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PLANT-BASED PROTEINS: SOURCES, EXTRACTION METHODS AND FUNCTIONAL PERSPECTIVES FOR THE SUSTAINABLE FOOD INDUSTRY

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Abstract. Current research in the food industry highlights the importance of alternative protein sources in the context of global population growth and the need to reduce the negative impact of raising animals, producing animal proteins, on the environment. Plant proteins represent a sustainable, affordable and beneficial option for health, with high potential to replace animal proteins in the food industry. Plant sources rich in protein include legumes, cereals, pseudocereals, oilseeds, green leaves, algae and, more recently, insects. They differ considerably in protein content and amino acid profile, and their strategic combination can ensure a balanced intake of essential nutrients. Legumes offer high protein content, but are deficient in sulfur amino acids. Cereals, although lower in protein, bring technological benefits and can complement the amino acids missing from legumes. Pseudocereals have a higher nutritional value than conventional cereals, while green leaves and algae offer a high proteins intake and compounds with antioxidant potential, although they require advanced technologies to overcome extraction limitations. Extraction methods play a decisive role in the yield and quality of plant proteins. Conventional techniques are efficient but can cause denaturation and provide low yields. On the other hand, emerging methods allow the recovery of proteins in larger quantities, preserving their functional and technological properties.

Keywords: *cereals, green leaves, green technologies, legumes, pseudosereals, techno-functional characteristics.*

Rezumat. Cercetările actuale în domeniul industriei alimentare evidențiază importanța surselor alternative de proteine în contextul creșterii populației globale și al necesității de a reduce impactul negativ al creşterii animalelor, producătoare de proteine de origine animală, asupra mediului. Proteinele vegetale reprezintă o opțiune sustenabilă, accesibilă și benefică pentru sănătate, cu potențial înalt de a înlocui proteinele animale în industria alimentară. Sursele vegetale bogate în proteine includ leguminoasele, cerealele, pseudocerealele, oleaginoasele, frunzele verzi, algele și, mai recent, insectele. Acestea diferă considerabil prin conținutul proteic și profilul de aminoacizi, iar combinarea lor strategică poate asigura un aport echilibrat de nutrienți esențiali. Leguminoasele oferă un conținut proteic ridicat, dar sunt deficitare în aminoacizi sulfurați. Cerealele, deși mai sărace în proteine, aduc beneficii tehnologice și pot completa aminoacizii lipsă din leguminoase. Pseudocerealele prezintă o valoare nutritivă superioară cerealelor convenționale, în timp ce frunzele verzi și algele oferă un aportului înalt de proteine şi compuşi cu potenţial antioxidant, deși necesită tehnologii avansate pentru a depăși limitările de extracție. Metodele de extracție au un rol determinant asupra randamentului și calității proteinelor vegetale. Tehnicile convenționale sunt eficiente dar pot cauza denaturări şi oferi randamente scăzute. Pe de altă parte, metodele emergente permit recuperarea proteinelor în cantităţi mai mari, păstrarea proprietăţile lor funcţionale şi tehnologice.

1. Introduction

Plant-based proteins offer a sustainable, economical and health-conscious alternative to animal proteins. They can be consumed directly as a source of protein or incorporated into various food products to balance amino acid profiles and improve nutritional values. Due to their rich nutritional content, including their more affordable price, plant-based protein sources have managed to become an attractive and innovative resource in the food industry [1-3]. Research aimed at meeting the rising demand for protein underscores the importance of alternative protein sources. Protein is increasingly recognized by consumers as a valuable nutrient, driving growing demand for both plant- and animal-based proteins [4]. According to Machovina et al., meat production is an extremely intensive and unsustainable activity that led to the deterioration of the planet's environment, leading to deforestation, pollution, negative impact on hydrogeological reserves and the disappearance of biodiversity [5].

Recently, there has been growing interest in developing food products that offer both physiological and nutritional benefits. The source of protein significantly influences nutrient availability: animal proteins generally exhibit higher digestibility and provide all essential amino acids, whereas plant-based proteins often contain anti-nutritional compounds that limit digestibility and are deficient in certain amino acids, such as lysine, tryptophan, cysteine, and methionine [6].

Health-promoting potential of plant-based meat alternatives is linked to dietary shifts, population growth, and the state of natural resources and biodiversity. In 2017, the global population was approximately 7.5 billion, and the United Nations projects it will reach 8.5 billion by 2030. To meet the protein demands of an estimated 9 billion people by 2050, meat production is expected to increase by 50–73%, highlighting the need for sustainable alternatives such as plant-based meat products [7]. According to Gerber et al. the livestock sector accounts for 14.5% of human-caused greenhouse gas emissions and consumes approximately 30% of the world's freshwater resources [8]. Population growth drives increased food production, placing significant strain on the sustainability of the current food system. The combination of rapid population expansion and the inefficiency of existing food patterns create a critical challenge: ensuring adequate nutrition for a growing global population.

Scarborough et al. reported that diets high in meat are associated with greater carbon dioxide emissions, averaging 7.19 kg CO_2 equivalent per day—more than twice the emissions from a vegan diet, which averages 2.89 kg CO_2 equivalent per day. The study concluded that reducing the consumption of meat and other animal products is an effective strategy to mitigate climate change [9].

Umerous factors contribute to the decline in meat consumption at both individual and global levels. A key concern is the rising global production of protein, particularly high-quality animal protein, which poses challenges for environmental sustainability. Nevertheless, despite growing consumer awareness, only a small proportion of the population has adopted a meat-free diet, with approximately 5% following a vegetarian diet and 3% adhering to a vegan diet [10-12].

One strategy to support the reduction of meat consumption is to offer meat substitutes. According of Ulhas et al. and Akyuz et al. where due to their rich nutritional content, affordable price, plant-based protein sources have become an attractive source in the food industry. Research efforts addressing ways to meet the growing demand for protein highlight the importance of alternative protein sources [1,2].

The study realized by Bouchard et al. highlighted the impact of the biological properties of plant proteins on overall health and well-being. These effects include antidiabetic, anticancer, antioxidant, and nephroprotective activities, as well as the ability to reduce risk factors for cardio-metabolic diseases, regulate appetite and lipid metabolism, and modulate the intestinal microbiome [13].

Despite all the benefits offered, the use of plant-based proteins in the food industry remains a challenge due to their technological properties, such as solubility, emulsification and gelling properties, and water retention capacity, which are lower than those of animal proteins. To overcome these disadvantages, various methods have been applied to modify and improve the techno-functional characteristics, thus enhancing their use as nutritional ingredients.

