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Muhammed Risvan K
Department of Food Technology and Nutrition, School of Agriculture, Lovely Professional University, Phagwara, Punjab, India

Annie Jenet Jacob
Department of Microbiology, School of Bioengineering and Biosciences, Lovely Professional University, Phagwara, Punjab, India

Corresponding Author:
Muhammed Risvan K
Department of Food Technology and Nutrition, School of Agriculture, Lovely Professional University, Phagwara, Punjab, India

Extraction of protein from non-conventional sources

Muhammed Risvan K and Annie Jenet Jacob

Abstract
This article reviews numerous extraction techniques involved in protein extraction from non-conventional sources. New feeding strategies have been created in recent years as a result of the current demand for increased food output and concurrently growing environmental concerns regarding livestock systems. One of these tactics, which tries to lessen rivalry with food, is to use byproducts from the agro-food sector as substitute feed for animals. Knowledge of feed quality in relation to its impact on animal productivity and the environment is essential because feed is a significant input in all animal production systems. Increasing our knowledge of the nutritional value of innovative feedstuffs with an emphasis on byproducts is the goal of this research.
Due to their positive health effects, plant-based proteins are becoming more and more popular and are being seen as a potential alternative to animal proteins in the creation of sustainable food systems. Plant leaves frequently have protein levels comparable to those of milk, making them an effective source of protein for use in global food production. Thus, we can have a comparison of overall utilization of plant protein extraction and an economic impact of its usage amongst human needs in daily basis. Relative analysis of protein quality and nutritional value of protein extracted from nonconventional source to traditional sources are also discussed.

Keywords: Non-conventional sources, protein content, sustainable food systems, Plant protein extraction

1. Introduction
1. Non-Conventional Sources
Natural products that already contain carbohydrates, proteins, and fats can be modified physically, chemically, microbiologically, or enzymatically to produce non-conventional food sources. These natural products can contain chemicals like carbohydrates, hydrocarbons, and industrial organic chemicals. The nutritional worth of non-conventional foods for people and animals, as well as their functional value in terms of food quality, social and cultural acceptance, are required (M Alongi and M Anese, 2021) [1].
They can also be created from natural products that already exist and contain carbohydrates, proteins, and fats by modifying them physically, chemically, microbiologically, or enzymatically (S Raveendran *et al*., 2018) [2].
Nontraditional foods include single-cell proteins, dried cells of microorganisms such algae, bacteria, actinomycetes, yeasts, moulds, and higher fungi, as well as protein isolates and concentrates made from them. Products made from plants and animals, such as isolates and concentrates of soy, cotton, and leaf meals and protein concentrates, as well as seed meals and protein from them (J H Lichfield *et al*., 2000) [3].
The term "non-conventional feed resources" (NCFR) refers to all feeds that are either traditionally not used in animal feeding or that are not commonly included in commercially prepared livestock diets. About 22 amino acids make up protein, eight of which are necessary because the body cannot make them on its own. Consequently, we must consume food to get them (Adetunji and Adepoju, 2009) [4]. Feeds having both animal and industrial origins are frequently included in NCFR, as well as a variety of feeds made from perennial crops. In order to ensure quality, social acceptance, and animal or human nutritional value, non-conventional meals must also have functional value. Additionally, they must adhere to the standards set by regulatory bodies for food safety, including being free of pathogenic microorganisms and poisonous, mutagenic, and carcinogenic compounds (Klostermeyer *et al*., 2000) [5].
Agriculture and forestry by-products are the primary sources of these feeds. One of the main issues influencing livestock output in underdeveloped nations is inadequate nutrition and bad management techniques (Dieumou *et al*., 2013) [6]. Adequate consumption and absorption of enough dietary minerals are necessary for the body to receive the proper nourishment and sustenance.

Due to the rise in popularity of vegetarian diets, plant-based proteins are currently becoming more and more popular. Additionally, there is a growing understanding of how producing animal protein harms the environment, and there are increasing initiatives to produce protein in a sustainable way (L H Litchfield *et al.*, 2000) [3]. Animal feed resources that aren't typically used to make feed for animals are known as non-traditional animal protein (NACP) sources (C N Ncobela *et al.*, 2015) [7]. Due to their high nutritional profile and ability to lessen feed-food competition, in comparison to traditional feedstuffs, insects offer a sustainable feed component. The majority of the cost of feed is attributable to the price of protein-rich ingredients including fish meal, soybean meal, and groundnut cake (O J Olarotimi and Adu, 2017) [8].

