FUTURE OF PLANT PROTEIN TECHNOLOGY: CHALLENGES AND OPPORTUNITIES

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July 14, 2022

Abstract:

According to an estimate, protein consumed by people globally constitutes 20% of their total calorie intake. However, the 'building blocks of life', proteins are not only lacking in the diet of people of most developed and developing nations but are often overlooked. Today there is an unprecedented challenge to produce and feed adequate protein to over 8 billion people in an environmentally friendly and affordable way. Interestingly, health and climatic conditions, especially the Covid 19 pandemic have led to a paradigm shift in consumer eating habits and mindset. They are reconsidering diets, viewing foods as medicine, and are inclined toward paying an added amount for sustainable healthy foods. Plant-based proteins present exciting opportunities to meet the food challenges of the future and deliver healthy and responsible food choices. They are a potential solution to our nutritional needs due to their long history of crop use and cultivation, lower cost of production, and easy access But, the poor techno-functional and bio-functional properties of plant proteins such as low solubility, poor foaming, emulsifying, and gelling properties, along with low bioactivity and digestibility limit their use in food products and formulations. Relative to animal proteins, including dairy products, plant protein manufacturing, and processing requirements are still at a nascent stage and small businesses or startups fail to find a steady start. To mitigate such issues, technological advances are required in the development of plant protein ingredients and foods. This review focuses on the challenges and opportunities in the process of implementing plant protein ingredients in foods. It elucidates the functional properties of plant-derived proteins, the technical challenges behind incorporating them in food systems, and some novel physical, chemical, and biological processing operations that can be employed to improve their extraction, functionality, nutritional profile, and sensory attributes. Finally, the science behind formulating innovative plant-based meat, egg, and dairy alternative products is also discussed to present a roadmap for creating future foods with plant proteins.

1. Introduction- Apart from meeting our calorie requirement, the most crucial nutrition imperative for body metabolism is protein. With the global population expected to rise above 9 billion people by 2050, we face an unprecedented challenge to manufacture and feed adequate protein to an ever-growing population. A 2017 survey shows that 73 percent of South Asians suffer from protein deficiency while above 90 percent are unaware of the daily protein requirement. Also, protein manufacturing and processing is a major concern because conventional animal protein production requires an intensive amount of land and resources [1]. Alternative proteins like plant-based proteins provide a viable solution to overcome these difficulties. Owing to their long history of crop use and cultivation, lower cost of production and easy access, the manufacturing of plant protein-based foods is also environmentally sustainable and affordable [2]. However, lower protein quality and poor functional properties of plant proteins like poor solubility, foaming, emulsifying, and gelling properties, along with low bioactivity and digestion problems limit their use as food ingredients and whole food products. Relative to animal proteins, including dairy products, plant protein manufacturing, and processing requirements are still at a nascent stage and small businesses or startups fail to find a steady start. To mitigate such issues, technological advances and knowledge creation are required in the development of plant protein ingredients and foods. This review focuses on the challenges and opportunities in the process of implementing plant protein ingredients in foods. It elucidates the functional properties of plant-derived proteins, the technical challenges behind incorporating them in food systems, and some novel physical, chemical, and biological processing operations that can be employed to improve their extraction, functionality, nutritional profile, and sensory attributes. Finally, the science behind formulating plant-based meat, egg, and dairy alternatives is also discussed to present a roadmap for creating future foods with plant proteins. Although the focus of this review on plant proteins is in a global scenario it is essential to recognize the manufacturing and commercialization challenges of plant-based protein foods in less developed nations, especially in South Asia and Africa. This review is therefore expected to stimulate food scientists in developing countries to consider creating low-cost and environmentally sustainable alternative protein sources for consumption.

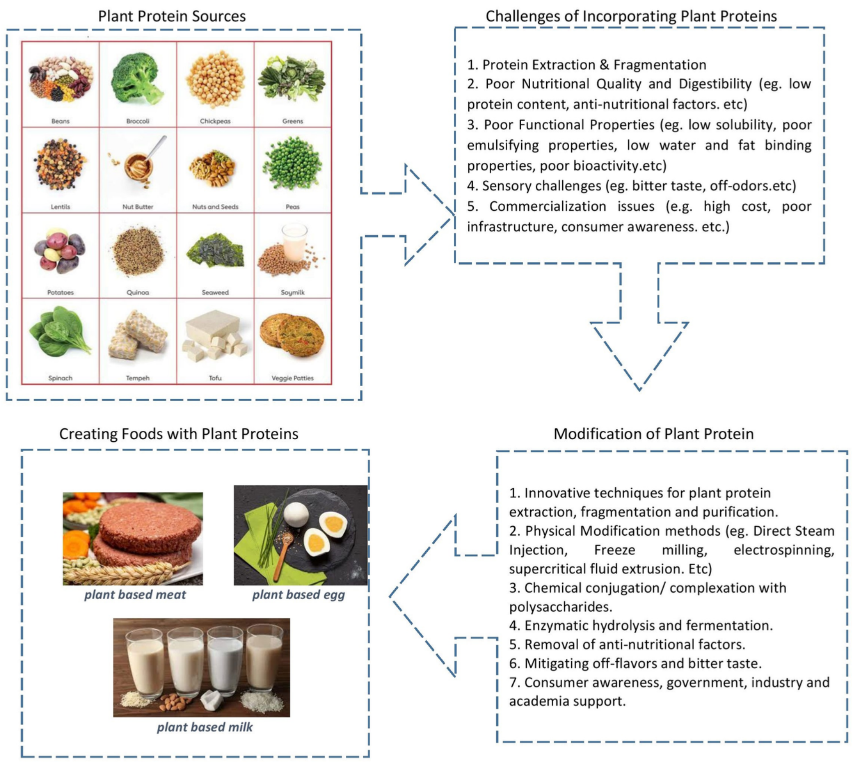

Figure 1. A roadmap to creating sustainable foods with modified plant proteins.