The purpose of this study was to identify and characterize protein-rich plant sources, conventional and unconventional extraction methods, and describe the physicochemical and functional properties of plant-based proteins.

2. Plant Protein Sources

Globally, approximately 80% of dietary energy and 65% of dietary protein are provided by foods of plant origin [14]. Plant-based proteins are complex and differ in quality characteristics.

2.1 Legumes

Legumes (such as beans, peas, chickpeas, and lentils) are a primary source of dietary protein and play a vital role in the food supply, particularly in developing countries [15-17]. Legumes contain approximately 21-25% protein. These values can vary slightly depending on genetic factors, the maturity of the plant, and environmental conditions [4]. Legumes are rich in lysine, leucine, aspartic acid, glutamic acid and arginine, but poor in sulfur-containing amino acids (cysteine, methionine and tryptophan). When consumed together with cereals, which are rich in sulfur-containing amino acids, they provide a balanced profile of essential amino acids to meet human nutritional needs [18].

Legumes are rich in bioactive compounds such as enzyme inhibitors, lectins, phytates, and phenolic compounds, which serve as part of the seed’s defense system and may influence various biological functions when consumed. Certain substances, including enzyme inhibitors and lectins, are regarded as antinutritional factors due to their ability to decrease protein digestibility and impair nutrient absorption [19]. However, this effect can be reduced by cooking food before consumption [20]. On the other hand, health benefits have been associated with the same bioactive compounds. Regular consumption of legumes is associated with reduced cholesterol levels and a reduced risk of cardiovascular disease, cancer, and diabetes [16,21-23]. Given these characteristics, there is increasing interest in incorporating legumes into the development of health-oriented ingredients, such as flour blends and extruded snacks, with the goal of producing functional and nutritious foods. Soy and pea proteins are among the most widely utilized plant-based sources, valued for their favorable functional properties, including water retention, gelation, fat absorption, and emulsification capacity. Other legume proteins—such as those from lentils, lupins, chickpeas, peas, mung beans, and fava beans—are also being investigated for their physicochemical properties, particularly foam stability, emulsification capacity, and gel formation [24]. Soy protein is historically the best-known alternative to animal proteins [25]. The study by Zhou et al. showed that soybean seeds contain approximately 42.95 to 46.32% of proteins which include several amino acids (g/100 g protein), such as alanine (3.59), arginine (6.67), aspartic acid (10.2), cystine (1.46), glutamic acid (17.45), glycine (3.6), histidine (2.3), isoleucine (4.25), leucine (6.78), lysine (5.33), methionine (1.13), phenylalanine (4.59), proline (4.96), serine (4.59), threonine. (3.14), tryptophan (1.12), tyrosine (3.22) and valine (4.1) [26].

2.2 Cereals

Cereals (wheat, corn, rice, barley, sorghum, etc.) provide 62% of the world's food supply, being the main staple foods consumed globally, especially in developing countries, where the average protein intake is below recommended values [27]. Compared to legumes, cereal grains contain little protein, averaging about 10–12% on a dry weight basis [28], however, they are widely used in the food industry due to their functional properties. More than 50% of the total protein content of mature cereal seeds consists of storage proteins (prolamins and glutelins), which are rich in glutamine, proline, leucine and alanine. On the other hand, the essential amino acids lysine, tryptophan, methionine and histidine are limiting [29,30]. Wheat is the largest source of plant-based protein in the diets of western consumers and is widely used in the manufacture of everyday food products. Wheat protein is not utilized as efficiently as animal protein, and when consumed as the sole protein source, relatively large amounts are required to meet human physiological demands for both total protein and specific essential amino acids. In particular, lysine, threonine, and tryptophan are frequently limiting in wheat-based foods. Nevertheless, when combined with other dietary proteins such as those from legumes, oilseeds, or animal products, wheat proteins demonstrate excellent nutritional complementarity [31]. The digestibility of wheat proteins is reported to be approximately 90% [32]. Another study conducted by Juliano et al. [33] showed that the protein content of different varieties of brown rice ranges from 6.8 to 11.2%. According to Kalman [34] the predominant amino acids in brown rice include glutamic acid (13.9 g/100g), aspartic acid (6.94 g/100g), leucine (6.41 g/100g), arginine (6.32 g/100g), valine (4.56 g/100g), alanine (4.47 g/100g), phenylalanine (4.41 g/100g), and tyrosine (4.26 g/100g).

2.3 Pseudocereals

Pseudocereals are dicotyledonous species rich in starch and exhibiting properties similar to those of true cereals. Their protein content generally ranges from 10% to 20%, and compared with cereals and other legumes, their protein quality is relatively high. The most commonly consumed pseudocereals include buckwheat, amaranth, quinoa, and chia. They serve as valuable sources of essential amino acids, essential fatty acids, phenolic compounds, vitamins, flavonoids, and minerals.

Buckwheat, a dicotyledonous crop belonging to the Polygonaceae family, is cultivated and adapted to withstand extreme climatic conditions in marginal areas. Native to China and Central Asia, it is an annual herbaceous plant with protein profile rich in diverse amino acids. Notably, buckwheat is the only pseudocereal known to contain rutin [35]. Buckwheat contains a wide range of proteins that are of high quality. Buckwheat proteins are significantly rich in lysine, aspartic acid and arginine and have lower levels of glutamic acid and proline than cereal proteins [36]. The protein content is 12.0–18.9% for buckwheat [37]. Furthermore, buckwheat also contains essential amino acids like lysine and arginine that ensure the full range of amino acids required for the proper functioning of the human body [38]. Buckwheat is the pseudocereal with the highest phenylalanine content (862 mg/100 g). Buckwheat proteins are gluten-free. Buckwheat proteins have a strong additional impact on other proteins to improve nutritional and amino acid balance. With special bioactivities such as cholesterol-lowering effects and constipation improvement, antihypertensive effects, as well as obesity situations, acting similarly to dietary fibers and interfering with metabolic processes in vivo [39]. Due to its protein composition, buckwheat is considered a healthier option compared to cereals.

