reproduced with permission)

Nonetheless, this method has not been thoroughly used in protein extraction and component formulation, especially concerning plant-based proteins, resulting in a knowledge deficit regarding the optimal deep eutectic solvent protein extraction conditions for various plant sources. The recovery of DES after protein extraction is a significant barrier to industrial scalability (Sharma et al., 2023; Zhou et al., 2022).

2.4 Reverse Micelles Extraction

Reverse micelles (RM) are characterized as "nano-sized spherical structures comprising an inner aqueous core surrounded by surfactant molecules in an organic solvent." Figure 5 shows the schematic presentation of extraction of proteins using reverse micelles method. The surfactants have a polar head group directed towards the micelle's inner aqueous core, while a hydrophobic hydrocarbon tail is directed towards the organic solvent. Micelles facilitate the solubilization of certain biomolecules inside the inner aqueous core, hence enabling the effective extraction of the targeted biomolecule.

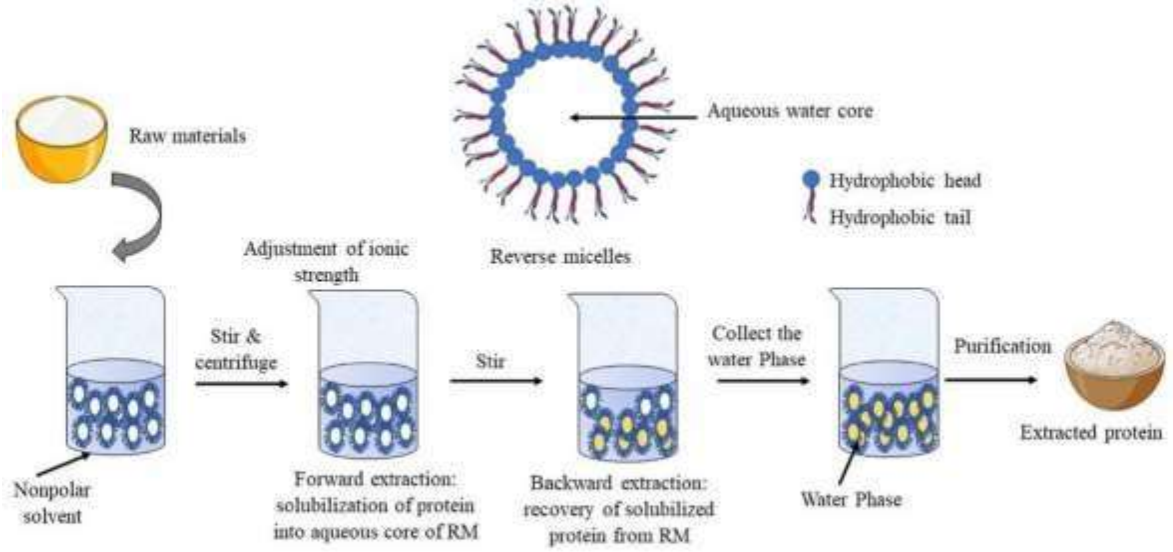

Figure 5 Schematic presentation of extraction of proteins using reverse micelles method (Hewage Et

al., 2022). (Reproduced with permission from, Elsevier).

The varying water content inside the RM results in the inner core water being physiochemically distinct from the bulk water. RM secures the biomolecule inside the core for solubilization without inducing conformational alterations (Fatima, Singh, Pandey, et al., 2024; Gul et al., 2023). This distinctive feature of RM has been used in protein extraction and purification. The extraction of protein by RM involves two steps: forward extraction (FE) and backward extraction (BE). FE encompasses the solubilization of proteins inside the inner aqueous core via three stages: RM formulation, protein encapsulation in RM, and phase separation; soluble proteins in the aqueous core are then retrieved from the reverse process. The protein extraction by the RM approach is affected by several factors, such as water content, pH, ionic strength, temperature, surfactant type and concentration, and solvent type. Moreover, the characteristics of RM features, including size, shape, and structure, are contingent upon the solvent used and influence the quantities of biomolecules transported. Ionic surfactants, such as sodium salt of sulpho-succinic acid bis (2-ethylhexyl) ester (AOT), are often used in food-related applications (Milica Pojića,∗, Aleksandra Mišana, 2018; Prandi et al., 2022). The primary mechanism behind FE is the electrostatic interaction between ionic surfactant molecules and protein molecules. Consequently, pH and ionic strength, which influence protein charges, are critical components in the RM extraction process. The combination of microwave-assisted extraction has been shown to enhance the effectiveness of RM extraction from plant materials. The research conducted by Cao et al., (2023) optimized the process parameters for isolating walnut protein through the combination of RM and microwave extraction using response surface methodology. The optimal conditions were determined to be an extraction time of 30 minutes, a temperature of 45 °C, and a solvent ratio of 3:1 (v/v), resulting in an extraction yield of 95%. Likewise, the capacity, oil retention capacity, foaming, and emulsifying capabilities of the walnut protein extracted by the combination technique were superior to those obtained via the RM-only method.

