

An overview of protein powders and their use in food formulations

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Abstract

Protein quality, as well as quantity, must be taken into account when designing healthy and sustainable diets and food systems. This is particularly crucial for protein powders since there is a wide range of sources and production methods affecting powder and particle properties, and protein functionality and quality. Proteins can be derived from numerous raw materials and converted into powder or particulate form for several reasons, such as ease of transportation and storage stability. Therefore, it is key to understanding the sources, processing methods, powder, and particle properties of protein powders to provide high-quality products. This study reviews the origins of proteins, extraction techniques, and drying methods employed to produce protein powders. In addition, functional and powder properties, their use in foods, and regulatory issues are highlighted.

Practical applications

Proteins are found in different foods and in food processing side streams such as de-oiled oilseed meals. Most seeds are typically discarded as food waste worldwide, even though they have high protein content. This review provides an insight into protein sources, extraction methods, powder production techniques, and use in food formulations. It also assessed the effect of powder properties on the functional properties of proteins. To our knowledge, no reviews have been conducted on protein powders. By providing information and insights, this article should contribute to better understanding the protein powders and their use in the production of functional foods.

KEYWORDS

plant proteins, powder production, protein powders, protein-rich foods

1 | INTRODUCTION

Proteins are valuable components during food processing providing structural, nutritional, and functional properties, including water binding, viscosity, surface activity, foaming, coagulation, gelation, emulsification, and heat stability (Hettiarachchy & Ziegler, 1994). The traditional sources of protein in the human diet are both plant-based

and animal-based. Proteins are found in various kinds and forms and have a diverse range of chemical, biological, functional, and dietary properties (Amagliani, Regan, et al., 2016). Some properties of proteins, protein-based hydrolysates, and specific peptide fractions are recommended for their hypoallergenic, anti-obesity, anti-oxidative, anti-cancer, and anti-hypertensive functionalities (Balakrishnan & Schneider, 2022; Karaman et al., 2022).

Proteins, in general, are produced in powder form to ease transportation and to increase the shelf-life by reducing their water activity. This way, the physicochemical, organoleptic, and nutritional properties of protein powders (PPs) can be preserved during storage (Amagliani, O'Regan, et al., 2016). Animal-based or plant-based protein extracts (i.e., milk proteins, meat proteins, seed proteins, protein hydrolysates) are converted into powder form using different drying techniques (Sánchez-Oliver et al., 2018; Saxton & McDougal, 2021). PPs are widely used in the production and stabilization of foods and beverages (i.e., cream liqueurs, infant milk formula, and protein supplementation). In addition, PPs are also used by athletes and individuals for sporting and non-sporting purposes (Knapik et al., 2016; Sánchez-Oliver et al., 2018).

Recently, there has been an increasing interest in proteins of plant origin rather than animal origin (Agarwal et al., 2015; Lagrange et al., 2015). The main reasons for this trend are environmental sustainability, animal welfare, ethical reasons, animal protein allergies, and increased risk of cardiovascular diseases (Hochwallner et al., 2014; Lappi et al., 2022; Satija & Hu, 2018). Almost one-third of the land resources are allocated for livestock production, resulting in severe environmental drawbacks (e.g., water pollution, land degradation, water pollution, desertification, and overgrazing) (Weindl et al., 2020). Therefore, increased demand for fresh water in agriculture, irrigation, and domestic use is widely affected by increasing loss of natural ecosystems, greenhouse gas emissions, and declining biodiversity (Henchion et al., 2021; Kaur et al., 2022). In parallel, the demand for protein also grows while the agricultural land and water significantly reduce due to climate change and the over-use of agricultural sources. Although meat and seafood production is expected to increase and is rich in high-quality proteins and other nutrients, novel protein sources and their valorization need to be investigated due to environmental and climatic issues (Schweiggert-Weisz et al., 2020).

Therefore, plant protein extraction, enrichment, purification, functionalization, and improving powder properties have gained significant attention in recent years (Brown et al., 2021). Powder properties, such

as physical and physicochemical attributes of particles and the bulk behavior of PPs, play important roles during storage, handling, and processing. The use of protein powders in different food formulations has recently gained attention, and no review paper was found in the literature on protein powders covering the essential aspects. Therefore, this study provides an overview of protein powders and their use in food formulations, specifically looking at their sources, extraction and drying methods, afunctional and powder properties.

2 | SOURCES OF PROTEINS

There is a need for a dietary transition toward novel sources of proteins (Munialo et al., 2022). Protein-rich food sidestreams and wastes (around 32%–57%, wet basis) could be utilized to meet the increasing protein demand (Sweers et al., 2022). The protein source mainly influences the nutritional composition of food, and the proteins could be used to replace carbohydrates and fats in foods that end up a waste of the sources (Richter et al., 2015; Thirunavookarasu et al., 2022; Yashini et al., 2019). The primary protein sources are outlined in Figure 1. The protein content of foods depends on their origin, that is, either plant or animal. The quality of plant-origin protein is susceptible to genetic and environmental stressors rather than animal-origin protein. In addition, the essential amino acids of plant-sourced proteins (20% to 22%) are generally lower than those of animal proteins (ranging from 38% to 43%) (Munialo et al., 2022).

A broad range of plants and their diverse parts, including leaves, seeds, and roots, contain varying amounts of protein. Some plants, such as soy and wheat, have a lengthy historical background in human nutrition, whereas there are some currently emerging ones. Recent studies have focused on novel protein sources of plant origin (e.g., pseudocereal, green biomass), recycling of food sidestreams (e.g., a defatted meal of oil processing), and non-plant sources (e.g., mycoproteins, cell culture) (Pérez-Vila et al., 2022; Schweiggert-Weisz et al., 2020).