Quinoa seeds have an average protein content ranging from 12 to 23%, which is higher than conventional cereals, although lower than oilseeds and legumes [40]. Previous research on quinoa protein has confirmed that the main proteins in quinoa are globulins, which account for approximately 37% of total protein, and albumins account for approximately 35%. Quinoa has a low prolamin content (0.5-7.0% of total protein) [41]. The protein content of quinoa is considered highly valuable, owing to its well-balanced composition of essential amino acids. These proteins contribute not only to the nutritional quality but also to the functional and structural properties of quinoa, including solubility, gel network formation, emulsification, and foaming capacity [42]. Quinoa has almost all the essential amino acids, as suggested by standard-setting bodies such as Food Agriculture Organisation (FAO) and World Health Organisation (WHO) [43]. Quinoa has a high concentration of lysine, which ranges mainly from 2.4 to 7.8 g/100 g of protein. The methionine content of quinoa ranges approximately from 0.3 to 9.1 g/100 g of protein. The threonine content ranges from 2.1 to 8.9 g/100 g of protein [44]. Analysis of the composition of quinoa has shown that its proteins are mainly deposited in the embryonic tissue, which also contains lipids, fiber and saponins, while the perisperm contains a high starch content [45].

Quinoa seed flour has a higher total protein content than grains such as barley (11%), rice (7.5%), or corn (13.4%). Additionally, the protein content of quinoa is higher than the protein content of peanuts (8.8-11.6%) and cowpeas (8.8-12.1%) [42,46]. The amino acid composition of quinoa protein isolate includes histidine (2.76), leucine (4.60), isoleucine (1.30), lysine (17.13), methionine with cystine (1.70), phenylalanine with tyrosine (9.34), threonine (1.47) and valine (2.03) as essential amino acids, while the non-essential amino acids include alanine (5.34), glycine (9.60), proline (0.10), serine (2.57), tyrosine (2.88), glutamic acid (12.80), aspartic acid (8.54) and arginine (0.03) (g/100 g protein), as reported by Elsohaimy et al. [42]

Amaranth and its varieties fall into the class of traditional crops with high nutritional and medicinal value, but their true potential has not yet been realized. The nutritional and bioactive components found in these plants contribute significantly to their ability to provide health benefits [47]. The protein content in amaranth varies and reaches maximum accumulation during the blossoming phase, ranging from 17.2 to 32.6% on a dry weight basis for various samples. Amaranth has twice as much lysine as cereals, such as wheat, and three times the lysine concentration of corn [48]. According to the literature, amaranth protein fractions contain albumin, globulin, prolamin and glutelin which are approximately 65%, 17%, 11% and 7% respectively. The amount of distinct fractions in the separated protein as well as its functional and nutritional qualities depends on the extraction process used [49]. The primary storage proteins in amaranth are albumins and globulins. Albumins are abundant in sulfur-containing amino acids, as well as lysine and valine, whereas globulins are rich in valine, leucine, glutelin, and histidine, but remain limited in lysine. Amaranth is a notable source of essential amino acids, particularly lysine, containing approximately twice the amount found in wheat and three times the concentration present in corn [48]. In addition, it contains significant amounts of sulfur amino acids that are limiting in legumes. Amaranth is a gluten-free pseudocereal, making it an effective choice for a gluten-free diet. The nutritional characteristics of amaranth have attracted the attention of researchers investigating the use of amaranth as a functional ingredient. Previous studies have shown that there is a significant difference in nutritional attributes between amaranth species, which includes amino acid composition, fatty acid composition, and mineral content [50].

2.4 Oilseeds

Oilseeds are the seeds of plants primarily used for oil extraction and include varieties such as peanuts, rapeseed, sesame, and sunflower. The protein content of oilseeds typically ranges from 6% to over 60%, with specific types like soybean meal containing high protein levels (e.g., 33-56%), while canola/rapeseed meal and sesame meal also offer valuable proteins. The oil extraction process generates a by-product known as meal, which is rich in protein and other valuable nutritional and bioactive components. Although traditionally used for animal feed, the growing demand for plant-based proteins has led to an increased utilization of these by-products for human nutrition. Oilseeds can contain up to 40% protein and possess a relatively well-balanced amino acid profile, particularly in sulfur-containing amino acids, though they are generally deficient in lysine. Additionally, bioactive peptides in oilseeds may offer health benefits, including the prevention and management of diabetes, obesity, and cardiovascular diseases [51].

Rapeseed protein exhibits a balanced amino acid composition except for methionine. Sesame protein is composed primarily of globulin (67.3%), albumin (8.6%), glutelin (6.7%), and prolamin (1.3%). It is nutritionally notable for its high content of methionine and tryptophan but is limited in lysine, threonine, isoleucine, and valine. Sesame is widely processed into protein-rich products, including flakes, flour, concentrates, and protein isolates, with the latter containing up to 80% protein [52].

2.5 Green Leafy Crops

Leafy greens occupy a prominent position in this industry due to their nutritional value, abundance, sustainability and are renowned for their high protein content, essential vitamins, minerals and phytochemicals. Furthermore, leafy greens are characterized by their low environmental impact, requiring minimal inputs of land, water and energy compared to conventional protein sources. The reason for focusing on leafy greens as a promising solution lies in their multiple benefits and their potential to address key challenges facing the global food system. By harnessing the nutritional potency and sustainability attributes of leafy greens, stakeholders can promote environmental stewardship, public health and food security [53].

Green leaf biomass represents one of the largest underutilized nutrient sources globally. Whether deliberately cultivated or recovered as a by-product from large-scale agricultural crops, it can serve as a viable alternative source of plant protein for food and feed formulations. The nutrient composition of green leaf biomass differs markedly from that of plant seeds, particularly in terms of protein quality, vitamin and mineral content, and fatty acid profiles, including omega-6 and omega-3 fatty acids [54]. In contrast to cereal proteins, which are often deficient in lysine and/or tryptophan, and legume seed proteins, which are limited in methionine and/or cysteine, leafy green proteins nearly meet the FAO criteria for a complete protein. Additionally, unlike edible seeds that are typically rich in omega-6 fatty acids, leafy greens are a significant source of omega-3 fatty acids, particularly α-linolenic acid, a key precursor of the metabolites eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) [55].