Mainly the focus is on the non-conventional sources of protein including plant-based sources, algae, insects, and microbial sources; and include several factors in comparison with protein quality and nutritional value of protein extracted from non-conventional sources to traditional protein sources. The effect of extraction and various parameters are also a rising feature in protein extraction (Zsedely *et al.*, 2023) [9].

1.1 Extraction of protein from plant-based sources

Humanity has traditionally used traditional plant proteins like soy, beans, and pea as a source of protein. Brazilians refer to the local cactus *Pereskia aculeata* Miller as "ora-pro-nobis" (OPN). The access of the current method was to evaluate protein content, protein digestibility in vitro, proximate mineral, vitamin, and other nutrient composition of the nutritional components. OPN leaves have outstanding amounts of total dietary fibre, minerals (Ca, Mg, Mn, Zn), and vitamins (vit A, vit C, and folic acid) (39.1% dry basis) (Takeiti *et al.*, 2009) [10].

Table 1: The amount of amino acids that are present in various food sources of protein

Food source	Threonine	Tryptophan	Lysine	Sulfur amino acids
mg/protein	38±3	12±4	64±10	25±3
Legumes	32±4	12±2	31±10	37±5
Cereals	36±3	17±3	45±14	46±17
Nuts, seeds	29±7	11±2	45±12	27±6
Animal foods	44±6	12±6	85±9	38±7

- This table lists the amount of amino acids that are present in various food sources of protein.

1.2 Dairy-based protein sources

Dairy products play a significant role in the diets of many individuals. Many delicacies, including cheese, yoghurt, milk, butter, and ice cream, are made with milk from cows, sheep, and goats. Due to their higher and occasionally comparable Crude Protein (CP) content to conventional plant-derived dietary protein sources used in bovine feed, Marula nut meals may be employed as dietary protein sources in feeds (Oladimeji *et al.*, 2008) [11].

A few non-conventional protein sources as algae, grains/cereals, fresh produce, insects, mycoprotein, nuts, seeds, and legumes are a few examples. There are considerable health advantages of non-traditional protein sources over conventional proteins (meat/poultry, soy, eggs, dairy), according to data from humans, animals, and in vitro trials. The health-related endpoints with the most often reported benefits were those in glycaemic management, muscle synthesis, lipid metabolism, and cardiovascular health (Caroline Bull *et al.*, 2022) [12].

- **Eggs**- A nutritious powerhouse, eggs are. They give the body 13 vitamins, minerals, a good source of protein, and crucial antioxidants. Eggs are a great addition to a wellbalanced diet since they are tasty, practical, and affordable.
- **Milk**- While some products lack the calcium and vitamin D fortification that makes dairy milk comparable to other sources. Vitamin B12 may also be added to some substitute milks. Among other things, milk can be taken as a beverage, added to smoothies, or poured over cereal. A item that is rich in calcium, carbs, and protein is milk, which is also nutrient-dense. In reality, 1 cup (237 ml) of whole milk has 146 calories, 8 gms of fat, 8 gms of protein, and 13 gms of carbs.
- **Yogurt** – Particularly versatile is yoghurt. Per cup (236 ml), whole-milk yoghurt has 149 calories, 8 gms of fat, 9 gms of protein, and 11 gms of carbs. 149 calories, 8 gms of fat, 9 gms of protein, and 11 gms of carbs are found in one cup of whole-milk yoghurt.
- **Cottage cheese** - Dairy-free substitutes typically have lower protein contents, and some varieties include as many as 8 gms of carbohydrates per ounce (28 gms), compared to dairy cheese, which rarely has more than 1 grammes per ounce.

1.3 Soy and plant-based protein sources

Milk, yogurt, and dairy cheeses all include complete proteins. But soy is the only plant-based substitute which replicates the quantity and calibre of protein. The inclusion of soy proteins in hypocaloric diets for weight loss in obese people has also been demonstrated to be effective (van den Berg *et al.*, 2020) [13]. To extract the Marula oil from the nuts (kernels), various pressing techniques are used is one of the plant-based technique and source obtained as a byproduct, nut meal is produced. The research chemically investigated the nutritional content of Maula nut meal and assessed its potential as a source of nutrients in livestock feeds since it have biomass content and may be a source of nutrients (J. Gandure *et al.*, 2011) [14].

Based on a review of studies on growth and nitrogen balance in newborns, children, adolescents, and adults, the nutritional value of processed soy protein (Isolated soy proteins and soy-protein concentrates) in human protein and amino acid nutrition is assessed (van den Berg *et al.*, 2020) [13].