2. Protein Structure and Functionality

Proteins are macromolecules consisting of linear polymers of amino acid residues joined together by peptide bonds which have various structural, functional, and nutritional properties that are useful for the food industry in food formulations [3]. The understanding of protein functionality starts at the level of its structure. Biological structural-functional relationships are often revealed when the three-dimensional structure of a protein is determined but that is not the complete picture of food protein functionality because food applications are usually accompanied by structural changes at the intramolecular or at the interface between two molecules rather than the original structure. [4] Protein functionality is often associated with changes in secondary and tertiary structure (heat denaturation for gelation; unfolding at an interface), so treatments such as high pressure have been used to alter the structure and functional properties [5-6]. For example, the amino acid lysine contains an e-amino group that contributes to the net charge and nucleophilic properties that allow for reactions with carbonyl compounds found in foods. The most used strategy is to utilize the beginning of the Maillard reaction to either: 1) manipulate protein charge and/or 2) form a conjugated molecule by attaching a sugar or oligosaccharide molecule [7-8] which in turn affects functionality. Protein charge and isoelectric point can also be altered by deamidation [8-9]. The intrinsic chemical and physical properties of protein molecules such as hydrophobicity, net charge, and the presence of reactive groups of a protein depend on various factors like the type, number, order, the orientation of its amino acids, and mutual interactions among them [10]. Additionally, the functional properties of protein including solubility, gelation, thermal stability, and emulsification are governed by its shape, molecular weight, physicochemical properties, and processing conditions. This in turn stimulates the protein's interaction with other micro and macromolecules, processing, storage, and degradation. [11, 12]. For manufacturing plant-based foods and ingredients, it is necessary to select a suitable plant protein with good functional properties than can mimic animal proteins. The simplest approach to understanding protein functionality in a food system is to examine single protein solutions/dispersions. For example,β-lactoglobulin is one of the most studied food proteins regarding functionality and a recent review presents a comprehensive model for denaturation and aggregation at temperatures ranging from 20 to 150º C [13]. However, protein ingredients are usually a complex mixture of proteins (e.g., whey or egg white proteins) and other macromolecules such as sugars and minerals. Hence, a protein ingredient added to a formulation contains more than just protein in it and therefore must "function" in a complex system. Modifying or structuring plant proteins for enhanced functional properties such as those supplied by animal proteins is also a key problem in this field. Proteins can be informally divided into three main classes, which correlate with typical tertiary structures, depending on their physicochemical properties such as amino acid residue quantity and sequence on the polymer chain: globular proteins, fibrous proteins, and flexible proteins. Globular proteins which are mostly enzymes are soluble. Fibrous proteins constitute structural properties such as collagen, the major component of connective tissue, or keratin, the main protein component of hair and nails. The fibrous proteins are generally water-insoluble due to their structure, whereas most globular structures are soluble in water, acids, and bases [14]. Plant proteins are generally constituted of globular proteins, present as covalently linked multimers, and can be classified as albumins (soluble in water), globulins (soluble in dilute salt solutions), prolamins (soluble in aqueous alcohol), and glutelins (insoluble in water but soluble in dilute acid/alkali) [15]. While albumin and globulins are mostly found in all pulses (>50%) [16] and some pseudo cereals (quinoa, and amaranth), prolamins (wheat, maize, barley, and rye) and glutelins (wheat) constitute 85% of total protein in the cereal [17] as well as in the pseudo cereal family [14, 18]. Fibrous or meat proteins have a complex structure of fibrous protein bundles positioned inside connective tissue formed of triple helices of collagen, and can be classified as sarcoplasmic, stromal (elastin, collagen), and myofibrillar protein (actin, myosin, tropomyosin, troponins) [19,20]. Lastly, filamentous proteins have flexible and disorderly structures; for instance, casein has a random coil structure, with hydrophobic and hydrophilic patches [21]. Due to its structure, casein links to calcium phosphate molecules to form casein micelles [22, 23]. Casein has excellent functionality including surface-active and stabilizing properties which are due to the following factors: a) high proportions of prolyl residues that allow open and flexible conformations; and b) random coils of hydrophobic, hydrophilic regions, and phosphate groups [22]. Mimicking the structural and physicochemical characteristics of gelatin and casein proteins has been challenging because most known natural plant proteins do not have flexible random-coil structures or micellar structures, respectively. Hence, assembling plant proteins into these superstructures that can simulate characteristics of animal proteins needs more attention. This can be achieved by introducing random coils in globular plant protein structures or the assembly of various plant proteins to mimic casein micellar structures.

Functional Property	**Functional Term**
Organoleptic, kinesthetic	Color, flavor, odor, texture, mouthfeel, smoothness, grittiness, turbidity, etc

Functional Property	Functional Term
Hydration	Solubility, dispersibility, wettability, water absorption, swelling, thickening, gelling, water holding capacity, syneresis, viscosity, dough formation, etc.
Surface	Emulsion, foaming, aeration, whipping, protein/lipid film formation, lipid binding, flavor binding, stabilization, etc.
Structural/textural/rheological	Elasticity, grittiness, cohesion, chewiness, viscosity, adhesion, network cross-binding, aggregation, stickiness, gelation, dough formation, texturability, fiber formation, extrudability, elasticity, etc.
Bioactivity	Antioxidant, antimicrobial, ACE inhibiting activity. etc of protein hydrolysates and peptides.
Other	Compatibility with additives, enzymatic, inertness, modification properties

Table 1. General classes of functional properties of plant proteins are important in food applications (Adapted from Kinsella & Melachouris, 1976).