Alfalfa (*Medicago sativa* L.) is recognized as a versatile and nutrient-rich source in modern food systems, offering a wide range of benefits and applications for human health. The health benefits of alfalfa, including antioxidant, anti-inflammatory, cholesterol-lowering and blood sugar-regulating properties, are highlighted along with its potential to combat chronic diseases and oxidative stress [56]. Alfalfa is rich in valuable nutrients, including proteins with a high content of essential amino acids, minerals, vitamins and dietary fiber. The proteins contained in alfalfa have a major impact on techno-functional properties, including water retention, emulsification, foaming and gelling properties, useful for obtaining next-generation products. Alfalfa is beneficial in alleviating some health disorders, including diabetes, atherosclerosis and hypercholesterolemia, due to its antioxidant properties. Chloroplasts in plant leaves contain almost 80% protein, with most of the remaining protein being located in the stroma and thylakoid membranes [57]. About half of the leaf proteins are white and hydrophilic, while the other half are green and hydrophobic. The hydrophobic green fraction comprises chloroplast lamellar proteins, which are associated with lipids, fat-soluble vitamins, and chlorophyll [58]. The crude protein content of alfalfa is typically between 15 and 22% and is mainly distributed in the leaves. Crude protein is divided into two major categories: protein proper and non-protein nitrogen (NPN), based on chemical properties. NPN includes free amino acids, amides, purines, pyrimidines, and alkaloids, accounting for about one-third of the total nitrogen content of alfalfa. L-glutamic acid and glutamine are the structural units of protein. They play a vital role in nitrogen metabolism and are biosynthetic precursors of a variety of amino acids, purines, and pyrimidines in the body [59,60].

2.6 Algae

Algae biomass has low water consumption (including seawater growth), no competition for arable land, and carbon neutral emissions, and has become an alternative to global food security issues. Macroalgae and microalgae species have protein amounts comparable to protein sources such as milk, eggs, and dairy products. Microalgae, such as Chlorella and Spirulina, have high potential for functional food processing [61]. Over 30% of the world's microalgae biomass is derived from spirulina *(Arthrospira platensis* and *Arthrospira maxima)*, which contains approximately 60% protein. Among *Chlorella* species, the most commonly used are *Chlorella pyrenoidosa*, *Chlorella sorokiniana*, *Chondrus crispus*, *Scenedesmus acutus*, and *Chlorella vulgaris*, with protein contents ranging from 51% to 58%. This high protein content makes microalgae a valuable nutrient source and an attractive option for enhancing the nutritional quality of foods. Moreover, various cultivation and extraction techniques have been developed to maximize the recovery of beneficial compounds from microalgae, thereby exploiting their full nutritional and functional potential [62].

The main factors limiting the development of microalgae as a protein alternative are pigmentation, difficulty in harvesting and production. Pigmentation and the fishy smell of microalgae make the product unpalatable. This is one of the strongest barriers to the use of microalgae as a food ingredient. In addition, microalgae protein contains less methionine, cysteine, lysine and tryptophan than meat protein and has a lower degree of digestibility. Nevertheless, microalgae protein remains a useful ingredient for meat alternatives and has great potential to improve the quality of plant-based food products [63].

2.7 Insects

Insects are gaining recognition as a promising food source due to their considerable nutritional value [64]. Insect protein, also called entomophagy, involves consuming insects as a food source [65]. It is nutritious, sustainable and offers an alternative to traditional protein sources. Insects are rich in protein, vitamins and minerals and can be consumed whole or processed into various food products. Queiroz et al. also reported that edible insects offer a promising solution as an alternative source of protein. In particular, they meet all the criteria necessary to promote the main sustainable development goals highlighted by the WHO, because unlike conventional primary protein sources used for human food and animal feed, insects present a high protein content and well-balanced amino acids. Furthermore, the integration of insect farming into traditional food practices heralds a more sustainable and efficient food system. Research by de Castro et al. [65] elucidated that insect peptides are potential antihypertensive, antimicrobial, and antioxidant agents, demonstrating their broad utility. Research has indicated that insect protein hydrolysates possess bioactive characteristics, such as anti-inflammatory, antihypertensive, antidiabetic, and antioxidant effects [66]. Lipoxygenase and cyclooxygenase inhibitors exhibit anti-inflammatory properties [67]. However, these protein sources remain consumed more in tropical and subtropical regions, and less in Western countries [68]. However, insects have significant potential as a sustainable solution to future nutritional demands, as they are renewable natural resources available year-round and have a low environmental impact, with increased food security [69,70]. The protein content in different sources of plant origin is presented in Table 1.

Table 1

Protein Content of Different Plant Protein Sources

Protein type	Protein resource	Protein content (%)	References
Legumes	Lentils	23-31	[71]
	Chickpea	17-22	[72]
	Lupin	32.0-55.3	[73]
	Faba bean	26.4-39.7	[74]
	Cowpea	23.6-33.0	[75]
	Black bean	22.9-23.2	[76]
	Pea	14-31	[18]
	Mung beam	25.8-39.7	[74]
	Soybean	42.95–46.32	[26]
Cereals	Wheat	7-22	[77]
	Rice	6.6-8.4	[78]
	Oat	8.7-16.0	[79]
	Sorghum	6-20	[80]
Pseudo-cereals	Hemp seeds	20.17–25.06	[81]

Continuation Table 1

	Amaranth	11.7-18.4	[79]
	Quinoa	13.0-14.0	[44]
	Chia	19.0-23.0	[82]
	Buckwheat	21.6-25.3	[83]
Oilseed protein	Groundnut	18.92-30.53	[84]
	Sun flower	10-25	
	Mustard	18-24	[85]
	Almonds	10-35	[86]
	Sesame	12-16	[84]
	Rapeseed	34-40	[87]
Green leaves	Alfafa leaves	15.3–32.27	[88]
	Spinach leaves	30	[53]
	Cabbage leaves	39.76–53.33	[89]
	Broccoli leaves	12	[90]
	Cassava leaves	33.6	[91]
Algae	Spirulina platensis	50-65	[92]
	Chlorella sp.	51-58	
	Scendesmus sp.	50-56	
	Spirogyra	6-20	
	Spirulina maxima	60-71	
Insects	Tenebrio molitor	46-69	[67, 93-95]
	Hermetia illucens	40.0-60.8	[96-99]
	Gryllodes sigillatus	61.3-70.0	[67,100]

3. Established Methods of Plant Protein Extraction

Extraction of plant-based proteins with increased quality indices are obtained from ecological and sustainable protein sources with the use of emerging technologies for their extraction, cost-effectiveness and incorporation as food supplements in the manufacture of food products.

The most important phase in obtaining plant-based protein is the appropriate extraction technology. Pojić et al. [101] reported the results of a study that reviewed the techniques for extracting proteins from plant sources. The most important ones remain conventional methods using different solvents and unconventional methods using microwaves, high-pressure processing, pulsed electric field and ultrasound (Figure 1).