Since the total number of raw materials available is considerably more important thus alternative protein-containing source materials could either have a high (like legumes or insects) or decisive factor. A substance with a lower protein concentration may have more protein overall than a source with a higher protein concentration, if that substance is abundant. Pricing and availability are also impacted by cascading effects, or the usage of additional material fractions as primary extraction targets. For instance, several other proteins are now made from grains or oils that were formerly utilised in lower-value applications like animal feed (Such as potatoes or soybeans). (Grossmann & Weiss, 2021) [15].

- Tofu is a high-protein food that contains every necessary amino acid that the body requires. It also includes a range of carbohydrates, lipids, and vitamins.

- **Soybeans** - A cup (172 g) of boiling soybeans has about 29 grammes of protein, one cup of soy milkmilk contains 7-9 gms of protein
- **Beans and legumes** -Fiber & B vitamins are abundant in beans and other legumes. They provide an excellent meat substitute for vegetarians and are also a great source of protein.
- **Chickpeas or other dried beans**- As much plant-based protein is found in one-half cup of beans as there is in one ounce of grilled steak.
- **Quinoa and other grains**-Cornmeal, kamut (wheat berries), teff, quinoa, whole-wheat pasta, wild rice, millet, couscous, muesli and buckwheat are among the grains high in protein.
- **Seeds (chia, flax, sunflower, pumpkin, etc.)** – Plant-based milk alternatives can be created using a variety of grains, legumes (soy), cereals (oats, rice), nuts (almond, coconut), seeds (flax, hemp), and other grains.
- Importance of various food groups' per capita of intake is given as follows:

Table 2: Importance of various food groups per capita of intake

	Proteins (g)	Proteins (%)	Energy (Kcal)	Energy (%)
Total Plant	46.1	65	2277	84
Cereals	33.7	47	1385	5551
Pulses, nuts	6.0	8	109	4
Starchy roots	2.0	3	141	5
Other vegetables	2.5	4	46	2
Fruits	0.8	1	65	2
Total animal	25.0	35	433	16
Total	71.1	100	2710	100

1.4 Algae based protein sources

Other than the usual ones like diet and health, people are consuming more algae and there is a growing demand for macroalgal and microalgal meals on a global scale. Although there is ample proof that foods made from algae are healthful, it is still exceedingly challenging to quantify both these benefits and any potential drawbacks. (M L Wells *et al*., 2017) [16].

Efficient extraction techniques can raise the protein content in macroalgae. Various techniques are possible, including traditional and cutting-edge processing technologies like enzyme- or Pressurised liquid extraction, supercritical fluid extraction, pulsed electric field, and microwave-assisted extraction (Bleakley *et al*., 2017) [17].

Microalgae are a rich source of nutrients and bioactive substances. Microalgae have the power to treat or prevent a number of acute and chronic illnesses. Why because it requires a lot of investment, microalgae are underutilised as a crop. A microalgal biorefinery strategy can promote market expansion that is sustainable (A K Koyande *et al*., 2019) [18].

Algae have an attractive possibility for satisfying the rising need for protein as a source of bioactive substances such as amino acids, polyunsaturated fatty acids, vitamins, and minerals for the enrichment of typical algal diets. (Matos *et al*., 2022) [19].

1.5 Animal-based protein sources

Protein and energy sources in food are costly. Ingredients in feed are needed to increase the productivity of animals. The economic performance of many fields is currently challenged by the global price fluctuations of conventional feed ingredients. Due to processing industrial hemp byproducts has turned into a huge obligation in addition to the expansion of the industrial hemp growing area and the relaxation of planting restriction policy. To fill in the data gaps for industrial hemp by-products and offer theoretical support for their use in the production of dairy cattle, they compared the chemical composition, carbohydrate and protein composition, in situ ruminal degradability, intestinal digestibility, and available energy values of industrial hemp by-products and conventional feeds. (Wang Y *et al*., 2022) [20].

Finding diverse and inventive protein substitutes can be crucial for ensuring food security. Over the past ten years, many protein sources have been used in food and livestock feed. Though the potential of a protein shortage has become more widely recognised as a result of the abuse of these sources (I Saadaaoiu *et al*., 2021) [21].

Because eating meat has related health advantages, the market for animal protein will continue to grow. The usage of dairy and other animal proteins in food and dietary supplements has a considerable impact on consumer demand for these ingredients. Plant proteins' usage in culinary goods has expanded as vegan, vegetarian, and flexitarian diets have grown in favour. Additionally, plant proteins are used to make a number of natural products. (B P Ismail *et al*., 2020) [22].