3. Plant-Based Proteins: Definition and Types

In recent years, the benefits of a plant-based diet have been widely popularized. Being low in calories, plant-based foods can help reduce calorie consumption whilst providing adequate nutrition. Plant protein simply is a meaningful food source of protein that is extracted from plants. This group includes pulses, soya tofu, tempeh, seitan, nuts, seeds, certain grains, and even peas. Plant proteins, mostly globulins have been obtained mainly from protein-rich seeds of cereals and legumes. Based on their sources plant proteins can be classified into the following-

- Soy-based: Tempeh, tofu, edamame, soy milk, and soy crumbles [textured vegetable protein].
- Bean or legume-based: lentils, beans, rice, chickpeas, black beans, vegan eggs.
- Pea protein-based: Pea protein isolate, pea protein milk
- Grain-based: Seitan, whole wheat flour, spelt, teff.
- Nut and seed-based: Almonds, cashews, pistachios, chia seeds, flax seeds, rapeseeds
- Vegetable-based: Potatoes, sweet potatoes, broccoli, asparagus
- Others: Mycoprotein, algae

4. Challenges of Incorporating Plant Proteins in Foods

The concept of altruistic health and wellness is no longer new. Today, for consumers in India and around the world, their baseline expectations are for food products to have clean, simple, and sustainable ingredients and are driven by heightened consumer awareness of how their product consumption influences living in a healthy world. The increasing popularity and widespread acceptance of plant-based diets have led to significant technological and commercial advancements in the field of plant-based proteins. This includes the discovery of novel sources, sustainable protein extraction methods, and improved fractionation approaches. Food scientists are aiming to develop techniques to produce plant protein-rich ingredients, optimize their nutritional profile, and understand their techno functionality in food products and formulations. However, the food and beverage manufacturers who still heavily depend on animal-based ingredients face several technical and commercial challenges in incorporating plant proteins in a good quality product. These include:

- Difficulty in extraction and purification of proteins derived from plants as compared to animal proteins. This is partly due to the indigenous structural features of plant proteins and partly because proteins are often biologically complexed with other macromolecules in the plant matrix.

- Nutritional challenges related to low protein content, poor quality, and digestibility of plant proteins arising because of unbalanced amino acid composition, anti-nutritional factors, and allergens
- Limitations in product development as the functionality of plant-based protein ingredients do not match the same in ingredients sourced from animal protein. These include poor water solubility, poor emulsifying, gelling and texturizing capacities, low water and fat binding properties, and inadequate bioactivity [e.g. antioxidant and antimicrobial properties]. This is a major drawback as these functional properties contribute to the texture, mouthfeel, and consistency of a food product.
- Challenges related to undesirable taste, flavor, and color contributed by plant-based protein ingredients.
- Higher cost of raw material and processing which affects sales.
- Lack of consumer awareness and regulatory hassles affecting brand acceptability.

In the following section, the above-mentioned challenges have been described in detail and new research directions in plant protein technology are explored that could improve the functionality of plant-based proteins and mitigate the techno-commercial challenges faced by food manufacturers in incorporating plant protein-rich ingredients in foods.

5. Modifications in Plant Protein extraction and Fractionation

To fully utilize the potential of plant-based proteins, they have to be extracted and fractionated intact. This is a major problem because as compared to animal proteins, extraction and purification of plant proteins are difficult. Until now, acid/alkali treatment and precipitation have been used extensively to isolate and purify proteins. However, research shows that the alkaline treatment of protein leads to the production of lysinoalanine, a toxic amino acid [24]. Also, the harsh conditions used in the wet extraction and fractionation techniques for producing plant protein isolates [alkaline extraction–isoelectric precipitation] cause' extensive protein denaturation and aggregation, severely affecting functionality [25]. For example, up to 75% of proteins present in pea protein isolates are insoluble and nonfunctional, and hence, unutilized [26]. In addition, endogenous phenolic compounds during processing may form complexes with plant proteins, affecting the functional and nutritional properties of the proteins [27]. All these problems, therefore, necessitate the development of different strategies and novel methods of protein extraction and isolation, some of which are discussed below.

5.1. Extraction

As discussed, proteins are isolated through solvent treatments (acids, alkalis, organic solvents) depending on the protein source followed by precipitation. Such solvents may cause protein damage and loss of functionality. As mentioned, the treatment of proteins with alkalis can also lead to the formation of lysinoalanine. A promising alternative is to use food-grade deep eutectic solvents (DES) for protein extraction and isolation. DES has been effective in extracting various food components such as phenolic compounds and sugars [28]. There are also recent proofs of using DES to extract oilseed cake protein [29] and oat protein [30]. As a green and mild solvent, DES also helps food manufacturers and legal bodies with the clean labeling of foods. However, more work is required to understand the extraction chemistry of DES for optimizing the extraction process and to obtain higher extracted protein content (currently ~50%). To improve the extraction efficiency, it is necessary to weaken or disrupt the plant cellular matrix, notably, the disruption of the polyphenol–protein, and fiber–protein complexes, so the extraction solvent can penetrate effectively. For this, some novel value-added physical and enzymatic techniques including ultrasound, pulsed electric field, microwave, high pressure, pectinases, and proteases can be employed [31]. Additionally, supercritical fluid extraction can further remove lipids and polyphenols bound to proteins [32]. However, these processes are expensive and can also damage the protein structures, rendering the extracted proteins less functional. In that respect, non-thermal processing methods may be favored. Thus, a balanced approach to these processing conditions can stimulate the affordable, sustainable, and efficient extraction of intact and functional plant proteins.

5.2. Fractionation

After extraction, the protein is purified and isolated from the extraction solvent. In alkaline extraction, the proteins precipitate at their isoelectric points and are thereby separated by decanting or centrifugation. However, this process has detrimental effects on protein structure and functionality, and typically only the globulin fraction is obtained. A promising alternative to isoelectric precipitation is membrane filtration e.g. Ultrafiltration and/or dialysis, whereby specific protein fractions, including albumins, can be isolated by molecular weight without compromising on protein concentration and functionality [33]. For ensuring maximum efficiency, food scientists will need to find novel membranes that prevent fouling and are safe for use in food applications. On the other hand, wet extraction and fractionation techniques are typically energy- and water-intensive processes and also generate proteinaceous effluents [25]. To improve sustainability, dry fractionation techniques such as air classification and electrostatic separation are currently explored [34–36]. These techniques make use of the density and particle size differences between protein and other components to obtain protein-rich concentrates. These methods are energy efficient and solvent-free, the proteins are obtained structurally intact, and hence, functional. Another aspect is that most commercial protein ingredients are a complex mixture of several protein sub fractions like albumins, globulins, glutenins, and prolamines. These fractions each have different techno-functional properties which affect the overall performance of the native protein based on its proportion in the ingredient. For example, pea albumins form stronger heat-set gels than pea globulins [37]. Within pea globulin subfractions, legumin is detrimental to acid gel formation [38] and has poorer emulsifying properties than vicilin [39]. It is therefore necessary to isolate different protein subfractions for specific applications or for obtaining a mixture of subfractions with different concentrations for tailored functionality. Separating the subfractions is difficult because of their similar molecular weights, isoelectric points, and solubility. Chromatographic techniques have been used to separate and isolate specific protein subfractions, but the separation efficiency is too low on a commercial scale. Hence, novel fractionation techniques must be developed to improve bulk separation efficiency.