3.1 Conventional Methods of Plant-Based Protein Extraction

Conventional methods refer to commonly used techniques, which can sometimes result in lower extraction yields due to protein degradation. This decrease in protein yield is governed by a number of factors, including extraction time, solvent type, pH, and temperature. Conventional methods used for extracting plant proteins include wet extraction methods and dry fractionation.

Wet extraction refers to the recovery of proteins using aqueous solvents or chemical agents such as alkalis, acids, or salts, followed by protein precipitation or isolation. Among conventional techniques, alkaline extraction is the most widely applied due to its simplicity, cost-effectiveness, and efficiency. Under alkaline conditions, proteins acquire a negative charge, and their solubility increases when the pH rises above the isoelectric point; insoluble residues are subsequently removed by centrifugation [102]. In acid extraction, solutions containing butanol, pentanol, hexane, or acetone are typically employed. The addition of acid gradually lowers the pH below the isoelectric point, resulting in the generation of a positive charge and enhanced protein solubility. The pH is then adjusted back to the isoelectric point, leading to the aggregation of soluble proteins, which are further separated by precipitation, centrifugation, or filtration. Salt extraction generally employs neutral pH saline solutions, such as sodium, calcium, or potassium chloride.

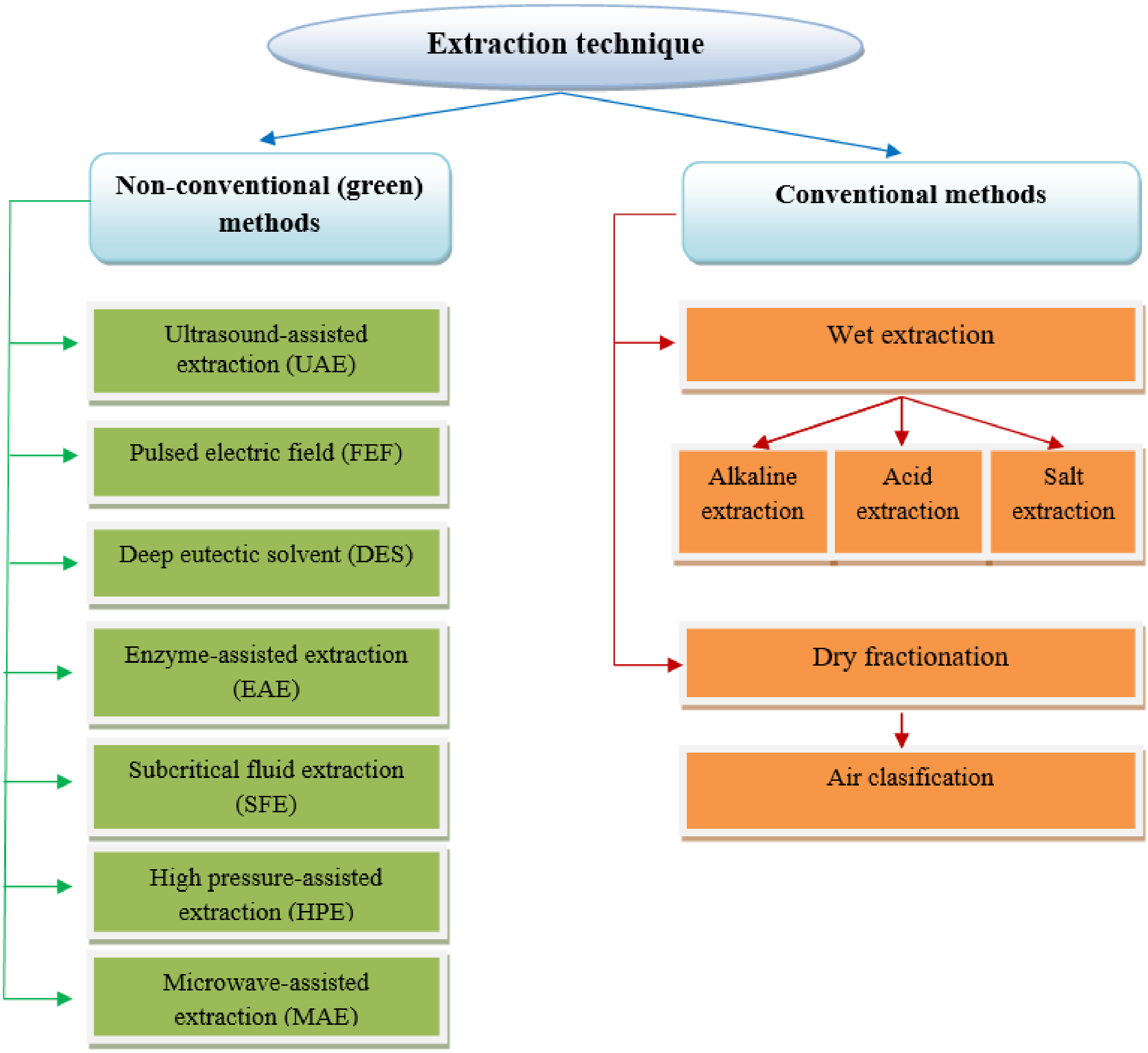

Figure 1. Methods for extracting proteins from plant-based raw materials.

This method relies on protein precipitation through salting-out and protein destabilization, followed by the removal of insoluble fractions by sedimentation, decantation, sieving, and centrifugation. The supernatant is subsequently desalted and dried to obtain the protein fraction. Compared to other methods, proteins extracted with saline solutions exhibit lower degrees of denaturation and aggregation, thereby retaining higher functional activity in terms of emulsifying, foaming, and gelling properties [103].

3.2 Non-Conventional Extraction Methods for Plant-Based Protein Sources

Nowadays, researchers are currently focusing more on non-thermal or non-conventional green technologies with the aim of improving extraction efficiency and reducing protein degradation during extraction. These methods do not have any harmful effects on the environment, and the proteins obtained are also safe for consumption due to the minimal use of harmful chemicals and solvents. Exploiting innovative protein extraction techniques can improve protein yield, nutritional and techno-functional properties.

Ultrasound-assisted extraction, enzymatic extraction, supercritical fluid extraction, pulsed electric field, deep eutectic solvent extraction, high pressure-assisted extraction andmicrowave-assisted extraction are modern processing techniques developed [24].