1.6 Microbial sources

Single-cell proteins and other alternatives might also be viable options for satisfying the demand for protein. The SCP production is helpful in resolving garbage disposal issues by illuminating a green and sustainable path that also lowers production costs. Microbial proteins have several benefits over traditional proteins when used as sustenance. Proteins from microorganisms are a good supply of vitamins, carotenoids, and carbohydrates. Single Cell

Protein (SCP) refers to the "source of mixed protein extracted from pure and mixed culture of bacteria, fungi, algae, and yeast" and is the name given to the protein derived from microbial sources (Tewari *et al*., 2016) [23].

With a clearance effectiveness of 91% for dispersion Violet HFRL, extracellular polymeric substance (EPS) from bacteria was successful in flocculating several disperse dyes in aqueous solutions. Proteus mirabilis' EPS was successful at removing dangerous dye (basic blue 54) from aqueous solution in batch setups. Basic blue 54 had the highest dye uptake rate at 2.005 g/g of EPS. According to Zhang *et al*. (2009), dye adsorption happens as a result of the high molecular weight EPS matrix's multiple binding sites and stronger van der Waals forces that bond with dye molecules. (T. T. More *et al*., 2014) [24].

2. Overview of methods used for extraction of protein from non-conventional sources

These include Microwave-assisted extraction method (MAE), Ultrasound Assisted Extraction, Super Critical Fluid Extraction Method

2.1 Microwave-assisted extraction method (MAE)

A benefit over other technologies and a successful tactic, microwave-aided extraction gives this. To extract several volatile and non-volatile compounds from various sources, the MAE method was created. The use of microwave radiation is the main principle of MAE. (Preethi *et al*., 2018) [25].

The findings of ultrasound-microwave-assisted extraction showed that the highest protein yield of M. oleifera leaves at the conditions (solvent-solid ratio 91:1 ml/g, extraction duration 148 s, extraction temperature 41 °C, microwave power 81 W) was 82.07 mg/g. Compared to the standard solvent extraction method, UAME is more efficient, saves time, and yields a better protein yield. (Lai *et al*., 2001) [26].

This innovative, promising microwave-aided extraction (MAE) technology has a number of enticing qualities, including the capacity to execute extractions quickly and efficiently while using less solvent and the ability to safeguard compounds that are thermolabile. At 9,000 g for 15 minutes at 4 °C, mixture was centrifuged, thus obtained preserved supernatant. Following the stages of conventional solvent extraction, the leaves' proteins were then extracted using acid freeze drying, precipitation and ultrafiltration (Fangfang *et al*., 2021) [27].

2.2 Ultrasound-Assisted Extraction

Improving mechanical impact, which in turn increases mass transfer and solvent penetration into the sample matrix, is the primary notion behind UV extraction. Extraction factors include solvent concentration, solvent/material ratio, temperature, extraction duration, and ultrasonic power that have an impact on how effectively the UAE extracts. An often employed instrument for parameter extraction optimisation is response surface methodology the RSM is an effective mathematical and statistical instrument that bases its analysis on the fitting of experimental data to a polynomial equation. RSM examines the impact of both independent and interdependent parameters (Kleekayai *et al*., 2023) [28].

Traditional solvent extraction methods yielded 68.99 mg/g of protein at 0.15 M Tris-HCl concentration, 38 min of extraction time, and 128:1 (v/w) solvent/solid ratio at 34 °C. Additionally, protein from leaves were extracted using ultrasound-microwave assisted extraction (UMAE), and according to the findings, the optimal extraction parameters were 91:1 (v/w), 148 s, 41 °C, and 81 W of microwave power, with a protein yield of 82.07 mg/g. UMAE has a much higher extraction efficiency than the standard solvent extraction method (Fangfang *et al*., 2021) [27].

2.3 Super Critical Fluid Extraction Method

At its critical point, Superfluid is a material that has both gaseous and liquid-like physical characteristics. CO_2 is a prime illustration of a superfluid because it behaves like a gas while also having liquid-like properties for solvating problems. Due to its outstanding performance as a non-polar analyte solvent and low cost/toxicity, CO_2 is used in extraction. (Preethi *et al*., 2018) [25].