2.5 Subcritical Water Extraction

The supercritical water extraction (SWE) technique is a recent innovation in the extraction of chemicals from agricultural biomass. SWE technology provides reduced manufacturing costs, is devoid of chemicals, and has abbreviated production durations compared to traditional procedures (Qin et al., 2024). Figure 6 shows the principle of Subcritical water extraction for proteins extraction. Subcritical water refers to water maintained at enough pressure (1–22.1 MPa) to preserve its liquid form at temperatures ranging from the boiling point (100 °C) to the critical point (374 °C) of water. At elevated temperatures and pressures, hydrogen bonds in water are broken, resulting in reduced viscosity and surface tension; nonetheless, the enhanced diffusivity facilitates improved penetration into matrix particles.

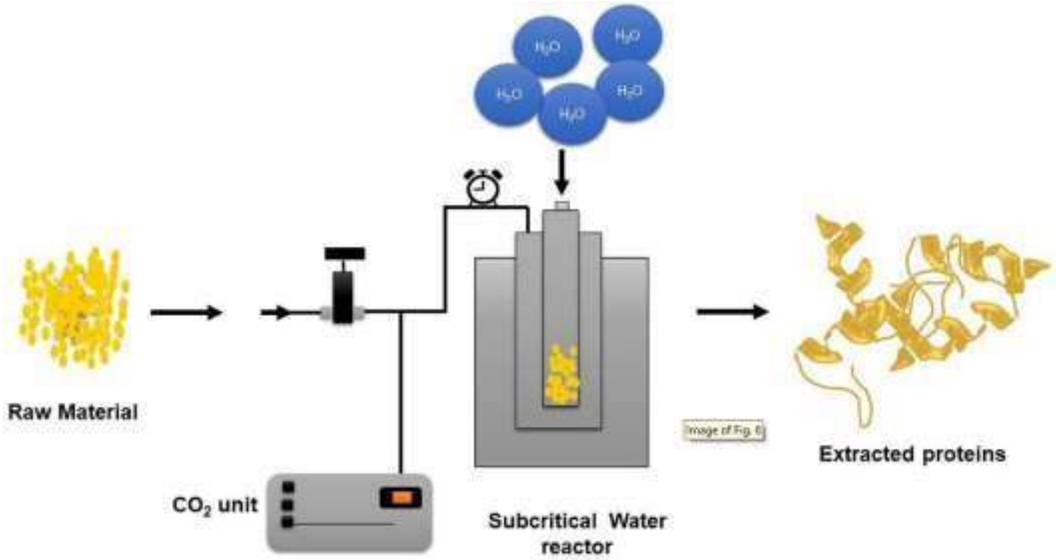

Figure 6 Principle of Subcritical water extraction for proteins extraction (Hewage et al., 2022) (Reproduced with permission from Elsevier).

Maintaining water in a liquid condition at temperatures beyond the boiling point necessitates the application of enough pressure, which also enhances the solubility of analytes inside the matrix's pores (Chatterjee et al., 2015; Fatima, Singh, Pandey, et al., 2024). Interactions between solute-matrix and solute-solute may be affected by energy provided by subcritical water, hence decreasing the activation energy required to perform the desorption process. Furthermore, increased pressure might facilitate the transport of water into the matrix. Elevated temperatures lead to decreased polarity of subcritical water, enabling the segregation of compounds into polar, medium-polar, low-polar, and non-polar categories. Numerous parameters, such as temperature, solvent rate, particle size, extraction duration, and solvent-to-sample ratio, are recognized to affect the effectiveness of supercritical water extraction (SWE) in isolating chemicals. The effectiveness of SWE for protein extraction from pumpkin, flax, and hemp seed cakes at 2 MPa under three different conditions (N2, CO2, and N2 + catalyst) was reported by Kumar et al., (2021). The extraction efficacy was highest with SWE in the CO2 atmosphere, followed by SWE in the N2 + catalyst atmosphere. Proteins separated using SWE (200 ◦C, 20 min, pH 7, and 4 MPa) from cyanobacteria (Arthrospira platensis), pasture grass, and brewer's leftover grain were compared to those isolated by alkaline extraction and aqueous extraction in terms of yield and purity.