3.2.1 Ultrasonic Asisted Extraction (UAE)

UAE represents an emerging technique for the extraction of plant-derived proteins, as it is capable of disrupting the cellular matrix, thereby enhancing extractability and tailoring the functional properties of these biomolecules. The efficiency of protein recovery and the overall extraction yield are strongly influenced by operational parameters such as sonic energy density, duration of treatment, and the substrate-to-agitation ratio. Beyond extraction, UAE is also employed to induce modifications in the physical, structural, and functional characteristics of protein ingredients. Notable alterations in protein physical properties induced by sonication include particle size reduction, changes in rheological behavior, electrical conductivity, and zeta potential. These modifications are attributed to cavitation-induced shear forces, which provoke alterations in secondary and tertiary structures, such as protein aggregation and oxidative crosslinking. Consequently, both the functionality of the protein ingredients and their nutritional quality may be affected. Changes in functional properties—particularly hydrophobicity, solubility, emulsification, and foaming capacity—are closely dependent on the intensity of ultrasound energy applied. A key challenge in this context lies in optimizing ultrasonic parameters, including frequency, power, and treatment time, to maximize protein extraction efficiency while minimizing structural degradation that may impair functionality. The beneficial effects of high-power ultrasound in enhancing protein recovery are primarily ascribed to acoustic cavitation, a phenomenon involving the nucleation, growth, and implosive collapse of microbubbles in a liquid medium exposed to ultrasound irradiation at frequencies above 20 kHz [104,105].

3.2.2 Pulsed Electric-Field Assisted Extraction (PEF)

PEF technology is a non-thermal approach, the effectiveness of which depends on several parameters, including electric field strength, total specific energy input and treatment temperature. PEF uses an electric field (electrical pulses of very short time of 10^{-4} to 10^{-2} s and high amplitude of 0.1–80 kV/cm) to create irreversible pores in the cell membrane, increasing membrane permeability. The PEF system works by cellular electroporation, whereby the application of electric fields induces the formation of positive and negative poles in the lipid bilayer of the cell membrane. Exceeding the critical electric field strength results in the irreversible breakdown of the cell membrane. Consequently, the disruption of the cell membrane improves the extraction efficiency [106]. This technique enhances protein extraction by inducing a critical transmembrane potential in plant cells. The application of PEF alters membrane permeability characteristics, thereby facilitating mass transfer processes and improving protein extractability [107]. The advantages of this technique consist of improved mass transfer, high extraction yields, with some authors showing an increase in protein extraction yield of over 28% compared to conventional extraction [108], short processing times, minimal protein degradation and reduced energy costs [109].

3.2.3 Deep Eutectic Solvents (DES)

DES are a mixture of hydrogen bond donor (HBD) and hydrogen bond acceptor (HBA) molecules that include natural plant metabolites (sugars, carboxylic acids, amino acids) and ionic molecules, usually represented by ammonium salts. DES represent versatile and sustainable media that can be specifically applied to the extraction of bioactive compounds, owing to their favorable physicochemical characteristics (recyclable, non-toxic, non-inflammable, thermally and chemically stable, easy to prepare, highly pure, highly biodegradable, low cost, and availability). The concept of natural deep eutectic solvents was first introduced by Choi et al. [110], where primary plant metabolites were employed to form DES that may naturally occur in biological systems. In addition, they serve as alternative solvents for protein extraction, offering a more environmentally friendly option due to their natural origin [111]. DES-assisted protein extraction can be carried out through both solid–liquid and liquid–liquid extraction methods. In solid–liquid extraction, solid materials are dispersed in a liquid medium, while liquid–liquid extraction entails the separation or partitioning of two compounds within an immiscible solvent system [112]. However, not all DESs are compatible with the protein extraction process, considering that the viscosity and conductivity of DES are temperature dependent. The temperature dependence poses significant challenges for the recovery and isolation of proteins from the DES extraction medium, thereby limiting the scalability and industrial applicability of this method. However, proteins extracted by this method may exhibit improved solubility, emulsification, foaming and gelation [109].

3.2.4 Enzyme-Assisted Extraction (EAE)

EAE is regarded as an environmentally sustainable technique for isolating proteins from diverse plant sources, owing to its mild processing conditions, reduced energy requirements, and lower waste generation compared to conventional chemical and physical extraction methods. Various food-grade carbohydrases and proteases have been applied to promote protein solubilization and release by hydrolyzing structural components of the plant cell wall as well as associated proteins [113,114]. The efficiency of EAE is determined by several factors, including incubation temperature, pH, enzyme-to-substrate ratio, extraction time and the solvent system employed. Beyond enhancing protein recovery, EAE can also improve nutritional quality (e.g., digestibility), as well as the techno-functional and bio-functional properties of the recovered proteins, particularly when protease-assisted strategies are used. To further enhance protein extraction efficiency, EAE has been increasingly integrated with complementary technologies to improve overall protein yield. One of the primary advantages of enzymatic treatment lies in its high selectivity and efficiency, as enzymes specifically target protein–cell wall interactions or intracellular structures without requiring harsh chemicals or extreme processing conditions. This approach allows for better preservation of protein functionality and structural integrity, while also representing a more sustainable and environmentally benign alternative, generating fewer undesirable by-products [115]. Moreover, enzymatic degradation of cell wall components facilitates the release of endogenous proteases, which may further promote protein hydrolysis, conformational modification, and the formation of short-chain bioactive peptides with potential physiological activity [116]. Proteins obtained through EAE are typically characterized by higher purity and improved nutritional quality, making them particularly suitable for human consumption. Despite its relatively higher cost and longer processing time compared with conventional alkaline extraction, enzyme-assisted extraction remains a promising, eco-efficient approach for producing high-quality plant proteins with superior functional and nutritional properties.

3.2.5 Supercritical Fluid Extraction (SCF)

SCF is a viable alternative to conventional solvent extraction systems. Supercritical fluids are substances for which both pressure and temperature are higher than their critical values, assuming physical characteristics intermediate between those of a liquid and a gas [117,118]. Therefore, the synergism between density, low viscosity, diffusivity, near-zero surface tension, and pressure and temperature dependence allows supercritical fluids to easily penetrate a microporous matrix to extract intracellular compounds [119].

One of the most widely used supercritical fluids is carbon dioxide (CO_2), it is an excellent solvent that has received special attention in SFE because it is chemically inactive, economical, easily accessible, separable from extracts, non-toxic and is an approved food grade solvent. [120,121]. However, subcritical water extraction is widely used in the protein extraction industry. Subcritical water (SCW) serves as an effective medium for the extraction of less polar compounds within relatively short processing times (approximately 30 min). SCW is defined as water maintained in a liquid state at temperatures between 100 and 374 °C and pressures below 22.064 MPa. At elevated temperatures, the dielectric constant of water decreases and hydrogen bonding is weakened, rendering SCW similar in polarity to organic solvents such as ethanol and methanol. The efficiency of subcritical water extraction is governed by several factors, including extraction temperature, processing time, water-to-solid ratio, physical and particle characteristics of the feed material, water flow rate, and the type and concentration of catalyst employed. Given the non-toxic nature of water, subcritical water extraction is considered a green technology for the recovery of plant proteins, offering a rapid, clean, and cost-efficient alternative to conventional extraction methods. Previous studies have reported extraction yields ranging from 70% to 78% using this approach [122].