Enzyme-aided extraction of Protein

Protein extraction from different plant sources is aided by an ecologically friendly green processing method known as enzyme-assisted extraction (EAE). This is so that it can be used instead of chemical and physical extraction procedures, which utilise more energy and have harsher working conditions. It generates less garbage as well. By hydrolyzing the plant cell wall and the proteins contained therein, a range of food-grade carbohydrase and protease formulations have been employed to aid in protein extraction/solubilization from various plant sources (Berwal *et al*., 2021) [29]. To get the maximum protein recovery, it is possible to adjust enzyme treatment parameters including the enzyme-to-substrate ratio, pH, incubation temperature, and hydrolysis time using a variety of statistical tools. EAE speeds up protein recovery, but it may also improve the biological, technological, and dietary (digestibility) aspects of the process, and capabilities of the proteins that were extracted, particularly when protease-assisted extraction was employed (Kleekayai *et al*., 2023) [28].

3. Factors affecting Protein efficiency such as type of Non-Conventional Sources

3.1 Protein stability during protein expression and purification

It is crucial to give the expression host the ligands, cofactors, and prosthetic groups that the target protein requires in order to guarantee proper protein folding and stability. During the expression process, the host cells scavenge many of these that are supplied by the expression medium. When expressing mammalian proteins in bacterial cells, host-cell selection is especially crucial because the codon usage of the two species differs. The AGA codon for arginine, for instance, is extremely uncommon in E. coli and can lead to premature chain termination, frame-shifting, and erroneous amino-acid insertion (Deller *et al*., 2016) [30].

3.2 Processing condition and extraction methods used

A) Protein separation through biochemical means

Using a single enzyme or a combination of several, protein is extracted using this method.

These enzymes aid in the release of the proteinaceous components into the neighbouring medium by hydrolysing the cell wall barrier. The physical techniques for extracting proteins from plant materials are B, C, D, and E.

B) Protein separation with ultrasound assistance

The energy waves from the ultrasound cause cavitation bubbles to form at the cell wall's surface, which ruptures the cell's membrane and cell wall (B1) and releases intracellular protein into the surrounding solvent. (B2).

C) Protein extraction with pulsed electric field assistance (PEFAE)

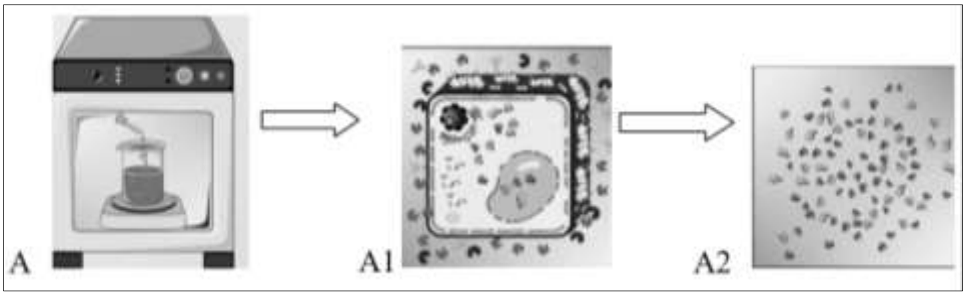
In this method, plant samples are introduced to a strong electric field for a brief period (micro to milliseconds). (C1). The extraction rates are improved because the electric field makes pores in the cell membrane and helps release intracellular proteins into the extracting solvent. (C2).

D) Protein separation with microwave assistance

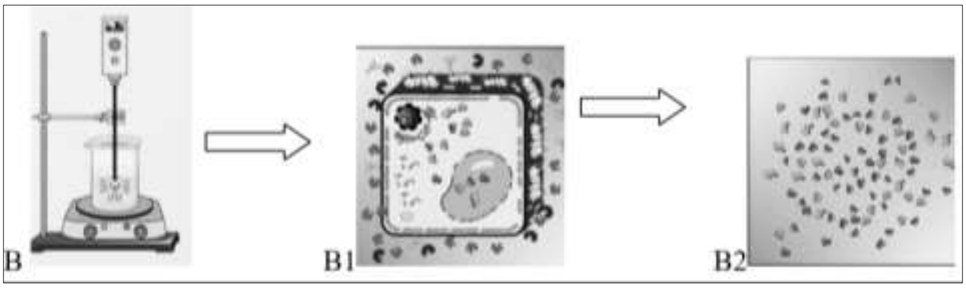
The microwave device's electromagnetic radiations increase the solvent's penetration into the plant matrix (D1) and facilitate the movement of soluble proteins into the solvent, boosting the protein recovery. (D2).