6. Modification of Plant Proteins for Enhanced Functionality

Plant proteins generally have poor techno-functional properties compared to animal proteins. Additionally, harsh protein isolation conditions like temperature and use of solvents lead to denaturation, and aggregation of protein molecules ultimately causing a loss in functionality. While most cereals proteins are barely soluble at neutral pH, pulse proteins form weak gels [40,41]. On the other hand, dry fractionated plant protein concentrates have better functionality. However, the lower protein content and presence of other impurities limit their applications in product formulations. The general strategy for the modification of plant protein functionality involves the application of physical, chemical, and enzymatic techniques or a combination of these at the molecular, mesoscale (reduction of molecules into sizes less than 300 µm, e.g. emulsion droplet, micelles), and macroscale of protein ingredient development [42]. The stresses in the process, also known as extrinsic factors and include various factors like temperature, pressure, shear, freezing and thawing, pH, ions, electrostatic, covalent, noncovalent, hydrophobic, electric field, electromagnetic field, surface tensions, hydration, and solvent force. Such process-induced disturbances will change the thermodynamic state of the protein, including its structural and conformational characteristics. For instance, a modification could change the size, surface charge, hydrophobic/hydrophilic ratio, and molecular flexibility of the protein. Overall, the modification could improve or create entirely new protein functionality [43]. The next section explores some of the research progress on protein modification for improved techno-functional properties and bioactivity with a focus on the use of physical, chemical, and enzymatic processes for plant proteins. Another research direction in protein modification that can be explored is the transformation of denatured and/or aggregated non-functional plant proteins into functional plant proteins. Some methods employed to transform non-functional proteins include thermal treatment [44] and micro-fluidization [45, 46]. There have been multiple reports related to physical, chemical, and biological methods aimed at improving the functionality of plant properties. Out of the physical methods, high-pressure processing [47-54], extrusion [55-62], and sonication [63-70] are the most commonly used techniques. The application of chemical methods for manipulating protein structure and functionality in the food sector is severely limited because of the use of many hazardous chemicals, and subsequently, the unknown food consumption credibility of the modified proteins or their by-products. However, chemical glycation is the most desired method for food applications because it does not need the use of hazardous chemicals and produces no by-products. As a result, this technique may be a suitable chemical modification strategy for plant-based proteins in terms of consumer preferences, clean labels, and commercialization [71-77]. This review focuses on some rare and novel technologies of modifications that have not been previously studied or are at a nascent stage of application.

6.1. Direct Steam Injection

Steam injection or hydrothermal cooking is a process where a product is subjected to very high temperatures of up to 155 c for a short period (1-180 secs). The temperature and pressure are precisely monitored and controlled by thermocouples and pressure gauges. The use of this technology for the functionalization of protein concentrates and isolates was first proposed by Wang and Johnson, 2001. [78]. Protein powders were subjected to steam injection at 154 º C for 11 to 42 s followed by rapid cooling and moisture removal. Hydrothermal cooking restored the solubility of soy concentrate to nearly that of the original protein. Additionally, there was an improvement in foaming properties, emulsifying properties, oil absorption capacity, hydration properties, and bulk density of the protein flour. Ganjyal et al (2011) reported an improvement in solubility, emulsifying, foaming, and gelling properties of different protein blends subjected to direct steam injection[79]. Heat treatment may result in the breaking of existing disulfide bonds, unfolding of the protein, and possibly activation of highly reactive sulfhydryl groups [80]. Similar findings were reported by Petruccelli and Anon (1995) in a study on soy protein isolates [81]. Yang et al (2014) investigated the effect of a combined treatment of steam injection and enzyme-assisted ultrafiltration for preparing soy protein isolate with improved functionality. The process resulted in an isolate with high protein content with improved solubility and lower content of anti-nutritional factors than the native protein [82]. It is assumed that the high temperature and steam pressure decreases the particle size of protein aggregates leading to improvement in solubility. However, the exact mechanism of protein modification during the direct steam injection process is not yet understood. Also, high temperatures may lead to the development of undesirable colors and flavors due to the Maillard reaction. More research is required to investigate the mechanism of action of direct steam injection on protein structure, quality, and amino acid profile.

6.2. Freeze milling

A novel chemical-free method for modifying protein flours and isolates is freeze milling. This method has previously been utilized for improving the solubility of rice protein isolates [83]. In this process, protein suspensions are frozen at very low temperatures -20 º C to -30º C before milling. One or more cycles of freeze milling significantly improve the solubility of protein isolates. In a follow-up study, it was reported that the emulsification and foaming properties of rice protein isolate improved after the freeze milling process as compared to untreated protein [84]. It was postulated that the milling technique causes protein unfolding and rearrangement of three-dimensional conformation, however, the effect of freezing pretreatment on the functional behavior of protein is not studied.