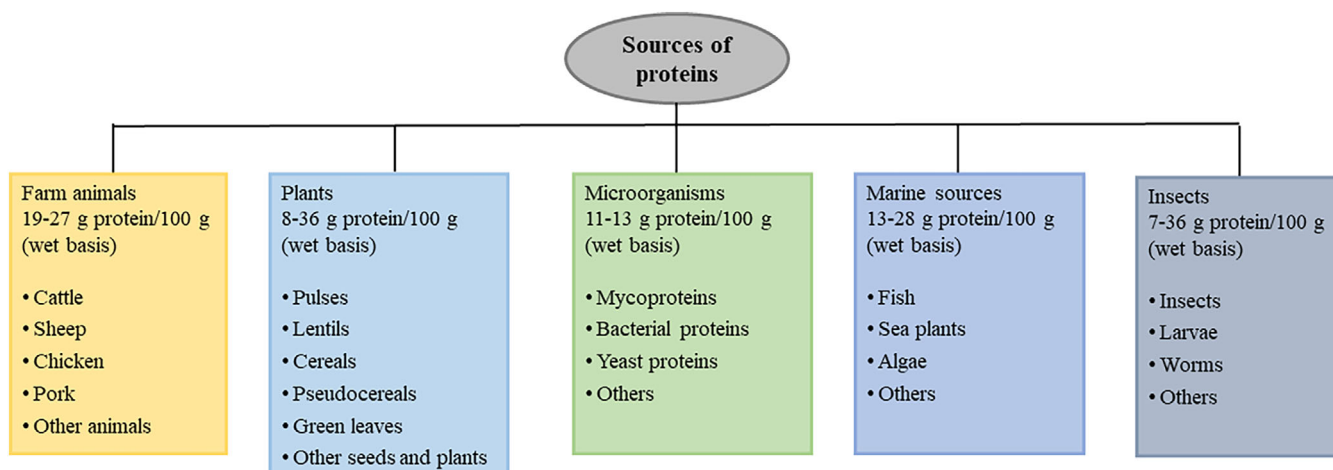


FIGURE 1 Primary sources of proteins. Source: Adapted from Munialo et al. (2022).

Animal-based proteins from meat and dairy products, nutritionally well-balanced protein sources, are still the most preferred. However, their growing demand will not be sustainable due to their high carbon footprint and low conversion efficiency. Therefore, sustainability is obligatory to consider novel protein alternatives other than animal-based sources (Geada et al., 2021).

There is an increasing interest in other potential dietary proteins from non-plant sources such as insects, fungi, mycoproteins, and other microorganisms (both unicellular, like yeast, bacteria, and multicellular, like filamentous fungi and algae) (Matassa et al., 2016). An example of a protein source can be mycoprotein from the filamentous fungus *Fusarium* (Szepe et al., 2021). Marine resources such as algae and seaweed have recently gained significant attention. Algae biomass can be considered an essential and sustainable protein source containing around 70% protein (Agarwal et al., 2022; Nadeeshani et al., 2022; Samarathunga et al., 2022).

2.1 | Extraction

The rising awareness about high-quality proteins from different sources in the human diet has motivated researchers to investigate eco-friendly and sustainable novel protein sources. This approach needs cost-effective and practical technologies of extraction (Kumar et al., 2021; Reshan Jayawardena et al., 2022).

The recent studies have focused on protein extraction from diverse biomasses using traditional (e.g., alkali and solvent-based methods), advanced green biochemical extraction with enzymes, and physical extraction approaches (e.g., electric pulse field-, ultrasound-, high pressure-assisted extraction, and microwave) (Amagliani et al., 2017; Munialo et al., 2022). There is a demand for innovative technologies to overcome the disadvantages of traditional extraction techniques, such as lower yield and a long extraction time (Ampofo & Ngadi, 2022). For instance, recent research works have revealed that novel cell disruptive techniques are significantly efficient with a higher recovery of protein and are environmentally friendly (Kumar et al., 2021). Carbohydrate-digesting enzymes (alpha-amylase, glucoamylase, pullulanase, arabinose, cellulase, beta-glucanase, hemicellulase, pectinase, phytase, xylanase) are often used to separate proteins from the milled plant seeds (Amagliani et al., 2017).

The alkaline extraction of isoelectric precipitation (IEP) at pH 4.0–4.5 effectively extracted proteins. Despite the advantages of alkaline extraction (i.e., higher digestibility and bioavailability), alkaline conditions may also cause the extraction of non-protein components, denaturation, hydrolysis, lower protein purity, increased Maillard reactions (due to high pH), and formation of toxic compounds (lysinoalanine) (Amagliani et al., 2017).

The subcritical water treatment method (pressurized water at temperatures from 100 to 374°C), which facilitates dissolving hydrophobic fractions, was used by Náthia-Neves and Alonso (2022) to extract proteins. According to their findings, the highest recovery rate was 133.5-mg protein/g raw material at 150°C with a solvent-to-feed ratio of 20 after 15 min of extraction.

The physical methods have superiority over chemical or enzymatic methods since they induce fewer modifications to protein structure, are more economical, easily adaptable, and are suitable for up-scaling production in the industry (Shih, 2003). Additionally, sonication, high-speed blending, freeze-thaw, hydrothermal cooking, microfluidization, and subcritical water can be examples of physical approaches employed to extract proteins. The extraction efficiency is increased when amylolytic and proteolytic enzymes are used together with these physical treatments (Amagliani et al., 2017). Patil and Rathod (2022) could recover up to 86% of protein from industrial turmeric waste powder using ultrasound intensified three-phase partitioning extraction technique. Although ultrasound is an eco-innovative technique, there exists less information about its performance in extracting of plant-based proteins (Ampofo & Ngadi, 2022). A flow diagram showing the extraction and drying processes is given in Figure 2.

2.2 | Powder production

The next step after protein extraction is drying to produce PPs. The functional properties, extraction process, and drying techniques play vital roles in successfully utilizing plant PPs in food formulations. Diverse drying and extraction methods would result in different protein yields having different functional properties. While the drying process provides PPs with improved storage stability, it causes partial protein denaturation, resulting in the formation of irreversible, insoluble aggregates, and shifting their functional properties (Vojdani, 1996). The drying process affects the powder and particle properties of protein extract depending on the degree of hydrolysis, distribution of hydrolysate's molecular weight (MW), and amino acid composition (Yu et al., 2011). The type of proteolytic enzyme used (i.e., papain, trypsin, and bromelain), physicochemical conditions, and the degree of hydrolysis impact the functional properties (solubility, foaming, and emulsifying capacity) of PPs.

On the other hand, a high degree of hydrolysis may cause the development of bitterness due to the accumulation of low MW hydrophobic peptides (Hadidi et al., 2022). The physical properties of the powder, such as particle size distribution (PSD), morphology, and moisture content (MC), should be analyzed after drying. In addition, functional characteristics (i.e., reconstitution, solubility, bioavailability, and shelf stability) and consumer acceptance must also be analyzed. For instance, increasing the particles' porosity improves water solubility (Amiza et al., 2013; Sarabandi et al., 2018). In addition, the flowability, stickiness, thermal properties (i.e., glass transition temperature), and storage stability of the PPs are important technological properties and need to be analyzed (Paul et al., 2022; Wang & Selomulya, 2020).