E) Protein extraction with high pressure assistance (HPE)

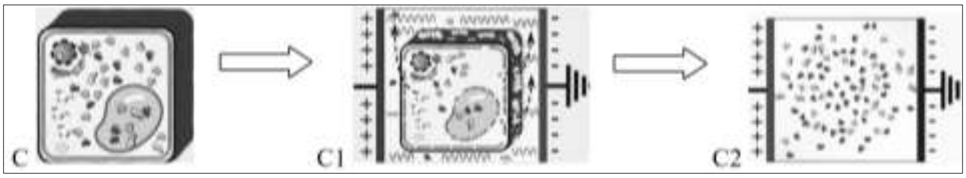
Plant tissues and cells are destroyed by pressure by rupturing the cell wall (E1); this improves the mass transfer of outside liquids into the plant cell wall and increases the efficiency of protein extraction (E2).



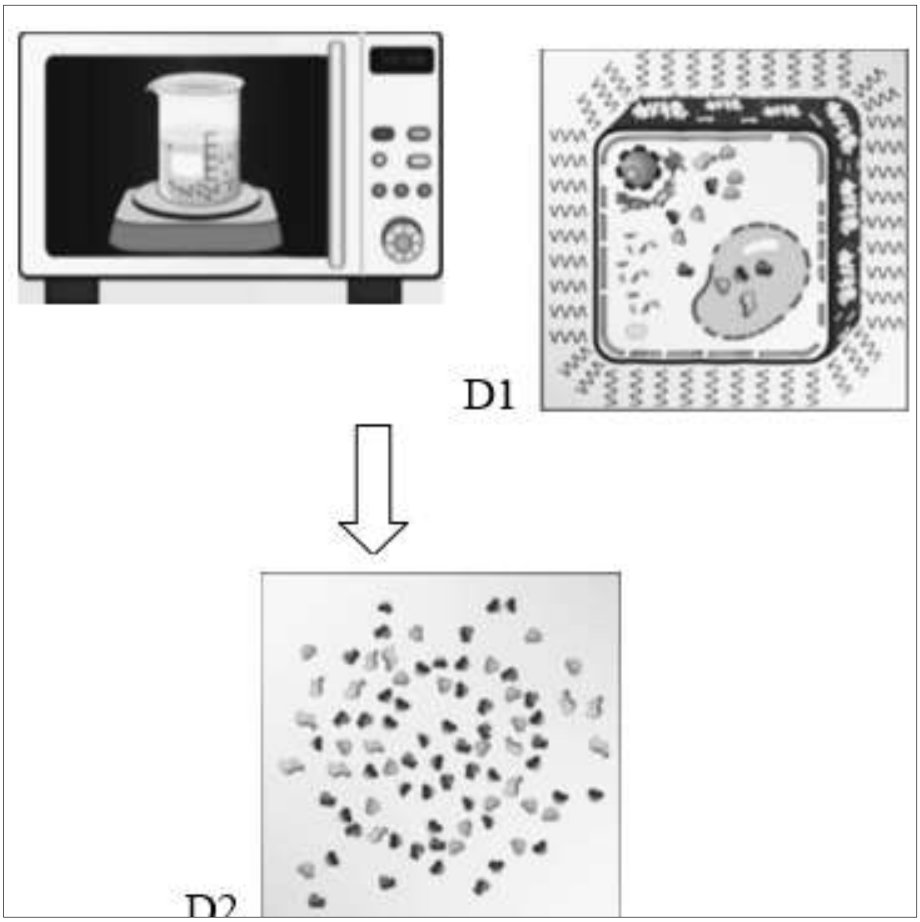
Biochemical technique



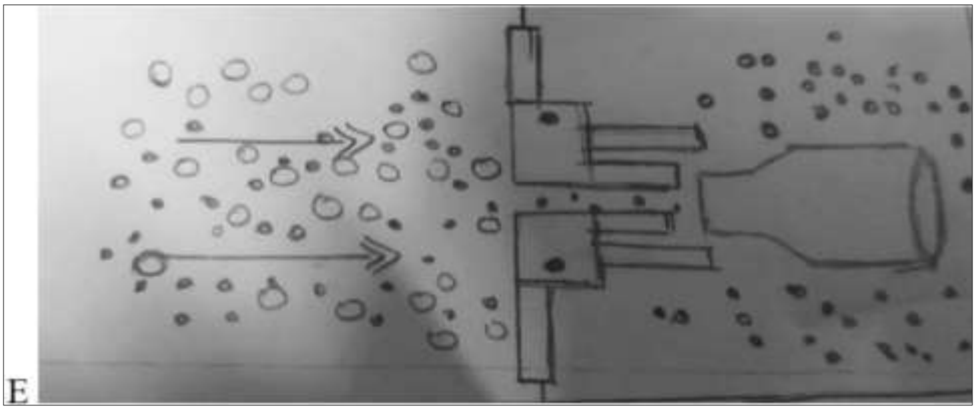
Ultrasound setup



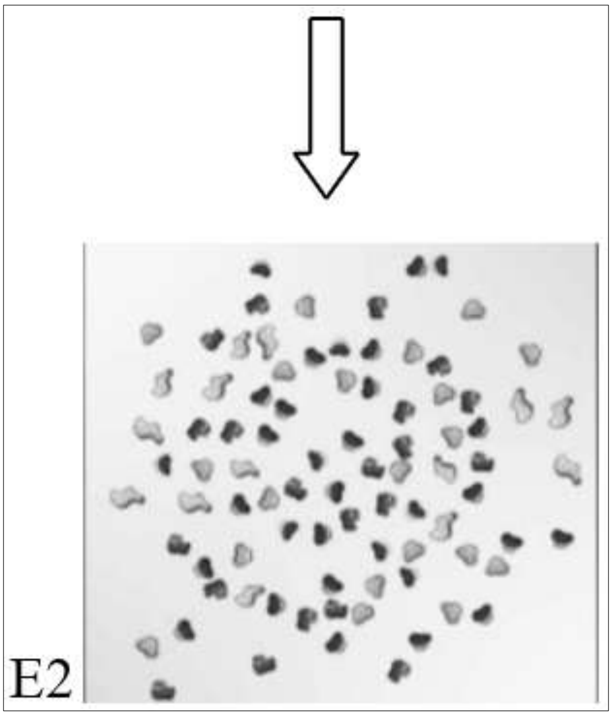
Plant cells with intercellular proteins



Microwave setup (D)



High-Pressure Extraction Technique showing Homogenized product on right side and Homogenized product on left side (E1)



4. Comparison of Protein Quality and Nutritional Value of Protein extracted from Non-Conventional Sources to traditional sources

The primary dietary protein sources are vegetation and animals, and there are notable distinctions between the types of proteins that each provides. A few of the variables include molecular make-up technical food characteristics such the capacity to gel, emulsify, bind water, etc., as well as the content of amino acids and digestibility. Both the sensory appeal of foods containing either animal or plant proteins as well as their bioavailability in terms of human nutrition are impacted by these intrinsic differences. These basic differences mean that substituting one ingredient for another is not enough to produce plant-based diets that are similar to animal foods. (L Day *et al*., 2022) [31].

4.1 Nutritional Composition of Plant Protein and soy protein

Animal feed production has a limited supply of protein, and the primary source of protein is a vegetable; 97% of the 334.56 million tonnes of soybean meal produced worldwide is used as animal feed. Many plant proteins, such as those in legumes, cereals, seeds, grains, and leaves, have undergone substantial investigation to produce novel food items with higher nutritional properties because of their rich mix of protein, fibres, minerals, and other bioactive chemicals. Plant proteins are frequently used in dietary systems as functional ingredients and nutritional supplements (M Kumar *et al*., 2022) [32].