To extract protein from heat-denatured soybean meal, Fatima et al., (2024) examined the effectiveness of protease M hydrolysis (concentration at 4% w/w, pH 4.5, temperature of 50 ◦C for 10, 30, 60, 90, and 120 min) followed by SWE. Comparing SWE treatment (120 ◦C for 20 min) to alkaline-acid precipitation (16.40%) at pH 9 and 25 ◦C, a significant improvement in extraction yield (59.3%) was seen. However, there were no alterations seen in the isolated proteins' 80% purity. However, compared to its untreated counterpart, the protein extracted utilizing combination treatments exhibited a notable increase in emulsifying capacity (Parandi et al., 2024). To improve the extraction yield and techno-functional characteristics of the isolated protein, SWE is a useful and eco-friendly technology that can be used either alone or in conjunction with other extraction techniques. SWE does have several limitations, however; one of the main ones is the high implementation costs, especially during industrialization (Mathews et al., 2023; Qin et al., 2024).

2.6 Pulse Electric Field-Assisted Extraction

The food sector has recently paid close attention to pulsed electric field (PEF), an environmentally beneficial technology. PEF has shown encouraging promise in a wide range of applications, including the extraction of biomolecules, the breakdown of pesticide residues, food preservation, and the stimulation of active components in biosynthesis (Hewage et al., 2022). Furthermore, because of its nonthermal properties, shorter processing times, chemical-free processing, reduced energy consumption, increased yield, and environmental friendliness, PEF has a higher average than traditional solvent extraction procedures. Which, taken as a whole, marketed this technique as a cutting-edge method of extracting proteins from plants. Electric waves with a high voltage amplitude (typically 10–80 kV/cm) are employed in PEF technology to treat the product that is deposited between the electrodes in the chamber. These waves are comprised of electrical impulses that last from microseconds to milliseconds. Electroporation (100–300 V/cm- batch mode) and electro-permeabilization (20–80 kV/cm-continuous mode) can be induced by PEF, contingent upon the voltage applied. However, the efficacy of PEF is contingent upon the process conditions, including the electric field, strength (kV/cm), pulse frequency, pulse breadth, the shape of the pulse wave, and exposure duration (which is influenced by the flow rate and volume of fluid in the electrode chamber). The utilization of PEF for the extraction of plant-based proteins has been demonstrated. For the treatment of rapeseed stems and leaves, Yu et al. (2015) employed PEF at room temperature (20 ◦C) with high electrical field intensities (5 kV/cm and 20 kV/cm for 2 ms) and moderate (800 V/cm for 200 ms). The protein yield from rapeseed leaves could be enhanced by up to 80% by an electric field intensity of 20 kV/cm, according to the authors. It was proposed that a certain threshold of electrical field intensity must be exceeded to achieve a high protein yield (Chatterjee et al., 2015; Ravindran et al., 2024).

Figure 7 shows the schematic of Pulse Electric Field-Assisted Extraction of proteins. The protein extraction efficacy of PEF has been further enhanced through the investigation of its combined treatment with other technologies. Hewage et al., (2022) conducted a study on protein extraction from green microalgae Ulva spp using osmotic shock, PEF, and hydraulic pressure. The authors discovered that the highest protein yield of 53.8 µg/ml was achieved when PEF was used with 26 kV, 7.26 kV/cm, 50 pulses, and 2.3 µs. Nevertheless, there is not much information about the mechanism of action of PEF on protein functioning. Consequently, more study is necessary to thoroughly clarify the PEF mechanism associated with protein functioning from various plant sources. Despite its potential as a sustainable processing method, PEF has obstacles in scaling up.

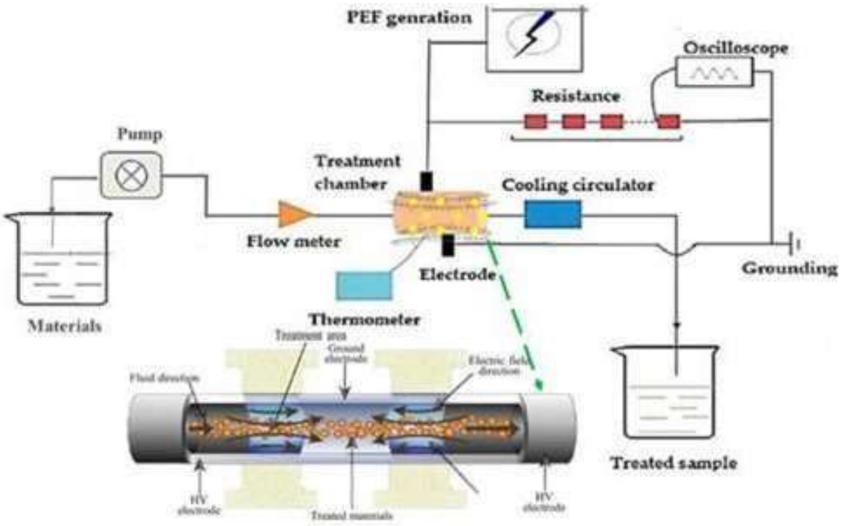

Figure 7 Schematic of Pulse Electric Field-Assisted Extraction of proteins (Fan et al., 2022)(Reproduced with permission)

There is a paucity of basic research on optimum process conditions for alternative cash crops. Furthermore, because of PEF's compatibility with easily flowing substances (liquid materials), it is necessary to design a specialized treatment chamber for PEF to handle solid materials, hence increasing installation costs significantly. This would be the primary impediment to the industrial implementation of this approach (Momen et al., 2021).