The most common techniques for PP production are freeze-drying, spray drying, spray-freeze drying, and oven drying. Among these techniques, spray drying is regarded as an economical technique that provides quality with rapid dehydration and low operational costs at a large scale (Özdemir et al., 2022; Vardanega et al., 2019). Freeze-

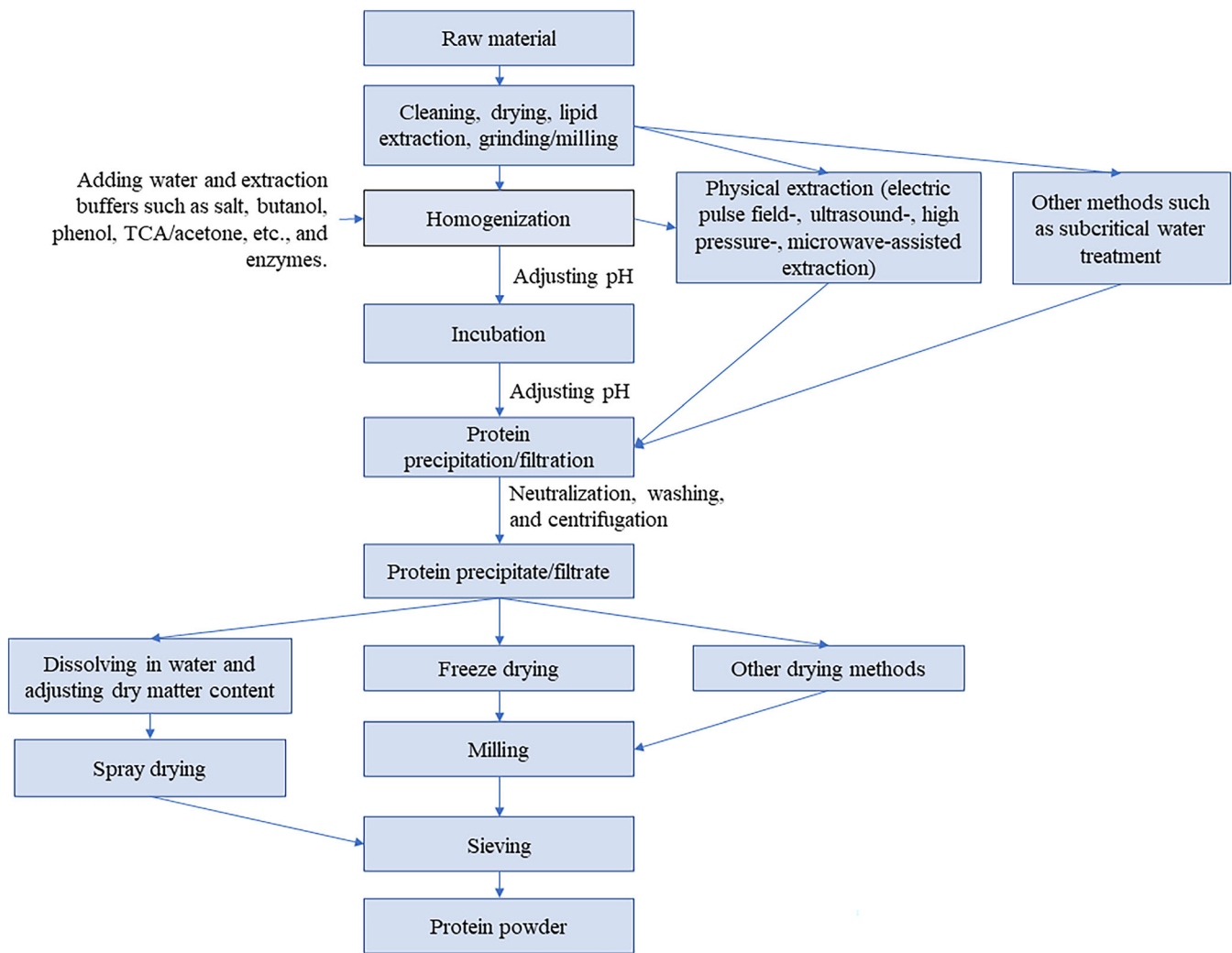


FIGURE 2 Flow diagram of extraction and drying of proteins.

drying is mainly used to characterize protein functionality. Even though oven drying and refracting window drying are less expensive than other methods, the residence time is much longer (Brishti et al., 2020; Feyzi et al., 2018).

2.3 | Spray drying

Spray drying (SD) is a widely used technique to convert the extracted proteins, their hydrolyzates, and peptide fractions into powder form for further applications (Maa et al., 1999). SD parameters should be optimized to maximize the nutritional endpoints (bioavailability and bioactivity) and functionality of PPs. Tsumura et al. (2005) reported the solubility of soy protein hydrolysate powder from a spray dryer more than that of soy protein isolate. The optimized SD conditions are 160°C as inlet temperature, 6 mL/min as feed rate, and 35% solid concentration to obtain better powder characteristics such as particle morphology, color, antioxidant capacity, and powder density (Wang et al., 2019). Using bulk agents such as maltodextrin or starch prevents the protein fractions from heat damage to some extent, even at

high inlet temperatures. For example, Akbarbaglu et al. (2019) used maltodextrin to obtain spray-dried flaxseed protein hydrolysate powder, and they reported good solubility (92%–96%) with high antioxidant activity of the powder. Lower inlet temperature could also help to reduce the denaturation of the proteins. Similarly, Ye et al. (2019) reported a decrease in inlet temperature by 20°C by adding ethanol to the protein extract while maintaining the same drying efficiency and powder quality.

The granulation process after SD may be needed to improve solubility, increase particle size, and reduce dust formation (Wang & Selomulya, 2020). In SD, while the atomized droplets' shape is spherical, the dried particles' shapes might change depending on the formulation and drying conditions.