While legumes are the first food crop that humans have ever produced and are rich in minerals, including a large quantity of proteins, fibres, and carbs as well as being an extraordinary source of minerals and vitamins, vegetables are the most accessible and abundant plant sources of protein. Additionally, some cereals and beans are gluten-free, making them a healthy substitute for vegetarians and those with coeliac disease (RD Semba *et al*., 2021) [33].

Soluble soybean proteins have a molecular mass distribution that runs from 19.3 to 81.3 kDa, which is similar to the pattern of the proteins found in M. oleifera leaves. M. oleifera leaves are rich in nutrients like vitamins, minerals, and biologically active substances. (Fangfang *et al*., 2021) [27].

Although soybean meal is frequently utilised as protein source in bovine diets, the protein in soybean meal is fast and thoroughly digested in the rumen (NRC 2001). As a result, numerous chemical and physical therapies have been created and put on the market which increases the quantity of amino acids (AA) that are available for absorption in the small intestine and decrease the ratio of protein that has been destroyed to protein that has not (Abdollahzadeh *et al*., 2020) [34].

Protein quality and its anti-nutritional factors

Bioavailability, digestibility, and amino acid composition are all included in the concept of "plant protein quality" (PPQ). The structure of the protein, prior processing, and the presence of substances that prevent digestion all affect how much protein is absorbed by an organism relative to the amount consumed. These latter elements, often known as "antinutritional factors" (ANF), include lectins, tannins, phytates, and protease inhibitors. Because plants have ANF, animal proteins are more easily absorbed than plant proteins (Sá *et al*., 2020) [35].