2.7 Alkali Based Protein Extraction

The most popular traditional technique for extracting plant-based proteins is alkali extraction. In order to maintain a basic pH and get a better extraction yield than organic extraction, alkali such as NaOH and KOH are often used. Protein recovery and yield were enhanced because the disulphide linkages in the protein were broken by the basic pH. Because acidic and neutral amino acids ionize at high pH values, proteins become more soluble as the solvent's pH rises (Fatima et al., 2024). Alkaline extraction is dependent on temperature, which helps stabilize the protein structure and folding and aids in maintaining covalent connections within the protein structure. Proteins are hydrolyzed into oligo-peptides by heat energy at temperatures over 140 ◦C. This study used an alkaline extraction method to extract proteins from sunflower meal. The optimal conditions were 67 g/L of sunflower meal to water, an extraction time of 1 hour, and a pH of 9. After that, they performed a second extraction using isoelectric point precipitation and spray drying, and the protein recovery was 70.4% on a dry weight basis. Several studies have shown that these variables can be optimized to achieve maximum protein yield at a low cost. Consequently, larger protein yields are obtained when protein is extracted in an alkaline environment (Ravindran et al., 2024).

3. Physical Extraction Methods

Techniques that use mechanical or physical forces to break cells and liberate proteins are known as physical protein extraction methods. The choice of method depends on the type of cells, the desired volume of protein extract, and the sensitivity of the proteins to shear forces or temperature changes.

3.1 Extraction using Water

Protein concentrates may be obtained by eliminating insoluble components such as sugars, small proteins, and flavours and odours using acidified water for sample extraction, with the pH adjusted to the isoelectric point of proteins. The residue included insoluble proteins, polysaccharides, and some minerals according to the extraction pH. A protein concentrate with a minimum concentration of 65% was created by neutralizing the extracted residue with an alkali solution and then freeze-drying it (Hewage et al., 2022).

3.2 Ultrasound-assisted extraction

The process in the UAE has attracted significant interest in the extraction of compounds from both plant and animal sources. The UAE technique involves a physical extraction process that enables rapid and efficient extraction of target compounds from food materials through the disruption of cells using ultrasound waves. Ultrasound waves (UW) with a frequency exceeding 20 kHz possess significant energy as they propagate through a medium (Özyurt et al., 2021). Alternatively, UW undergoes compression and stretching as it moves through a medium, resulting in the formation of localized negative pressure buildup. During the stretching phase, known as "rarefaction," the pressure generated is adequate to surpass intermolecular binding forces, leading to the formation of bubbles or small cavities within the medium. This occurrence is referred to as "cavitation". The growth of these bubbles or cavities occurs with each successive cycle, ultimately leading to a violent collapse; as a result, a significant amount of energy is released into the system (Prandi et al., 2022). Figure 8 shows the schematic of principle of UAE of proteins. The collapse of bubbles results in a localized pressure surge exceeding 400 MPa, capable of disrupting cell membranes. This disruption aids in the movement of solvent in and out of the cell, along with the release of target compounds. As a result, the disruption of biological cell walls will ultimately result in the release of cellular contents. The effectiveness of UAE in protein extraction is influenced by various factors, such as ultrasonic power, extraction duration, the liquid-to-solid ratio, and extraction temperature. Consequently, it is essential to optimize these parameters to establish optimal extraction conditions for each material (Bouloumpasi et al., 2024).