2.4 | Freeze-drying

Freeze-drying (FD), also so-called lyophilization, is an approach to solidifying protein mixtures for storing them in dry (solid) form with minimum structural damage (Chen et al., 2017). FD contains freezing,

TABLE 1 Selected studies focused on protein powder production.

| Protein source | Extraction method | Drying method | Key findings | References |
|------------------------------|--|-------------------------------|--|---------------------------------|
| Beef lung | - | Air drier at 50, 70 and 100°C | <ul style="list-style-type: none"> Superior quality product 50°C. Highest haem-iron content in the samples dried at 50°C. Increased glycine content with increased drying temperature. Decreased protein degradation in BLP samples dried at 50°C. | Reshan Jayawardena et al., 2022 |
| Sesame bran | Ultrasound-assisted enzymatic extraction | SD and FD | <ul style="list-style-type: none"> Improved wettability and dispersibility of FD PP. Higher foaming capacity of FD PP. Lowered bulk and tapped densities of FD PP. SD PP provided better color properties and higher water-holding capacity. | Özdemir et al., 2022 |
| Cottonseed | Alkaline extraction and precipitation | FD | <ul style="list-style-type: none"> Excellent functional properties of food (oil/water absorption capacity, emulsifying-foaming, and solubility) | Kumar et al., 2022 |
| Mussel meat | Enzymatic hydrolysis | SD | <ul style="list-style-type: none"> Increased glass transition temperature with carrier agents (maltodextrin 10 DE and gum arabic) addition and reduced powder hygroscopicity. Decreased powder moisture content with increased carrier agent concentration. Particles with a greater number of dents and larger size. | Silva et al., 2012 |
| Blue shark skin | Enzymatic hydrolysis | SD | <ul style="list-style-type: none"> Lowered moisture content and bulk density by higher inlet air temperatures. Reduced moisture content, higher bulk density, reduced hygroscopicity, and larger particle size by higher maltodextrin concentration. Higher antioxidant capacity as compared to hydrolysate (liquid). | Rodríguez-Díaz et al., 2014 |
| Fresh duck egg white | Vacuum ultrafiltration | FD and MW-vacuum drying | <ul style="list-style-type: none"> Decreased drying time by FD + MVD as compared to FD-only. Improved color (higher L* and lower b*) in PPs dried by FD + MVD, as well as lower apparent density and foaming stability, whereas higher emulsifying index than those dried by FD only. | Zhou et al., 2014 |
| Chicken meat | Enzymatic hydrolysis | SD | <ul style="list-style-type: none"> Inlet air temperature influenced the antioxidant activity of protein hydrolysate powder. | Kurozawa et al., 2011 |
| Milk | Ultrafiltration and diafiltration | SD | <ul style="list-style-type: none"> Increased calcium and protein contents lead to inadequate functional properties. Improved wettability, dispersibility, solubility, flowability, heat stability, buffer index, emulsification and foaming, and water and oil binding capacities in MPC powders by adding Na₂HPO₄, homogenization; DF of 5 × UF retentate with 150 mM NaCl solution. Developed milk protein concentrate powders with smooth surfaces, intact and separated smaller particles compared to the control sample. | Meena et al., 2019 |
| Coconut wet processing waste | Enzymatic hydrolysis | SD | <ul style="list-style-type: none"> Improved emulsifying properties of coconut PP. Coconut PP with better emulsifying properties than skimmed milk powder and defatted soybean powder. | Naik et al., 2012 |

(Continues)

TABLE 1 (Continued)

| Protein source | Extraction method | Drying method | Key findings | References |
|------------------------------|---|-----------------------|---|----------------------------|
| Cricket and mealworm | - | Roasting and grinding | <ul style="list-style-type: none"> • Low solubility at pHs 3.0, 5.0, and 7.0 • Cricket powder with a foaming capacity of 82% and foam stability of 86%, whereas mealworm powder was non-foaming. • Insect proteins have similar protein quality as pulse proteins and higher solubility at pH 5.0, while less soluble at pH 7.0. | Stone, Tanaka et al., 2019 |
| De-oiled sunflower seed cake | Isoelectric precipitation | SD | <ul style="list-style-type: none"> • Micro-spherical particles with irregular surface properties. • PPs showed poor fluidity. • Poor solubility, sufficient emulsifying and oil-binding capacity. • Moderate wettability. • Denaturation temperature of PP at around 80°C. | Ermis & Karasu, 2020 |
| Insoluble pea proteins | High pressure homogenization and centrifugation | FD | <ul style="list-style-type: none"> • Disruption of large protein aggregates into smaller particles increased their solubility and surface activity at pH 3. | Moll et al., 2022 |

primary, and secondary drying stages (Wahl et al., 2016). Typical protein quality depends on the amount of intact protein, degree of protein aggregation, and protein functionality. Wahl et al. (2016) present various physicochemical properties of lyophilized proteins in the solid state. Inefficient FD operation may reduce dispersion, wetting, capillarity, undesired particulate formation, agglomeration, and prolonged reconstitution time. Lyophilized proteins are amorphous structures based on their intrinsic glass-forming properties. Faster reconstitution of lyophilized PP is also enhanced by amorphous and disordered states (Grossmann et al., 2018). Brishti et al. (2020) investigated the effects of spray-, freeze-, and oven-drying techniques on PPs isolated from mung bean on a pilot scale. Based on their findings, FD provided porous particles with better solubility, while SD and oven drying yielded wrinkled-shaped particles. Grossmann et al. (2018) used FD to obtain PP extracted from microalgae *Chlorella protothecoides*.

2.5 | Other drying methods

Among the other alternative drying techniques, Spray-freeze, oven, MW (Melgar-Lalanne et al., 2019), supercritical anti-solvent, and fluidized bed (Kröncke et al., 2018) can be given as examples. Different drying methods and their effects on the functional and physicochemical properties of PPs have been evaluated by previous research works (Abdul-Fattah et al., 2007; Emami et al., 2018; Huang et al., 2019; Reshan Jayawardena et al., 2022; Shen et al., 2021). Based on the data reported, supercritical antisolvent and spray freeze-drying methods could produce particles with light and porous structures. Maa et al. (1999) reported a higher fine particle fraction, larger median particle size, and larger specific surface area (around 40 times) for spray-freeze-dried particles as compared to SD ones. In addition, in spray-freeze drying, the spherical shape of the droplets could be maintained due to rapid freezing after atomization. The drying methods

and their effect on powder properties are summarized in Table 1. Grinding or milling can be applied after drying the proteins to decrease the particle size using milling techniques, including ball, roller, disc, and jet mills. Superfine grinding is reported as one of the novel techniques to improve the surface properties (i.e., absorption, solubility, and dispersibility) of protein particles (Yousefi & Abbasi, 2022).