5. Applications of Protein extracted from Non-Conventional sources

It is notable that extraction techniques often target the content of protein yield. While examining this area in the number of papers it shows that nutritive and functional alterations in post protein extraction is limited. Due to food safety concerns, both for human and animal consumption viability, this creates a loophole in the reutilization of extracted protein. Furthermore, it is notable that extraction technology models are frequently attributed for being environmentally greener, but without considering the cost component. A comprehensive approach to protein extraction is required, considering not only yield but also food safety, environmental effect, and affordability (Berners-Lee *et al*., 2018) [36]. The manufacturing of food must be high enough in both quantity and quality to feed the entire world's population while causing the least amount of harm to the environment. Food distribution must be effective enough to ensure that everyone has access to a wide variety of meals with required nutrition while minimizing negative environmental effects. (Adam *et al*., 2022) [37].

5.1 Products obtained from non-conventional sources

Starch from bark (*Caryota urens*)/Kithul starch

Kithul starch is an unusual starch. It is made from the flour collected from the trunk of the kithul palm. The gamma irradiation treatment resulted in significant modifications in the physicochemical, pasting, morphological, thermal, and functional properties of kithul starch. Irradiation at various dosages s (0.5 kGy, 1 kGy, 2.5 kGy, 5 kGy, 10 kGy) increased the solubility, acidity and carboxyl content (C Sudheesh *et al*., 2018) [38].

- Starch extraction techniques differ from the original techniques, demonstrating the need for new methodology that is optimised for unconventional starches.
- The lack of native starch patterns in the study species makes comparative studies difficult. The extracted starch may experience slight modifications that change its features when chemical agents and grinding processes are applied to it.
- Alkaline solutions, for instance, are used to facilitate extraction in tubers containing mucilage, while sodium bisulfite prevents bacterial growth, inhibits enzymatic browning, hydrolyzes proteins, and has a potent reducing effect, preventing oxidation.
- Extraction technique must be carried out efficiently using water and starch is allowed to decant through water and multiple washings until it reaches a high level of purity (less than 2% of other nutrients). This will make it possible to study starches in their most natural setting (Bruna Lago *et al*., 2020) [39].

5.2 Non-Conventional sources of protein served in various medicinal purposes and ailments

One of the most extensively covered subjects in the field of nanomedicine is proteins and peptides. In the pharmaceutical, medicinal, and food industries as well as in improving novel goods created using conventional techniques like alkali extraction, researchers view plant sources as abundant, affordable, and safe resources. Experts have lately examined the use of plant proteins to create nanocarriers for medicinal medications due to the low likelihood of disease transmission from plant sources to humans. (Kianfar E *et al*., 2020) [40].

5.3 Environmental sustainability of protein extraction from nonconventional sources

The development of novel food products that have less of an impact on the environment and can feed the world's rising population by using proteins from alternative sources like plants, microorganisms, and insects has attracted a lot of interest. Compared to many established proteins and protein fractions, for which a considerable amount of knowledge has been collected through time, far less is known about these growing proteins. (RD Semba *et al*., 2021) [33].

In order to gain novel techno-functional alternative protein constituents that help in the production of different dietary structures, particularly fibrous ones has been progress in the discovery and extraction of new protein sources. New molecular and processing technologies are constantly being created, setting the foundation for ground-breaking food designs based on these alternative protein sources. (Grossmann & Weiss, 2021) [15].

Conclusion

Proteins are complex molecules with a wide range of functions that are essential for maintaining the structure and operation of the living form. As a result, it has a wide range of uses, including in food, feed, and medication. Some unusual foods have been discovered to have great nutritional value. MAE has risen substantially in recent years. It is essential to take seriously the necessity for improving current separation techniques as well as the development of fresh, highly efficient ways. New effective phytopharmaceutical compounds will very definitely be discovered as a result of these developments. In order to better understand the extraction mechanism, remove technological limitations, enhance the design, and scale up novel extraction systems for better industrial applications, more study is required. Plant leaf protein is one of the sustainable and alternative protein sources. It can be cultivated in most agroclimatic conditions without a lot of agricultural inputs. Its functional traits are distinctive and are used in the formulation of novel food products.

However, the state of world food production today has forced certain major economies to reevaluate the potential of plant leaf proteins and to put out significant effort. Pilot-scale demonstration plants for protein extraction from agro-food residues have been established in order to meet the need for protein with product formulation.

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