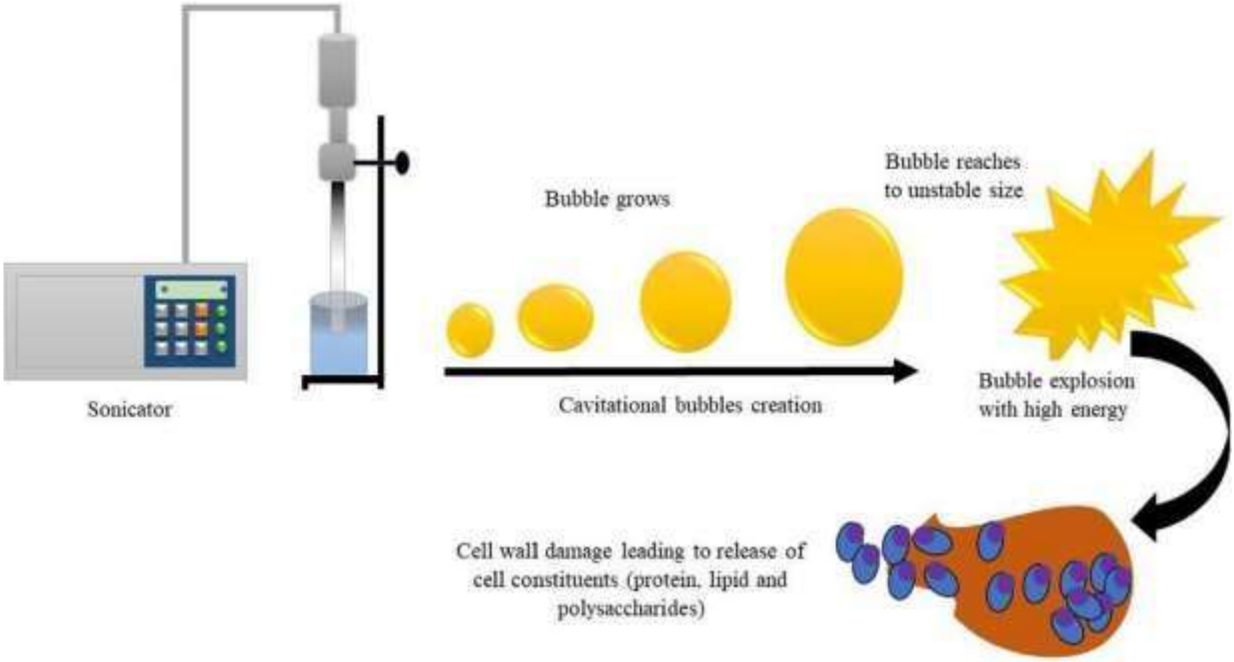

Figure 8 Schematic of principle of UAE of proteins (Hewage et al., 2022) (Reproduced with permission from Elsevier).

The utilization of ultrasound-assisted extraction for obtaining plant protein has been successfully demonstrated. In the investigation conducted by Prandi et al., (2022), the recovery rate of coconut protein extracted through UAE (24 kHz; 2.5 min; 6.85 W/cm2, 0.573 kW/kg) was analyzed in comparison to those obtained via MAE (4.31 kW/kg by pulse, 3 pulses of 20 s, 1 min, 2.5 GHz) and the conventional method (pH adjustment). The authors indicated that the protein recovery rate was highest for UAE, followed by MAE, with the conventional method yielding the lowest rate. According to Nuchdang et al., (2022), the ideal conditions for extracting watermelon protein through ultrasound-assisted extraction included a ratio of 1:50 w/v, a temperature of 30 ◦C, ultrasound power of 90 W, a frequency of 25 kHz, and a duty cycle of 75% for pH, powder to solvent ratio, temperature, ultrasound power, frequency, and duty cycle, respectively, achieving an extraction rate of 87%. The influence of the UAE on the techno-functional characteristics of plant- based protein has been recorded. The protein obtained from watermelon seed through UAE demonstrated superior water-holding capacity, oil-holding capacity, emulsifying capacity, and stability compared to that extracted via conventional methods. In a similar vein, the functional properties and protein digestibility of protein extracted from cold-pressed sesame seed meal showed enhancement when utilizing UAE, in contrast to traditional methods. Consequently, the UAE demonstrated significant effectiveness in enhancing the yield, functionalities, and digestibility of the extracted plant proteins. Nonetheless, the utilization of UAE for the extraction and modification of plant proteins remains in its nascent phase. The significant installation cost, along with the unintended structural changes caused by ultrasound, further complicates its path to industrial adoption (Singh et al., 2022). Table 2 presents the summary of protein extraction using Ultrasound-assisted extraction method.

Table 2 Summary of protein extraction using Ultrasound-assisted extraction method.

Sr.No.	Natural Material	Extraction Conditions	Yield of Proteins recovered (%)	Reference
1	Sesame	Ultrasound intensity- 55 W/cm^2 pH- 9.5 Time- 98 min Temp- 45 °C Sample to solvent ratio- 1:10 (w/v) Pressure- 2 bar	59.80	(Görgüç et al., 2019)
2	Spirulia	Ultrasound intensity- 55 W/cm^2 Time- 15 s Temp- 49 °C Sample to solvent ratio- 1:20 (g/g) Pressure- 2 bar	28.42	(Vernès et al., 2019)
3	Rice Bran	Ultrasound intensity- 20 kHz pH- 7 Time- 2 min Temp- 55 °C Sample to solvent ratio- 1:10	64.5	(Liu et al., 2019)