3 | EVALUATION OF THE PROPERTIES OF PROTEIN POWDERS

The use of PPs in food formulations depends on their functional properties (i.e., viscosity, gelation, foaming, oil-binding, water-binding, flavor-binding, and emulsifying), which are directly linked to the molecular structure, composition, and performance (i.e., protein-protein and interactions with water and other ingredients) in foods through each step of the food supply chain such as processing, storage, and consumption (Amagliani et al., 2017; Brishti et al., 2020). Composition, processing methods, additives, and pH value of the vicinity are the major factors affecting protein functionality (Yashini et al., 2019). Wahl et al. (2016) provided a brief review of the various conventional and novel analytical techniques which can be used to characterize the properties of the PPs. Factors affecting the quality properties of PPs are summarized in Figure 3.

One of those quality attributes of PPs is solubility which is affected by the factors such as particle size, particle surface properties, pH, temperature, the dielectric constant of the solvent, and salt concentration, respectively (O'Flynn et al., 2021; Shevkani et al., 2019; Wang & Selomulya, 2020). Solubility is an important attribute that affects the food processing quality of the proteins (i.e., rheological, hydrodynamic, and surface activity characteristics) in preparing food formulations (Cha et al., 2020). A high degree of

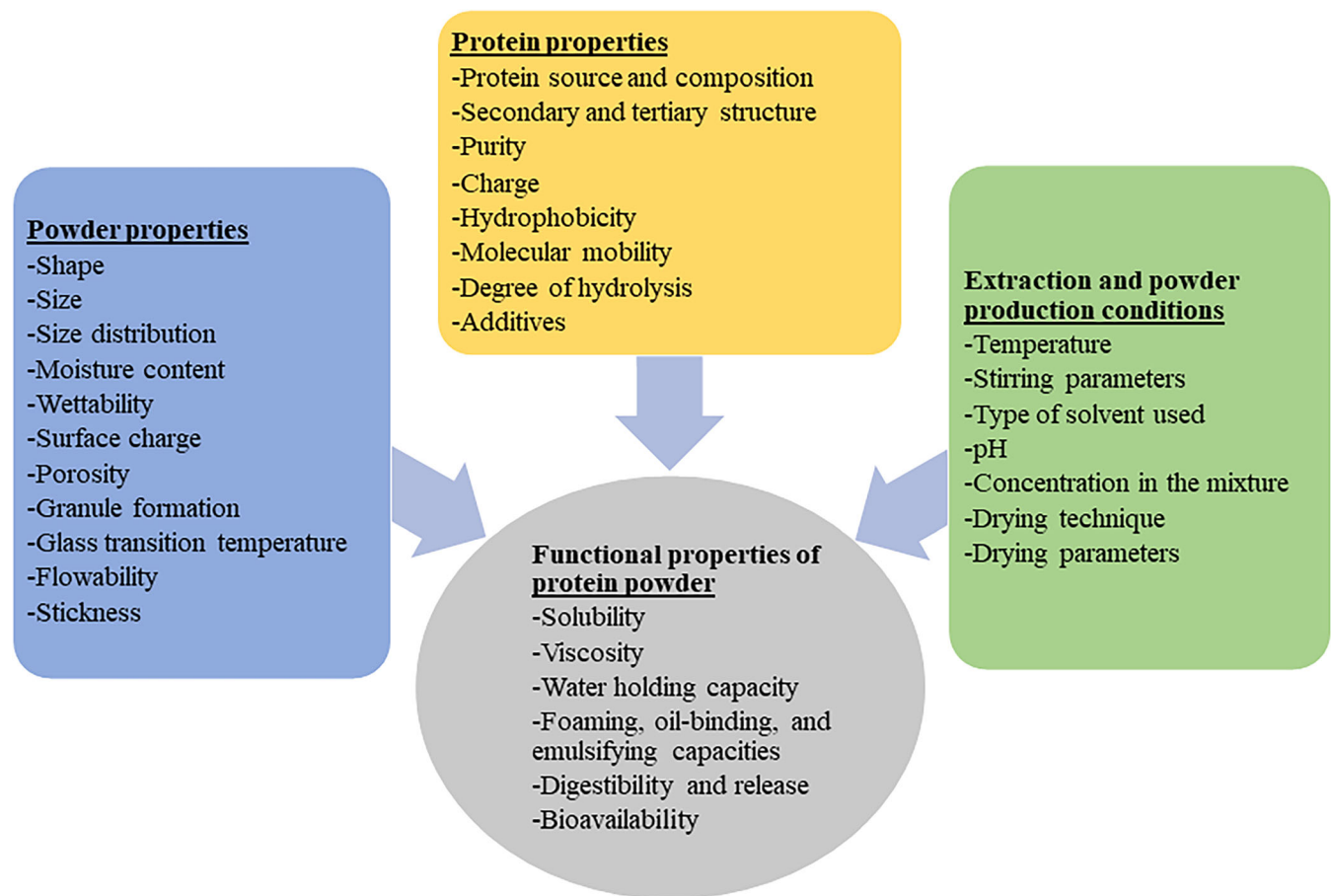


FIGURE 3 Factors affecting the properties of protein powders. Source: Adapted from Wahl et al. (2016).

solubility is required in liquid or semi-liquid foods, including sauces, soups, coffee creamers, salad dressings, fortified beverages, whipped toppings, and enteral nutrition products for clinical purposes. In contrast, the solubility of the protein ingredients is not a big concern for the products such as meat analogs, protein bars, bakery products, breakfast cereals, and pet foods (Amagliani et al., 2017).

Foaming capacity can be defined as the ability to form a cohesive viscoelastic film at the air-water interface via intermolecular interactions. Foaming capacity denotes the amount of interfacial area that proteins stabilize per unit weight or initial amount of foam created after stirring (Amagliani et al., 2017). Foaming stability represents the foam that remains after a specific time (Burger & Zhang, 2019; Hadidi et al., 2022). Other essential quality properties are given as emulsifying activity (max interfacial area per unit weight of the protein of a stabilized solution), emulsifying capacity (max amount of oil to be emulsified under specified conditions by a standard amount of protein), and emulsion stability (the ability of a protein to form an emulsion resist full to changes in its properties at a certain period, specific temperature and gravitational force) (Amagliani et al., 2017). PPs as surfactants could contribute to the emulsion activity and emulsion stability representing the resistance to structural changes over time (Hadidi et al., 2022). High oil absorption and water absorption capacity of PPs are needed to provide the food product with an optimal

texture, improved mouthfeel, and flavor retention (Chandi & Sogi, 2007; Hadidi et al., 2022).