6.3. Biological/Enzymatic Modifications

Protein modification using biological or enzymatic methods can be classified into enzymatic hydrolysis, enzymatic cross-linking, and protein fermentation. Enzymatic hydrolysis is carried out by breaking protein peptide bonds to improve their biological and nutritional value, creating hydrolysates of high-added value [85]. On the other hand, enzymatic cross-linking is achieved by forming covalent bonds using transglutaminase, by catalyzing acyl transfer reaction between the carboxamide group of protein-bound glutamine and the amino group of lysine. However, the most emerging biological technique for modification of plant protein functionality is protein fermentation. Fermentation has conventionally been used to improve the nutritional profile, sensory characteristics, shelf life, and health-promoting benefits of food products by increasing the bioavailability and bio-accessibility of bioactive compounds (isoflavones, Vitamin B1 and B2, Vitamin E and C) as well as the reduction of undesired anti-nutrients (phytates, saponins, tannins, inositol phosphates, and trypsin inhibitor) [86,87]. However, there is a renewed interest in fermentation for the development of functional plant protein ingredients and plant-based protein products. Fermentation has been applied to mask the beany flavor of pea/lupin protein isolates [88-90], increase the texture of vegan cheese (Li et al., 2017), to develop vegan yogurt with longer shelf life (Yazici, Alvarez, & Hansen, 1997), and increase the nutritional index of plant-based milk (Tangyu, Muller, Bolten, & Wittmann, 2019). Previous reports claim that fermented plant protein flours showed an increase in crude protein content and an increase in emulsion properties. This may be due to the proteolytic cleavage during fermentation and exposure of its hydrophobic groups leading to a balance of hydrophilic-lipophilic balance that favors emulsification [91]. Proper methods of fermentation have to be standardized on a commercial scale for effective protein modification on a larger scale.

6.4. Complexation with polysaccharides

Whole foods are a complex mixture of various components including proteins, fats, and carbohydrates. Apart from proteins and fats which make up the bulk of most food components, polysaccharides make up the predominant carbohydrate component in most plant-based ingredients. Polysaccharides or polycarbohydrates are long-chain polymeric carbohydrates composed of monosaccharide units bound together by glycosidic linkages. These include starch, cellulose, pectins, agar, carrageenan, alginates, and gums [92]. Polysaccharides form the major building blocks in food formulations as structuring and stabilizing agents because of their thickening, emulsifying, and gelling properties [93]. When used in combination with proteins, their functionality can be further improved through mutual biopolymer interactions [94]. Hence, there is great scope in designing plant-based foods such as vegan milk, ice cream, etc by understanding and modulating protein-polysaccharide interactions in food matrices. Plant proteins typically have low solubility, hence polysaccharides are often added along with a processing step to improve overall biopolymer solubility. Some modification steps include simple complexation [95, 96], sonication [97, 98] and conjugation [99,100,101]. The increase in protein solubility can be used for developing acidic beverages with high protein content, to reduce the precipitation of plant proteins. Apart from solubility, polysaccharides also improve the viscosity [102,103], foaming [96,104–106], emulsifying [107–115], and gelling [116,117–122] properties of plant proteins. Another interesting strategy to leverage the poor solubility of plant proteins is to create insoluble plant protein-polysaccharide particles as Pickering emulsion stabilizers [111,112]. However, the addition of polysaccharides in some cases might result in the reduction of the functionality of proteins. E.g. reduced solubility and foaming ability. Hence more work is required to understand the factors influencing plant protein-polysaccharide interactions like pH, temperature, ionic strength .etc.

6.5.Electrospinning

Animal skeletal muscles consist of mostly fibrous protein structures that form long, flexible fiber bundles. These fibrous structures contribute to the texture, mouthfeel, and appearance of conventional whole muscle cuts. On the contrary, plant proteins are typically made of globular proteins that are tightly packed, spherical, and require structuring and modulation to mimic fibrous animal proteins. Techniques to texturize globular plant proteins into fiber-like materials are classified as “bottom-up” or “top-down” methods [123]. Bottom-up methods assemble small individual components into a large matrix from nano- to macro-scale. Fiber spinning has emerged as the most viable bottom-up technology for plant protein texturization. This method form thinner protein fibers with enhanced aspect ratios compared to top-down methods, which texturize biopolymers on larger length scales. Despite the utility of bottom- approach, top-down strategies are currently mostly used due to their scalability and affordability. Consequently, top-down strategies such as extrusion and molding have more applications than fiber spinning technologies. But there are many novel strategies to improve plant protein fiber spinning to be more effective, commercially scalable, and affordable. In the fiber spinning technique, a polymer solution is ejected from a needle or spinneret with an external force, and the elongated polymers are collected as solid fibers. Factors affecting the final properties of the ejected fibers include the magnitude of the external force applied, environmental conditions, and intrinsic polymer characteristics. Fiber spinning technologies can be classified as wet spinning, electrospinning, jet spinning, and blow spinning. In the wet spinning method, the polymer solution is simply extruded through a spinneret into a non-solvent, causing the precipitation of polymer into a fiber. Electrospinning methods extrude polymer solution through a needle by applying an electric potential [124]. The electric repulsive forces ultimately cause the polymers to form thin fibers. Typically, electrospinning yields thinner fibers (˜100 nm) than simple wet spinning (˜10 µm) and be controlled easily. Jet spinning and blow spinning methods use rotational speed and high-pressure gas, respectively, to force polymers to fibrous structures [125]. But all these methods have been applicable for synthetic polymers and therapeutic biopolymers. The absence of suitable conditions for food-grade protein spinning has prevented their scalability and use in food industries. Until now, there have been very few reports on standardizing the electrospinning of plant proteins [126-128]. The major focus now should be to optimize experimental conditions for spinning plant-based proteins into textured proteins that can mimic animal protein fibers, including optimizing solution type, viscosity, conductivity [for electrospinning], and surface tension. The process parameters for each type of spinning method should also be standardized. For example, in the case of electrospinning, this would mean tuning electric voltage, flow speed, and distance between the ejection needle and fiber collector. There is also room to select particular proteins whose structure supports the aforementioned methods. For example, random coiled structures are best for spinning because of their flexibility. Specific proteins, particularly zein and gelatin, have better spinning efficacy than other proteins. On the other hand, a combination of poorly spinnable proteins with more easily spinnable proteins can be used to improve overall spinning efficacy. In this way, more plant proteins and their combinations should be tested for their optimal spinning conditions. As spinning technologies develop further, techno-economic models will have to be evaluated to understand the cost components and opportunities of texturizing plant proteins with various spinning technologies.