		(w/v)		
4	Wampee seed protein	pH- 12 Time- 64 min Sample to solvent ratio- 1:29 (w/v) Power- 240 W Power Intensity- 40 kHz	15.06	(Liu et al., 2019)
5	Plum seeds	pH- 7.5 Time- 1 min Sample to solvent ratio- 35 mg/ 5 mL (w/v) Amplitude- 30%	38.6	(González-García et al., 2014)
6	Coconut Milk	Time- 2.5 min Frequency- 24 kHz Power- 0.573 kW/kg	86.1	(Martínez-Padilla et al., 2022)
7	Sunflower Meal	Power Density- 220 W/L Temp- 45 °C Time- 15 min Sample to solvent ratio- 0.5/10 (w/v)	54.26	(Dabbour et al., 2018)

		pH- 8		
8	Ganxet beans	Ultrasonication Power- 40 kHz Time- 60 min Temp- 4 °C pH- 12.95 Solid to liquid ratio- 1.10 (w/v)	78.73	(Lafarga et al., 2018)

3 Microwave Assisted Extraction

Microwaves are a form of non-ionizing electromagnetic radiation, characterized by frequencies that span from 300 MHz to 300 GHz. The microwave heats the sample through the combined effects of dipole rotation and ionic conduction, resulting in the disruption of hydrogen bonds found in the cell wall of the plant matrix. This reaction enhances the porosity of the cell wall, allowing for improved solvent infiltration into the cell and more effective release of intracellular compounds into the solvent system. The conversion of microwaves into heat energy within the matrix leads to moisture evaporation, resulting in increased pressure on the cell walls (Prandi et al., 2022). MAE is conducted in either an open or closed vessel system, contingent upon the specific temperature and pressure conditions. An open system is appropriate for treatments conducted under ambient conditions, while closed systems are best suited for scenarios involving high temperature and high pressure. The optimal conditions for extracting protein from rice bran were determined to be a microwave power of 600–800 W and a duration of 100 seconds. The rotation of dipoles induced by microwaves disrupts hydrogen bonds and increases solvent penetration into the sample due to ion migration. This facilitates the release of intracellular materials (proteins) into the solvent media by disrupting the components of the cell wall. MAE produced 1.54 times more protein compared to a chemical method utilizing alkaline solvents (Fatima, Singh, Kumar, et al., 2024; Li et al., 2023). MAE offers numerous benefits over traditional thermal extraction methods, including uniform heating, enhanced extraction rates, reduced solvent usage, and decreased extraction times (Lee et al., 2017), which makes it an ideal choice for solid- liquid extraction. Figure 9 shows the schematic representation of the microwave-assisted extraction apparatus

The microwave treatment produces a significant amount of thermal energy, leading to the degradation of heat-sensitive bioactive compounds, rendering them inappropriate for protein extraction. Utilizing brief pulses of microwaves or optimizing microwave input parameters presents a viable alternative for the efficient extraction of plant proteins. The primary advantages of this technique include reduced extraction time and a lower need for solvents. The sequential application of microwaves alongside other physical or biochemical techniques can enhance the efficiency of protein extraction (Ravindran et al., 2024). Table 3 presents the summary of protein extraction using microwave-assisted technique.

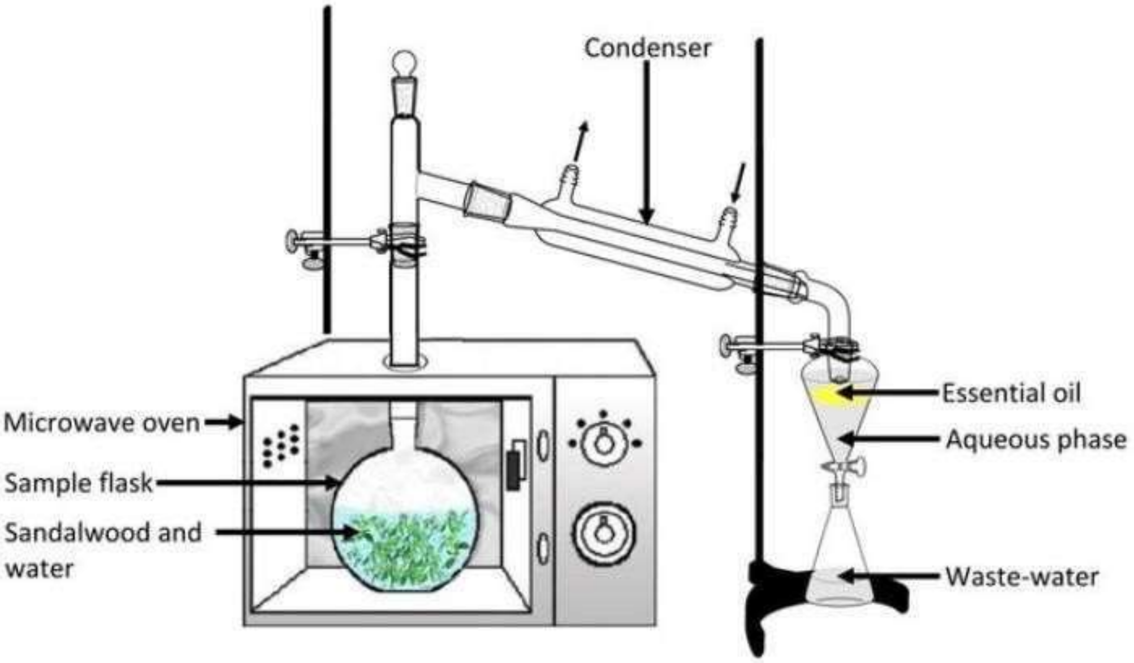

Figure 9 Schematic representation of the microwave-assisted extraction apparatus (Kusuma and Mahfud, 2016) (Reproduced with permission)

Table 3 Summary of protein extraction using microwave-assisted technique.