The thermal properties of PPs are also important in characterizing temperature-induced conformational changes (i.e., glass transition, denaturation, heat stability) in protein structure (Yang et al., 2017). The thermal properties of PPs play an essential role during processing and storage. The denaturation peak temperature indicates the thermal stability of proteins, while the denaturation enthalpy denotes the proportion of undenatured proteins (Arntfield & Murray, 1981).

The physical properties of the particles and the powder material (i.e., powder bulk density, powder flow, particle density, particle shape, size distribution) of the PPs affect the functionality and technological performance. In general, the size of PPs varies from 10 to 900 μm depending on the powder production method used. In some cases, nanosized protein particles might be needed to access the small dimension structures. In addition, an increase in the ratio of surface area to volume increases the ability of diffusion of bioactive compounds (Hadidi et al., 2022). It was reported that the bulk density of PPs ranged from 0.5 to 0.6 g/mL and particle density ranged from 1.0 to 1.2 g/mL (Carvalho-Silva et al., 2013). Alonso-Miravalles et al. (2020) studied the physical properties of protein-rich pseudocereal powders and reported irregular-shaped and rough surfaces having mean particle diameters from 96.5 to 215 μm . Their findings showed

that protein-rich pseudocereal flours exhibited cohesive behavior, higher compressibility indices, and lower bulk densities than regular protein-containing flours. Again, Babu et al. (2018) studied the bulk and shear flow properties along with the morphological and functional changes of milk protein concentrate powders after storage for 12 weeks at 25 and 40°C. Milk protein concentrate (MPC) powders with high protein content displayed higher permeability and higher flow rate index. In contrast, higher-protein-containing samples were poor in shear flow behavior. Samples kept at 25°C were flowable rather than those stored at 40°C. MPC powders were remarkably different in flow properties because of their compositional and morphological differences.

Amagliani, O'Regan, et al. (2016) studied the physical (particle size distribution and shape, surface characteristics) and flow properties of a broad of rice-origin PPs. They showed that the examined hydrolyzed PPs exhibited higher hygroscopicity linked to high relative humidity values compared to their intact counterparts and good flowability (i.e., easy-flowing or free-flowing behavior). On the other hand, the intact rice protein ingredients were higher in bulk density than their hydrolyzed counterparts. For instance, microstructure changed significantly within the samples depending on several factors, including the origin of protein ingredients (i.e., bran, endosperm), the type of processing, and drying techniques. Additionally, chemical composition, surface characteristics, and particle shape were the major factors influencing the powders' flowability rather than particle size.

According to Nishanthi et al. (2018), the particle surface of native, sweet, and acid-whey protein concentrate powders was dominated by proteins under 90 days of storage, while fat and minerals prevailed on the surface of salty-whey protein concentrate. According to their findings, WPC particles exhibited spherical shapes and dented- and smooth-surface with a free-flowing property at the beginning of storage. Agglomeration, aggregation, and rupture occurred between particles at higher storage temperatures and more extended storage periods.

The moisture adsorbed by PP affects its molecular properties and storage stability. The quality properties of PPs, such as storage stability, shelf-life, storage volume, taste, and nutritive value, are associated with the level of water content (Isengard, 2009; Yang et al., 2017). The water content of PPs may range from 2% to 10%, depending on the drying method and parameters used.

Ebert et al. (2020) investigated the appearance, aqueous solubility, and natural pH of 26 PPs of plant origin. They revealed that prolamins in wheat or glutelins in rice were poor in solubility than PPs isolated from peas and sunflowers, which are rich in globulins and albumins. The appearance was affected due to pigment degradation and/or non-enzymatic browning after extraction procedures. In addition, Murayama et al. (2021) investigated the associations among solubility loss of milk protein concentrate (MPC) through various surface characteristics and storage. At the beginning of the storage, the MPC's solubility was determined as 56% and 10% at the end of the 60-day storage. The fusion of casein micelles in the MPC powder particle surfaces is identified as a causative factor for the solubility loss of MPC through long-term storage. The findings suggest that the

solubility loss may be prevented by inhibiting a crusty surface formation on powder particles. Chen et al. (2017) treated myofibrillar PP with high-pressure homogenization to examine its effect on functional properties. They found that water solubility and emulsifying properties were significantly enhanced with increased pressure.

Ji et al. (2016) investigated the wettability and dispersibility of milk PP using different methods. The findings showed that the immersional wetting procedure is suitable for skimmed milk powder only, whereas capillary rise wetting is much more beneficial in the case of agglomerated milk PPs with porous structures. Another work exhibited that agglomeration caused an external structural modification in high-protein dairy powders. Therefore, it seems challenging to speed up the dispersion process of micellar casein (Hou et al., 2017; Kiewiet et al., 2018). The effects of SD, FD, and oven drying methods on mung bean protein isolate powder at a pilot scale were investigated by Brishti et al. (2020). Their findings revealed a porous structure from freeze drying while spray drying and oven drying formed wrinkled and compact crystals, respectively. In addition, freeze-drying yielded better protein solubility and more significant water and oil absorption capacities. Spray drying provided the smallest particle size and improved stability and emulsion activity index.

4 | APPLICATION OF PROTEIN POWDERS IN FOOD FORMULATIONS

Protein products from plant or animal sources are concentrated in PPs in three typical forms: protein concentrates, protein isolates, and protein hydrolysates. Protein concentrates are produced using heat, acid, or enzymes to extract protein from foods. These generally comprise 60%–80% protein, with the remaining 20%–40% lipid and carbohydrates (Hertzler et al., 2020). Approximately 90%–95% of protein is included in protein isolate powders because more fat and carbohydrates are removed by further filtration to increase the protein content. For protein hydrolysates, peptide bonds between amino acids are broken during additional heating with acids or enzymes to create hydrolysates to enhance their bioavailability and functionality (Kiewiet et al., 2018).