3.2.6 High pressure-Assisted Extraction (HPAE)

HPAE is a non-thermal technique for protein recovery, in which the feedstock is exposed to hydrostatic pressures ranging from 100 to 1000 MPa under controlled temperature and time conditions. The process facilitates protein release primarily through the disruption of cell walls induced by increased pressure [113]. HPAE has shown a faster extraction process for plant-based proteins, a high extraction yield (82% at 100 MPa) [123] compared to other methods, and the production of pure proteins with improved functional properties and digestibility [124].

3.2.7 Microwave-Assisted Extraction (MAE)

MAE offers simultaneous, rapid, and uniform energy transfer compared to conventional methods. This accelerated heating causes water to evaporate, generating internal pressure on the cell wall, which eventually ruptures and releases intracellular organic compounds into the solvent. However, precise temperature control is essential, as excessive heating may lead to protein degradation and altered functional properties. Therefore, defining the optimal processing conditions is critical when applying microwave-assisted extraction [125]. MAE demonstrates uniform heat distribution in the extraction feedstock, rapid extraction rates, lower solvent consumption, shorter extraction times, higher extraction yield, and proteins with improved quality properties [126]. According to Chao et al. the extraction yield for pumpkin proteins was over 90% [127].

4. Quality Characteristics of Plant-Based Proteins

Proteins are essential nutrients that support human growth, contribute to the repair of damaged tissues and cells, and help maintain muscle mass. Beyond their physiological and nutritional roles, the techno-functional properties of proteins play a key role in determining the appearance, texture, and stability of food products. Plant-derived proteins represent a sustainable, cost-effective, and health-conscious alternative, owing to their high nutritional value, affordability, and wide availability.

Plant protein sources have emerged as valuable resources in the food industry, largely due to their functional attributes. These include biofunctionality, which encompasses their nutritional and physiological roles, and technofunctionality, which relates to properties such as solubility, water- and fat-binding capacity, gel formation, rheological behavior, emulsification, foaming, and whipping abilities [128,129]. These properties play a crucial role in the development of innovative functional food products. Consequently, understanding and evaluating the factors that influence them, whether positively or negatively, is essential for optimizing extraction processes and producing suitable protein-based ingredients.

However, studies by Gentil et al., Chen and Campanella have concluded that the formation of protein complex systems with other food components (polysaccharides, lipids, polyphenols, etc.) can affect the behavior of proteins in food systems during processing, manufacturing, storage and preparation, e.g., absorption, solubility, gelation, surfactant, ligand binding and film formation [130-132].

The physicochemical and functional properties of food proteins can be influenced by several environmental factors, such as pH, temperature, pressure, and ionic strength, as well as processing conditions. In addition, protein structure-function relationships govern how proteins interact with each other and with other substances in complex food systems [132]. For example, soy protein isolate showed the lowest emulsifying activity at pH 5.8 and the highest emulsifying activity at pH 8.0. The results indicated that the emulsifying activity increases with increasing pH, which may be atributed to enhanced protein solubility at higher pH values [133]. At pH values near the isoelectric point (pH 5.0), pea protein exhibited the lowest emulsifying capacity, making the resulting emulsions more susceptible to coalescence and foaming. Interestingly, its emulsifying ability at pH 3.0 was superior to that observed at neutral (pH 7.0) or alkaline (pH 9.0) conditions. Furthermore, surface hydrophobicity—a key physicochemical factor influencing the surface functional properties of proteins—was nearly twice as high for purified legumin at pH 7.0 compared with purified vicilin, suggesting a markedly greater tendency for hydrophobic cluster formation.

Amaranth protein isolates showed high solubility at pH 2.0 (76–84%) but minimal solubility near the isoelectric point (pH 5.5–10%). The lowest emulsifying activity occurred at pH 5.0, while the highest was observed at pH 9.0. Foaming capacity was also lowest at pH 5.0 and peaked at pH 7.0. The lower solubility, emulsifying activity index, and foaming capacity observed at pH 5.0 can be attributed to the isoelectric point of amaranth proteins. At this point, the proteins carry no net charge, resulting in minimal repulsive interactions and promoting protein–protein interactions that are unfavorable for solubility [134].

The solubility of protein isolates from *Amygdalus pedunculata* seeds initially increased and then decreased with rising sodium chloride concentration, peaking at 0.4 mol/L. This behavior may be explained by the effect of ionic strength: at low salt concentrations, solubility is enhanced due to the salt’s solubilizing effect, whereas at higher concentrations, increased ionic strength diminishes the repulsive electrostatic interactions between charged protein molecules, thereby reducing solubility [13].

However, some technological treatments applied to proteins, such as high pressure and high-intensity ultrasound, can improve the functional properties of the protein. Although soy protein isolate exhibited low surface hydrophobicity, it demonstrated the highest emulsifying activity index following treatment at 400 MPa. This effect was likely due to the high pressure inducing partial or complete denaturation of the protein into monomers, thereby enhancing its emulsifying capacity [136].

The hydrophobicity of Bambara bean protein was significantly higher at pH 4.0 and lower at pH 9.0. At pH 4.0, the protein carried little net charge, favoring hydrophobic protein–protein interactions, whereas at pH 9.0, the protein was negatively charged, promoting protein–water interactions. Hydrophobicity decreased with increasing temperature up to 70 °C at pH 4.0, and heat treatment also reduced hydrophobicity at pH 9.0. These changes were likely due to protein aggregation through hydrophobic contacts (decrease) or partial unfolding that exposed previously buried regions (increase). The emulsifying capacity of the protein isolate peaked at 80 °C and pH 9.0, while emulsion stability was highest at pH 4. Protein solubility decreased with rising temperature at pH 4.0 and 7 but increased at pH 9.0 at 100 °C [137].