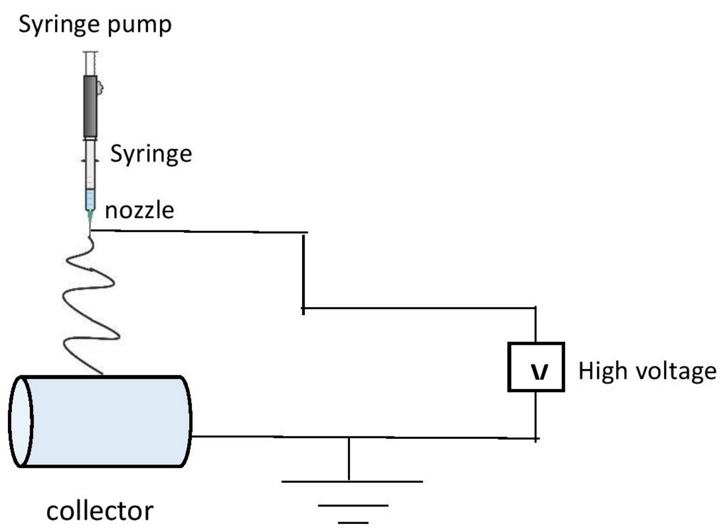

Figure 2. Typical Electrospinning Equipment

6.6. Supercritical fluid extrusion

Reactive supercritical fluid extrusion is a novel extrusion technology traditionally used for producing expanded starch foam and was patented by Rizvi and Mulvaney,1992 [129]. The extrusion process uses supercritical carbon dioxide (SC-CO2) as a blowing agent, a nutrient carrier, and an in-line process modifier instead of steam. SC-CO2 is environmentally friendly, chemically inert, physiologically safe, and easily recycled, which makes it an ideal solvent for food operations. The effects of SC-CO2 treatments on the functionalities of commercial whey protein were investigated by Zhong and Jin,2008 [130]. The authors reported that the gelling properties, surface hydrophobicity, and rheology were improved by SC-CO2 –treatments. The rheological behavior of modified whey protein was found to be improved as compared to unextruded control. A 20% (w/w) SC-CO2 extruded WP dispersion exhibited a highly viscous and creamy texture with particle size in the micron range which could serve as a thickening/gelling agent or as a fat replacer in food formulations over a wide range of temperatures [131]. In addition, the cold, gel-like emulsions prepared with texturized WPs by the reactive SCFX process could be beneficial for controlling the texture of emulsion-filled gel products and their derivatives. Modified WPs also yielded excellent emulsifying properties compared to the commercial WPC-80 [132]. It is possible that structural changes in modified WPs due to denaturation and polymerization induced by the reactive SCFX process lead to an increased surface hydrophobicity and molecular flexibility, allowing effective adsorption of protein molecules at the oil-water interface. Until today, there are not many reports available on the supercritical fluid extrusion treatment on plant-sourced protein. Despite its advantages, control of the reactive extrusion process is still very challenging due to the comprehensive effects of the thermal and mechanical energy stress, and complex physicochemical transformations in the extruded product. More work has to be done on a software simulation of the raw material properties, extrusion parameters, supercritical fluid temperature, and quality evaluation of the extrudates.

7. Strategies to improve the nutritional profile of plant proteins

Plant proteins have a lower nutritional profile as compared to animal proteins due to a lack of necessary amino acids and are less bioavailable or digestible due to a lot of factors. The major factor is the presence of anti-nutrients like phytates and trypsin inhibitors that impedes digestibility and absorption. Another factor is the presence of more α- helixes in plant protein structures that facilitate aggregation. Lastly, the presence of dietary fibers in plant proteins prevents proteolytic digestibility and reduces bioavailability. Some novel strategies have been reported for improving the nutritional profile of plant proteins for human consumption.

- Removal of anti-nutritional factors from isolated plant proteins eg. soy protein isolate by processing techniques and methods such as fermentation, debranning, autoclaving, and soaking. etc. or a combination of these methods.[133]
- Increasing the consumption of plant proteins per meal to compensate for their reduced anabolic response compared to animal protein [134].
- Fortification of plant proteins with essential amino acids can improve the nutritional quality of proteins. For instance, fortification of soy protein with leucine, isoleucine, and valine can has been reported to increase whole-body protein synthesis [135]
- Genetic engineering to improve the availability of essential amino acids in plant proteins.[136]

8. Mitigating protein off-flavors

Although plant protein concentrates and hydrolysates are gaining increasing attention for food applications, their use still results in low consumer acceptance primarily as a result of their “green”, “grassy”, or “beany” off-odor [137] as well as long-lasting bitter and/or astringent off-taste,[138] which limits palatability in human consumption. To comprehensively exploit the potential of plant proteins, these differences have to be minimized by taking advantage of increasing knowledge on the key drivers of undesired aroma and taste impressions. These are:

1. The study of the impact of food texture on aroma and taste perception as well as protein-odorant/taste interaction is necessary to understand the combinatorial flavor code and implement new strategies to bridge the flavor gap and enhance the flavor of plant-based proteins. [139]

2. Advanced downstream processing, including protein extraction, purification, functionalization, and final processing techniques, such as extrusion and three-dimensional [3D] printing,[140] will help to optimize the functional, sensory, and nutritional properties of plant protein-based food.

3. Targeted and controlled protein hydrolysis and fermentation techniques, including the use of specific food-grade enzymes [e.g., flavourzyme], bacteria (e.g., lactic acid bacteria), [141] molds (e.g., Koji type), [142] fungi, or germination, [143] can be used to tailor the flavor of plant-based proteins.

4. Moreover, in-process flavor generation during the production of, e.g., meat analogs by adding reducing sugars and/or increasing the amino acid levels (e.g., by partly hydrolyzing the protein) can help guide the formation of Maillard-derived flavor compounds known to evoke pleasant taste attributes.[144]. Choosing

suitable ingredients and possible flavor precursors, followed by thermal processing, promotes the formation of flavor-active compounds and allows for tailoring of the sensory impression.

5. Finally, molecular breeding can serve as a core strategy to meet the flavor challenge in the future. New breeding approaches of genotypes resulting in lower concentrations of off-flavor stimuli could help to minimize the number of adverse compounds, with a special focus on sensory characteristics, allergenicity, and optimal techno-functionality depending on the planned use.