The primary sources of plant PPs are reported as cereals (i.e., corn, wheat, rice), pulses (i.e., soy, lentils, beans), oilseeds (i.e., sunflower, cotton, peanut), green leaf proteins (i.e., alfalfa, duckweed), nuts (i.e., peanut, walnut, almond), pseudocereal (i.e., chia seed, amaranth, quinoa), and other sources of plant proteins (i.e., potato, mushroom) (Balakrishnan & Schneider, 2022; Hussain et al., 2021; Karaman et al., 2022; Mesfin et al., 2021; Nikbakht Nasrabadi et al., 2021; Sá et al., 2020). The proportion of protein groups and their molecular size differs considerably depending on the species or subspecies of the plants that originated. Variations in molecular structure exhibit different specific properties (Hadidi et al., 2022). In addition, even though plant proteins are known as incomplete proteins due to limitations in terms of several essential amino acids such as methionine, lysine, threonine, and sulfur, they offer 65% of the per capita consumption and/or availability of protein in the World and

TABLE 2 The application of protein powders in food formulations.

| Protein powders | Food products | Functions and significant findings | References |
|---------------------------------------|---|--|---|
| Whey protein | Dairy products such as yogurt, ice cream, and milk drink | Skim milk powder can be replaced by demineralized lactose-free whey powder to provide a desirable body and mouthfeel of milk products. | Boland, 2011 |
| | Sugar confectionery products such as lollipops, toffees, candies, caramels, chocolate, cotton candy | Color and flavor in candy products are increased by interacting with amino acids and lactose during heat processing under Maillard reactions. | Shinde et al., 2018 |
| | Sauces and soups | Acid solubility and water-binding capabilities during thermal processing are improved. | Królczyk et al., 2016 |
| | Infant formula | Human milk with nutritional benefits is simulated by modifying infant formula. | Fenelon et al., 2019 |
| | Confectionery and bakery products such as bread, biscuits, and cakes | Aeration in product structure can be enhanced | Królczyk et al., 2016 |
| | Dietetic foods for elderly | The bioavailability of salts like calcium and zinc is improved, and essential vitamins are provided in diets. | da Camargo et al., 2020 |
| | Dietetic foods for weight management | A satiety-enhancing dietetic diet with a high protein, low-fat content, and an ideal amino acid composition is developed. | Zemel & Zhao, 2009 |
| | Meat, seafood, comminuted meat, and fish products. | The properties of gelation, emulsification, and hydrophilic are improved. | Youssef & Barbut, 2011 |
| | Nutraceuticals | Providing many health benefits with significant bioactive proteins such as lactoperoxidase, lysozyme, lactoferrin, and numerous growth factors. | de Wit, 1998 |
| Soy protein powder | Dairy products, confections, candies, baking products, and baby formula | Gelation, water holding, absorption, emulsification, flavor absorption, foaming, fat permeability prevention and coagulation, and viscosity are improved. | Astawan & Prayudani, 2020; Singh et al., 2008 |
| | Dumplings | Lean meat can be replaced with soy protein powder, which helps absorb excess animal and plant fats while enhancing the taste and appearance of dumplings. | Cheng et al., 2021 |
| Soy powder and fermented lupin powder | Cakes | At all levels, higher antioxidant activity was present in cake samples containing fermented soy powder than in fermented lupin powder. Compared to fermented soy powder, fermented lupin powder had a less detrimental impact on the volume and hardness of the cake. When these two protein powders were added up to a 20% ratio, a positive general acceptance was observed. | Aslan & Bilgiçli, 2021 |
| Fish protein powders | Meat and poultry products | Strong interaction with other proteins and high gelling capability were provided. | Chung et al., 2000 |
| | Seafood such as herring roes and fillet blocks | Adhesion, dispersion, and emulsification are enhanced. | Pires et al., 2012 |
| | Ice cream | Nutritional and functional properties are enhanced. | Shaviklo et al., 2011 |
| | Surimi, the wet and leached mince product | Water binding capability and gelation to generate <i>kamaboko</i> gels are improved. | Carvajal et al., 2005 |
| High milk protein powder | Nonfat yogurt | Skim milk supplemented with this protein resulted in low-fat yogurts with the highest quality at 5.6% total protein content. Without using stabilizers, the additional protein enhanced the yogurt a solid appearance. | Mistry, 2002 |
| | Cheese | The cheese yield was improved by approximately 2.4 kg per 100 kg of milk with 2% high milk protein powder. The cheese had more moisture, more protein, and less fat content. | |

(Continues)

TABLE 2 (Continued)

| Protein powders | Food products | Functions and significant findings | References |
|--|---|--|-----------------------|
| Egg white protein powder | Geometrical shapes of 3D-printed mixture samples | Printing ink with a 5% egg white protein content is optimum for 3D printing. A specific concentration of egg white protein may enhance the firmness and flexibility of the gel sample. | Liu, Liu et al., 2018 |
| | Different geometric shapes of food formulation under extrusion-based 3D food printing | The ideal solution composition of the 3D-printed food formula was 12.98 g egg white protein powder, 8.02 g sucrose, 19.72 g cornstarch, and 14.27 g gelatin in 250 mL deionized water. | Liu et al., 2020 |
| Wholegrain rye powder and milk powder | High protein and dietary fiber-rich snacks produced from extrusion-based 3D printing | Adding whole-grain rye powder to the milk formulation significantly enhanced shape retention during baking. Adding more milk powder to the printing paste elevated the baked products' glossiness, sweetness, volume, and saltiness. | Lille et al., 2020 |
| Milk protein concentrate and whey protein isolate powder | A milk protein-based 3D printing food simulant | The printing performance of milk protein paste with a milk protein concentrate/whey protein isolate ratio of 5/2 is optimum. | Liu, Liu et al., 2018 |
| Protein extract powder from stale bread | Chickpea flour added to wheat bread | Increasing the addition of protein extract powder from 6.5% to 13% (wt/wt, based on flour) resulted in increased loaf volume | Şişman et al., 2022 |

their combination can meet the nutritional needs of healthy individuals (Alcorta et al., 2021; Lima et al., 2022; Mahmoodani et al., 2014; Yashini et al., 2019). Furthermore, plant-based proteins could replace carbohydrates and fats in food formulations and could be a choice for individuals allergic to animal proteins (Amagliani et al., 2017; Yashini et al., 2019). On the other hand, animal-based sources such as meat, dairy, fish, poultry, and eggs provide essential amino acids with superior biological value and good digestibility (Yashini et al., 2019). Some examples of PPs in food formulations can be seen in Table 2.