The sprouting process enhanced the physical and functional properties of faba bean protein isolates, including a water absorption index of 2.97 g/g and foaming stability of 140.13 mL/100 mL. Additionally, pressure-cooked faba beans exhibited superior functional properties compared to those subjected to conventional thermal processing, with higher water solubility (2.12 g/100 g) and water absorption capacity (2.02 g/g) [138]. Another study also showed that higher functional properties were observed in germinated amaranth flour than in raw amaranth flour. The improvement in the functional properties of proteins during germination is due to the activity of proteases that can form compounds (proteins) with lower molecular weight [139].

In addition, Yao et al. [140] examined the effects of γ-irradiation on the physicochemical and functional properties of rice protein. They reported higher physicochemical index values at lower irradiation doses compared to higher doses. This effect was likely due to the disruption of intermolecular bonds at low doses, resulting in smaller protein particles. At higher doses, however, protein aggregation occurred through cross-linking, electrostatic and hydrophobic interactions, and disulfide bond formation, leading to increased particle size and higher molecular weight. [141,142]. Rice protein exhibited the highest surface hydrophobicity at a gamma irradiation dose of 2 kGy, whereas increasing the dose to 5 kGy resulted in a decrease in surface hydrophobicity [140]. This could be because radiation treatment exposed hydrophobic residues in the protein, increased the number of hydrophobic sites, and improved the ability to contact the external environment, thus enhancing surface hydrophobicity [143]. On the other hand, when the γ-irradiation dose was excessively high, the hydrophobic sites of the protein were reduced due to the increase in the number of β-sheets in the protein secondary structure, thus reducing the surface hydrophobicity [144]. Also, the solubility of rice protein increased after γ irradiation treatment, as the irradiation dose increased, the solubility of rice protein initially increased and then decreased. The solubility of rice protein was 69.18% when the γ irradiation dose is 2 kGy. This could be a result of the γ-irradiation treatment, which contributes to opening the protein structure, exposing internal hydrophilic groups and facilitating the binding of water molecules, increasing the protein solubility [145]. At the same time, when the irradiation dose increased to 5 kGy, protein molecules either aggregated or protein residues were oxidized to form disulfide bonds, which decreases protein hydration and rice protein solubility [140,146]. Furthermore, the highest water and oil retention capacities of rice protein were 5.89 g/g and 3.45 g/g, respectively, at an irradiation dose of 2 kGy. [146]. This could be due to the fact that gamma irradiation treatment exposed internal groups and opened the protein structure, which led to increased contact with water molecules and, consequently, improved water-holding capacity of the protein.

Plant-based proteins exhibit excellent emulsifying and foaming properties, making them valuable for use in various food formulations. Emulsification is the process of dispersing one liquid phase into another, usually oil in water, creating a stable and homogeneous mixture. Foaming, on the other hand, involves incorporating air into a liquid to form a stable foam [147]. They contain both hydrophilic (water-attracting) and hydrophobic (oil-attracting) regions in their structure. This unique composition allows the protein to interact with both the aqueous and oily phases, facilitating the formation and stabilization of emulsions [148]. When plant-based protein is added to an oil-water mixture and stirred, it forms a stable emulsion by surrounding the oil droplets with a protein layer, preventing coalescence and separation of the two phases. This property is particularly useful in salad dressings, mayonnaise, and various emulsified sauces. Plant-based proteins can also generate stable foams by trapping air bubbles in a protein network. [149]. When proteinsare whipped or stirred, they form a stable foam with high volume and good stability. The protein film surrounding the air bubbles ensures the structural integrity of the foam, preventing the bubbles from coalescing and collapsing. Protein foams find applications in meringues, whipped toppings, and aerated desserts. [150]. The emulsifying and foaming properties of pland-based protein contribute to the texture, mouthfeel and stability of food products, improving their sensory characteristics and overall quality [151].

Proteins are capable of gel formation and can thicken solutions, making them valuable texturizing agents in a variety of food products. Chickpea protein, in particular, can form gels upon heat treatment or when subjected to changes in pH [152]. Protein molecules undergo conformational changes, leading to aggregation and the formation of a three-dimensional protein network. The resulting gel has a solid structure that traps water and other components, creating a firm and cohesive texture [153]. Chickpea protein gels can be commonly used in meat analogues, vegetarian sausages, and plant-based burgers to mimic the texture of meat. The protein can also act as a thickening agent in liquid systems. When added to a liquid, such as soups, sauces, or gravies, chickpea protein interacts with water molecules and forms a viscous solution [154]. The thickened solution improves the consistency and mouthfeel of the product, enhancing overall sensory attributes. The gelling and thickening properties of the protein contribute to the structural integrity and texture of food products, enabling the development of a wide range of plant-based food applications [155].

Proteins have high water and oil absorption capacities, essential in food applications where moisture retention and fat binding are crucial. They can absorb and retain large amounts of water [156]. When incorporated into food formulations, proteins can bind water, reducing its loss during cooking or processing. This property enhances the juiciness and tenderness of meat analogues and other plant-based products, while also improving moisture retention in baked goods. Likewise, chickpea protein can absorb and bind oil, which helps control oil migration, prevent separation, and improve emulsion stability. This oil-binding capacity is particularly valuable in formulations where fat replacement or imitation is desired [157]. Overall, the water- and oil-binding properties of proteins enhance texture, juiciness, and stability across a variety of food products, highlighting their value as functional ingredients in the food industry.

5. Conclusions

The integration of plant proteins into the food industry and the diet of the global population is an essential direction for the development of a sustainable food system. Given that the demand for protein is constantly expanding, and meat production involves a considerable ecological impact, plant sources, along with algae and insects, represent viable solutions for the future. The quality and functionality of plant proteins depend on multiple variables: plant variety, cultivation conditions, and extraction and processing methods. Adapting technological parameters to the characteristics of the raw material is crucial for obtaining an optimal yield and maintaining functional properties. Emerging technologies, such as microwave, ultrasound or electric field-assisted extraction, open promising perspectives by increasing efficiency and reducing energy consumption, while maintaining the nutritional integrity of proteins. In terms of nutrition, the strategic combination of different plant sources can counteract deficiencies in essential amino acids, providing an intake comparable to that of animal proteins. In addition, their bioactivity – including antioxidant, antidiabetic or cardioprotective effects – amplifies their value for consumer health. The integration of proteins from green leaves and microalgae requires innovative solutions to overcome technological and sensory barriers, so that they are widely accepted by consumers. The successful implementation of plant proteins in the food industry depends on the balance between sustainability, functionality and acceptability. Plant proteins can become the foundation of resilient food systems, able to respond to demographic and environmental challenges.

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