As highlighted in this perspective, the flavor challenge of plant proteins calls for further interdisciplinary research, combining and expanding our knowledge in food/biotechnology, food quality, analytical, food, and especially flavor chemistry as well as plant science, to provide healthy, protein-rich, sustainable, and pleasant food. First and foremost, it is essential to obtain deeper insights into the off-flavor sensometabolomes of protein products and the chemical mechanisms involved in their reduction or masking strategies.

9. Creating future foods with plant proteins

Plant-based protein ingredients can replace animal-based ones [such as meat, fish, eggs, and milk] in a variety of food products such as cheese, dressings, sauces, spreads, and yogurts. Some of the most common plant-based food alternatives that are created to replace animal-based ones include meat, egg, fish, milk, cheese, yogurt, creams, etc. In the remainder of this review, the science behind the formulation of plant-based alternatives to meat, fish, eggs, and dairy alternatives is discussed in detail. For each food category, the focus is on how plant-based ingredients can be used to assemble products with the required physicochemical, functional, and sensory characteristics.

Plant-based meat

Plant-based meat analogs are created by assembling plant-based proteins, polysaccharides, lipids, and functional ingredients, such as colorants, flavorings, minerals, vitamins, and preservatives that impart taste, texture, color, and aroma to meat [145]. The physicochemical and sensory characteristics of the final product are determined by the concentration of the ingredients and processing methods required to assemble them. Hence optimization of the ingredients is essential to simulate the exact characteristics of meat in a plant-based meat analog.

Texture- The textural properties of comminuted meat products, such as sausages, burgers, or nuggets, have been simulated fairly accurately using texturized vegetable proteins (TVPs) [146]. Researchers are investigating a variety of physicochemical or processing operations, which can be used separately or in combination [147] to assemble plant proteins in a product that can mimic the texture of meat. For instance, using appropriate compositions of plant-based biopolymers in different concentrations and solution conditions (pH, ionic strength, and temperature) a biopolymer mixture is fabricated that spontaneously separates into two phases. This phase-separated mixture can then be extruded to create fiber-like structures that are then gelled by changing environmental conditions [such as heating or cooling] or by adding crosslinking agents like enzymes. A similar high moisture extrusion has already been used to create meat-like fibers from a variety of different plant ingredients including peanut, hemp, soy, microalgae, pea, wheat gluten, faba bean, and lupin proteins. [148]. High-pressure shearing methods have also been used to produce chicken meat-like textures from blends of soy or pea proteins with gluten [149]. In another experiment, the adipose tissue in pork fat has been simulated by developing emulsion gels from olive oil and chia mucilage [150].

Water Holding Capacity - The WHC of meat analogs may be modified by altering the type of the biopolymers used, such as their molecular weights, polarities, or cross-linking densities. For instance, it was reported that the swelling and WHC of meat analogs formed from plant proteins decreased as their degree of crosslinking was increased by adding chemical agents, such as glutaraldehyde [151]. Alternatively, the addition of plant-based dietary fibers, such as carrageenan, pectin, or alginate may improve the fluid-holding properties of meat analogs, as in real meat products [152].

Aroma- The aroma of cooked meat depends on “meaty” flavor volatile notes such as 2-methyl 3-furantiol or bis-(2-methyl-3-furan) disulfide, as well as other flavor compounds that have “green,” “mushroomy,” “sweet,” and "earthy" odors. Meaty flavor notes can be created in meat analogs using a variety of approaches. For instance, leghemoglobin isolated from soybean roots or microbial fermentation creates the desirable "meaty" flavor of some commercial plant-based meat products, produced by Impossible Foods. Mycoprotein is also being explored as it has been reported to give a meaty aroma, a savory umami taste, and a meat-like texture [153]. Furthermore, meaty flavors that can be used in plant-based meat analogs can also be produced from vegetable oils or by carrying out controlled Maillard and oxidation reactions on plant-based ingredients [154].

Color- In commercial plant-based meat analogs, several techniques have been used to obtain a color that imitates real meat. For instance, Beyond Meat's products use a water-soluble pigment betalain obtained from beet juice extract in plant-based meat analogs to mimic the color of meat. Studies show that the chemical degradation of the betalain due to heating causes beet juice to change color from red-violet to orange-yellow [155,156]. A variety of other natural pigments, such as leghemoglobin, with different color profiles, can be used individually or in combination to obtain the desired product appearance in plant-based meat analogs [157].

Ingredient	**Sources**	**Function**
Non-animal proteins 20-50%	Plant-based: soy,pea,hemp,rice,lupin,legumes,and potato. Novel sources: Microalgae and seaweed.	Nutritional value, structure, color, and texture.
Lipids 0-5%	Saturated and unsaturated fatty acids: Coconut oil, cocoa butter, sunflower oil, canola oil, sesame oil, and avocado oil. Fat replacers: Oleo gels and fibers.	Flavor, texture, and mouthfeel
Polysaccharides 20-30%	Native starches, flours, and fibers.	Consistency and water binding.
Flavoring Ingredients	Savory yeast extracts, paprika, sugar, spices, and herbs	Flavor
Coloring agents	Lycopene, beet juice extract, or leghemoglobin	Meat color
Fortifying ingredients	Tocopherols, zinc gluconate, thiamine hydrochloride, sodium ascorbate. etc	Nutritional value