Sr.No.	Natural Material	Extraction Conditions	Yield of Proteins recovered (%)	Reference
1	Rice Bran	Microwave Power- 1000 W Time- 90 s Sample to Solvent Ratio: 0.89 g/ 10 mL	77.27	(Phongthai et al., 2016)
2	Sesame seed meal	Temp- 87.8 °C pH- 8.0 Time- 37 min Microwave Power- 850 W	43.53	(Sá et al., 2022)
3	Peanuts Flour	Time- 8 min Microwave Power- 725 W Sample to Solvent Ratio: 1:10 (w/v)	55.53	(Ochoa-Rivas et al., 2017)
4	Jackiopsis ornata roots	Liquid to solid ratio- 26:1 (v/w) Temp- 65 °C Microwave Power- 300 W Extraction Time-20 min	20.47	(Abugabr Elhag et al., 2019)

5	Coffee Silverskin	Solvent Concentration- 0.6 M Time- 10 min Microwave Power- 434.7 W Temp- 87.8 °C	43.53	(Wen et al., 2021)

3.4 High Pressure Extraction

The achievement of HPAE occurs in three distinct stages. The product is first combined with extraction media and then positioned within the pressure vessel. The pressure is elevated from ambient to the necessary level within a brief timeframe. The fluid pressure typically varies between 100 and 1000 MPa. With the rise in pressure, the differential pressure between the interior of the plant cell and its surroundings escalates, leading to cell deformation and potential damage to the cell wall. The solvent infiltrates the compromised cell wall and membrane, facilitating the mass transfer of soluble compounds within the cell. Should the compression force remain within the deformation limit of the cells, the solvent will permeate through the cell walls under pressure, rapidly filling the cells. The bioactive components subsequently dissolve directly in the solvent. When the compression of the product surpasses the deformation limit of the cell, the cell wall breaks, allowing active compounds to escape and dissolve in the solvent. During the pressure maintenance phase, the established pressure is sustained for a duration to equilibrate the pressure within and outside the cell. The solvent persists in its penetration through the cell wall, effectively dissolving the components present. Extending this stage can enhance the extraction yield (Fatima et al., 2024).

In the final stage, the release of pressure leads to a decrease in the pressure within the cell to atmospheric levels, resulting in the expansion of the cell and subsequent deformation. A shorter pressure release time leads to the formation of more pores within the cells, which enhances the surface area of the raw material and facilitates the diffusion of an active compound, ultimately resulting in increased extraction efficiency. The effectiveness of HPAE is influenced by various factors such as extraction pressure, duration of operation, characteristics and concentration of the extraction solvent, and the solid-liquid ratio. Recently, a variety of studies have been published regarding the application of high hydrostatic pressure for the extraction of bioactive compounds from food and herbs. Segatto et al., (2022) created a response model aimed at forecasting the concentration of protein extracted through high hydrostatic pressure, based on the applied pressure ranging from 100 to 300 MPa and the type of solvent utilized, such as phosphate buffer saline, trichloroacetic acid, and Tris-HCl for extraction. Figure 10 shows the high pressure extraction based static and dynamic configuration for protein extraction HPAE is a developing non-thermal method that holds significant promise in food safety, as thermal treatments can compromise the nutritional value of food. This leads to the availability of fresh, minimally processed food that aligns with the growing consumer demand. Applying high pressure can effectively inactivate microbes and enzymes, as well as modify cell structure, all while preserving the sensory quality of the food. This method can also be effectively applied for extracting bioactive compounds and biomolecules from plants and herbs. The extraction process demonstrates increased speed, achieving a high yield while ensuring minimal impurities in the final product. The extraction process occurs at ambient temperature, thereby preventing thermal degradation of heat-sensitive components and nutrients. HPAE is increasingly recognized as an eco-friendly extraction method, making it a popular alternative to traditional solvent-based extraction techniques. The utilization of HPAE in conjunction with enzymatic, microwave, or ultrasound treatment presents an opportunity to enhance the extraction of plant proteins (Qin et al., 2024).