Table 2. Main ingredients for Plant-based Meat Analogs

Plant-based egg

Compared to meat, creating plant-based egg analogs is quite difficult for food scientists. This is because the thermal transition temperature (63-90 C) at which plant proteins unfold and aggregate must be similar to real egg proteins which are challenging as normally plant proteins have higher denaturation temperatures. As a result egg protein alternatives have to be heated at a much higher temperature and for a longer time to form a gel that resembles the one formed by real eggs. It is therefore important to identify a combination of plant proteins that have a similar texture-temperature profile as well as produces similar texture and appearance in the cooking. Plant-based egg analogs are assembled from a group of globular plant proteins than can undergo a sol-gel transition when they are heated. Plant-based eggs come in two different forms- liquid and dried powder. The liquid counterpart is created by protein isolation and is useful for making scrambled eggs, and omelets. etc. whereas the dried powder is prepared by protein fermentation or isolation. One methodology for producing plant-based eggs from mung bean protein has been patented by the company Eat Just. In this method, mung bean is subjected to alkaline extraction followed by precipitation of globulin-rich fractions at isoelectric points (pH5-6). The extracted protein is fractionated and purified by ultrafiltration or ion-exchange chromatography and powdered by spray drying technique. The globular proteins in egg analogs may contribute to the emulsifying and foaming properties in products where these attributes are needed, such as dressings, sauces, or meringues. An emulsified plant-based oil may be included to simulate some of the functional properties provided by lipoproteins in real eggs. Additionally, thickening or gelling agents may be added to manipulate the texture and prevent the sedimentation or aggregation of particulate matter. The yellowish color of egg analogs is contributed by natural pigments, such as curcumin from turmeric and carotenoids from carrots. The opacity and textural characteristics are also controlled by the addition of emulsified canola oil. Various other ingredients may also be added to these products as texture modifiers/stabilizers (such as corn starch and gellan gum), flavorings (such as garlic, onion, sugar, and salt), pH modulators (such as bicarbonates, citrates, or phosphates), preservatives (such as nisin), and crosslinking agents (such as transglutaminase). The development of plant-based egg analogs is still at a nascent stage due to the challenges in production, cost, and lower protein content. Another factor is the presence of added preservatives, emulsifiers, and binders the prolonged consumption of which may be unhealthy. More work is required to create plant-based egg analogs with a minimum amount of additives without compromising on the texture and taste.

Plant-based milk

Milk analogs are colloidal dispersions consisting of a mixture of oil bodies, fat droplets, protein aggregates, and/or plant tissue fragments suspended in an aqueous solution containing dissolved sugars and salts. These colloidal dispersions can be developed by two major techniques. [158]. Firstly, they may be produced by disruption of whole plant materials, such as soybeans, coconut flesh, almonds, hazelnuts, oats, or rice by mechanical processing or enzymatic methods. Second, they may be created by pressure-assisted homogenization of plant-based oils (such as soy, sunflower, flaxseed, canola, corn, or olive oil) with water in the presence of a plant-based emulsifier (such as proteins, polysaccharides, phospholipids, or surfactants) [159]. While making milk analogs it is important to ensure that the colloidal particles dispersed in solution are of smaller size, or they will aggregate/sediment, cause an undesirable gritty mouthfeel, or lead to a heterogeneous appearance. This can be done by selecting appropriate size-reduction conditions during processing such as HPP, as well as by adding effective emulsifiers and other stabilizers, such as thickening agents such as plant-derived proteins (e.g., soy, pea, fava bean, or lentil proteins), phospholipids (e.g., soy or sunflower lecithin), or surfactants (e.g., quillaja or tea saponins) [160]. Plant-derived polysaccharides can also be used as thickening agents or stabilizers, such as pectin, locust bean gum, gellan gum, starch, methylcellulose, carrageenan, and alginate [161]. The taste, aroma, and mouthfeel of milk analogs are determined by the concentration of their constituents. For instance, soy milk has a beany flavor whereas hazelnut milk has a nutty flavor [162]. This is a limitation as most plant proteins have off-flavors, such as astringent, earthy, or vegetative notes [154] contributing to undesirable taste and odor in the milk analogs prepared from them. Hence, a great deal of research is being done to reduce the presence of these undesirable flavor notes using plant-breeding or processing methods. Researchers have also reported the stark differences in the storage stability of commercial milk analogs due to gravitational separation [163], which can mainly be attributed to differences in their particle size and rheology. As an example, coconut milk is unstable to heating, high salt levels, and pH values near the isoelectric point, which was attributed to increased particle aggregation [164]. Other milk analogs have also been reported to exhibit a similar stability profile, including those stabilized by soy proteins [165-166], faba bean, pea, and lentil proteins [167]. Furthermore, in milk analogs that contain polyunsaturated lipids [such as flaxseed oil], it is important to arrest their oxidation during storage and processing, to avoid rancidity [168]. Thus it is important to identify the physical and chemical instability mechanisms of each plant-based milk product and carefully optimize their composition for extending their shelf life.

10. Conclusion

A rapidly increasing number of consumers are transforming their eating habits to adopt a healthier, sustainable, and ethical plant-based diet, which has led to rapid growth in plant-based proteins and vegan foods. This review has presented a roadmap to accelerate alternative protein science and technology, focusing on plant protein ingredient development and the creation of delicious and nutritious plant-based future foods. However, there are still some hurdles that need to be overcome before a greater proportion of the population includes plant-based proteins in their diet. The areas for further research include improvement in plant protein extraction and fractionation techniques, and functionality modification. The potential impact that different forms of fractionation and improved functionality may have on the nutritional quality of the modified protein also requires attention. Furthermore, more research is needed in understanding plant protein-polysaccharide interactions and developing different structuring techniques. From a commercial perspective, effective strategies must be developed through education and training to encourage consumers to try, accept, and adopt these products. There is also a dearth of knowledge available to both small and large companies about the commercial roadmap of plant-based proteins, including the affordability and relative costs of different ingredients and extraction processes, regulatory frameworks, supply chain problems, and safety issues. The availability of this information would encourage the entry of companies into the plant-based protein sector. For instance, India can be an export hub of plant protein isolates for plant-based meat if policy, infrastructure, and industry favor it. For instance, while animal protein is tax-free, plant-based meat is taxed at 18% GST which requires major reforms. Nationalistic campaigns similar to NECC are required to promote plant-based foods in India. Though India is the largest consumer, producer and importer of lentils, it is still a protein-starved nation as lentils retain only $1/3^{rd}$ of nutrition after boiling. Also, soya and pea are currently the epicenters of the plant-based meat industry globally hence novel indigenous alternative proteins like faba bean, and mycoproteins need to be the focus of the alternative protein sector. Furthermore, fermentation-based products should be an area of focus and innovation in the plant-based protein segment as fermentation is the most sustainable and scalable technology available. To summarize, progress in the plant-based protein sector requires a multidisciplinary approach to overcome the technological and commercial hurdles, which will involve the integrated efforts of agricultural scientists, food scientists, nutritionists, engineers, social scientists, psychologists, economists, and environmental scientists.

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