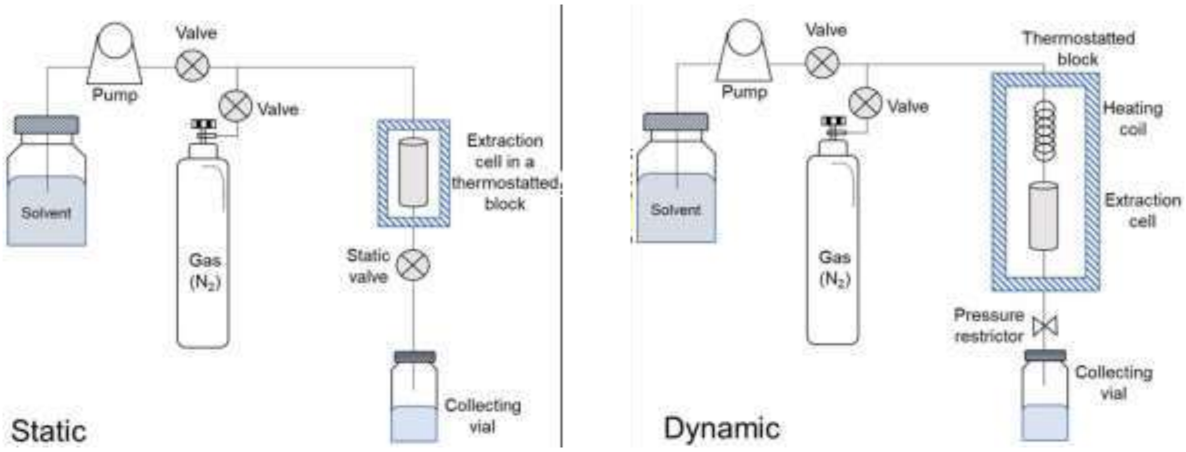

Figure 10 High pressure extraction based static and dynamic configuration for protein extraction (Barp et al., 2023) (Reproduced with permission)

4. Futuristic Prospectus

Protein extraction is an essential process across several sectors, including food manufacturing and medicines. Conventional techniques often use corrosive chemicals, substantial energy expenditure, and protracted processing durations. The future of protein extraction is dependent on the integration of innovative technology and sustainable methodologies. This prospectus presents an innovative strategy for protein extraction, combining advanced methodologies with an emphasis on environmental sustainability and economic feasibility. Tailor protein extraction techniques to meet individual dietary requirements and tastes, facilitating the production of specific protein products. Devise innovative extraction methodologies for the separation of certain proteins for therapeutic purposes, including personalized and regenerative medicine. Design compact and effective protein extraction devices for use in space exploration missions, facilitating the synthesis of food and other vital resources in alien settings.

The global protein concentrate market is showing strong support and is anticipated to experience significant growth in the upcoming years. Consequently, further methods and innovations are necessary. The global protein concentrate market is propelled by a growing interest among consumers. The market for protein concentrates is experiencing growth due to the increasing interest among young individuals in bodybuilding and fitness. The increasing prevalence of lifestyle diseases is raising health concerns among working professionals, which is contributing to the growth in sales of upstream protein concentrate products. The growing utilization of protein concentrates in various products, including cheese, biscuits, cereals, flavoured shakes, and beverages, stands out as a significant market driver. The demand for these downstream protein concentrates products is anticipated to rise during the forecasted period.

4.1 Why chosen ultrasound-assisted extraction process

Ultrasound-assisted extraction (UAE) is distinguished as a favoured technique for protein extraction relative to other methods owing to numerous significant benefits. The UAE employs high-frequency sound waves to produce small bubbles in the extraction medium. These bubbles swiftly enlarge and contract, generating locally elevated temperatures, pressures, and shear stresses. Cavitation destroys cell walls, enhances mass transfer, and facilitates the release of proteins from the source material. The UAE may modify the protein structure, enhancing its solubility in the extraction solvent. This results in increased protein production. UAE can be applied to a wide range of protein sources, including plant materials, animal tissues, and microorganisms. In summary, the UAE offers a compelling combination of efficiency, speed, and mildness, making it a highly attractive option for the extraction of proteins from various sources.

5. Conclusions

Recent advancements in eco-friendly extraction technologies offer benefits such as enhanced safety, improved extraction yield, and a reduced negative impact on the techno- functional properties of food proteins compared to traditional methods. Nonetheless, the majority of these approaches have primarily been executed at a laboratory scale, indicating that commercial adaptation remains in its early stages. Consequently, it is essential to engage in comprehensive investigation and development efforts to comprehend, enhance, and implement those intricate processes to boost their utilization in food industries, especially within the plant protein domain. Therefore, it is crucial to examine the economic feasibility and cost efficiency of scaling up processes for these innovative yet eco-friendly technologies. Additionally, the potential mechanisms or alterations in protein structures require investigation with novel extraction techniques, as these significantly influence the physicochemical and functional characteristics of proteins. Moreover, it is essential to focus on energy efficiency, environmental impact, and coproduct generation to advance new technologies that improve process efficiency, product quality, and environmental sustainability.

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