Whey protein, milk protein, soy protein, and fish PPs have been widely and commonly used in food formulations for decades. They provide various functional properties in food formulations, such as gel formation, water-holding, water absorption, emulsification, flavor absorption, foaming, the prevention of fat permeability and coagulation, and viscosity in various kinds of food products, such as meat, plant-based meat substitutes, candies, dairy and milk products, baking goods, baby formula, dietary supplements, and functional foods (Astawan & Prayudani, 2020; Cheng et al., 2021; Singh et al., 2008; Youssef & Barbut, 2011). Whey PPs have the most economical and practical potential in food industry applications compared to other PPs. In addition to their nutritional benefits, whey PPs have several functions during food processing: thermal stability, foaming/aeration, gelation, thickening, and emulsifying activity (Królczyk et al., 2016; Ramos et al., 2016). In addition, whey PP can enhance color and flavor. It has been reported that whey protein products containing optimal amino acid profiles have the potential in diets to promote weight loss (Ramos et al., 2016; Zemel & Zhao, 2009). The fish PPs providing solid connections with other proteins and high gelling capabilities make them ideal for usage in restructured beef, pork, and poultry products (Chung et al., 2000; Pires et al., 2012; Ramírez et al., 1999). The semolina of pasta was replaced with fish PP at different

concentrations to enrich its nutritional value (Ayyash et al., 2018). Low-fat yogurts were prepared using skim milk supplemented with whey protein isolate to improve the quality (Hashim et al., 2021).

5 | USE OF PROTEINS IN 3D FOOD PRINTING

The innovation in food manufacturing, extrusion-based 3D food printing, allows food production to be customized to each person's requirements and preferences. Recent studies revealed that the use of 3D printing is a promising processing method to provide individualized healthy eating solutions through the use of a variety of protein or dietary fiber-rich materials, including meat (Dick et al., 2019), oat and faba bean protein concentrate (Lille et al., 2018), milk protein (Liu et al., 2019), whey protein (Du et al., 2021), pea protein powder (Chirico Scheele et al., 2021), egg white proteins, and soy protein isolate (Chen et al., 2019, 2022; Mu et al., 2021), either alone or in conjunction with starch and other hydrocolloids.

A novel food formulation for 3D printing based on a complex system containing gelatin, cornstarch, sucrose, and egg white protein was developed by Liu et al. (2020). The raw material was egg albumen protein powder with an egg white protein content of 80%. The result showed that printing ink with a 5% egg white protein content is optimum for 3D printing. They also demonstrated that a specific concentration of egg white protein might enhance the firmness and flexibility of gel samples and perform well as supplementary material for 3D printing inks (Liu, Meng et al., 2018; Liu, Liu et al., 2018). Egg white protein powder can be utilized extensively in food 3D printing due to its favorable gelling characteristics. An optimal strategy for an innovative 3D-printed food formula was determined, and the ideal solution

composition was 12.98 g egg white protein powder, 8.02 g sucrose, 19.72 g cornstarch, and 14.27 g gelatin in 250 mL deionized water (Liu et al., 2020). The use of extrusion-based 3D printing to develop protein- and dietary fiber-rich snacks from whole milk powder and wholegrain rye flour was investigated. Grid-like samples were printed at room temperature using the aqueous pastes prepared from different ratios of raw ingredients. The printed samples underwent post-processing by being baked in an oven at 150°C. Adding wholegrain rye powder to the milk formulation significantly enhanced shape retention during baking. According to sensory analysis, adding more milk powder to the printing paste elevated baked products's glossiness, sweetness, volume, and saltiness (Lille et al., 2020). Another study exhibited that the viscosity of protein pastes would be reduced and softened by adding more whey protein isolate. It is too challenging to extrude milk protein paste from a printer nozzle by only using milk protein concentrate alone. The printing performance of milk protein paste with a milk protein concentrate/whey protein isolate ratio of 5/2 is optimum (Liu, Liu et al., 2018).

A movable syringe loaded with protein-based ink (i.e., collagen, silk, fibrinogen, keratin, and other food proteins) is often used to extrude through a nozzle on a flat surface. For outstanding printing efficiency, the rheological and viscoelastic properties of protein-based inks play a crucial role. In addition, the protein's structure and mechanical performance (i.e., extrudability, filament fidelity, and sol-gel transition) are also important (Mu et al., 2021; Townsend et al., 2019).

5.1 | Regulatory issues and risk assessment

In recent years, researchers have focused on alternative sources of proteins for developing novel ingredients suitable for food analogs (meat and dairy alternatives) and improving their technique and processing functionality. Most of the attempts have aimed to meet the need for matching their physicochemical properties, safety and risk assessments, nutritional endpoints, and technologies with those occurring from animal sources. For instance, plant-origin protein concentrates and/or isolates have been used to develop meat and dairy analogs, including protein bars, non-dairy beverages, and snacks (Stone, Nosworthy et al., 2019). The Allergenicity of some plant proteins, such as hazelnut proteins, gluten, and soy proteins, poses a health risk for sensitive consumers. Therefore, potential risk factors must be carefully monitored among the susceptible populations (Sha & Xiong, 2020). Various isolated proteins were examined for processing behavior, flavor, and ability in line with customer attitudes and sustainability issues (Jiménez-Munoz et al., 2021).

Food safety policies and legislative regulations provide reasonable certainty that food will not pose a risk to humans based on its intended use. To ensure future food security and safeguard our planet, the new goal of “sustainable alternatives to traditional proteins” has become increasingly important. Including novel proteins in human and animal diets is necessary to shift our food system to return and remain within planetary boundaries. However, it needs to be

understood by scientists and risk assessors that a situation of zero risk does not exist. In the EU, this certainty is also valid for new unconventional protein sources and products. The updated novel food regulation (NFR - 2015/2283) provides a centralized-generic authorization system with a renewed risk assessment procedure by describing novel proteins/foods with a new definition based on EFSA's scientific approach to make them safe for human consumption. The methodology should comply with the principles of the “do no significant harm” principle of Regulation (EU) No 2020/852”, European Green Deal (EGD) 2019, EU's Regulation 2015/2283, and UN FAO/WHO Codex (de Boer & Bast, 2018; Segatto et al., 2022).

6 | CONCLUSION

Recent research mainly highlights nutritional endpoints, safety, health risk assessments, and functional properties of PPs occurring from different conventional and novel plant sources. Due to numerous advantages, the interest in extracting and characterizing proteins from plant sources is growing. Therefore, there is an expectation of increasing plant protein utilization in food and pharmaceutical applications. Although previous works have provided significant data for understanding some criteria such as composition, distribution, processing, and functional properties of PPs, more research is needed to provide insight for the “PPs” quality, stability, and functionality characteristics to extend the range of technological applications and meet the needs of the food industry.

Further research should be conducted to improve the technological and material properties of PPs further, considering thermal protection during drying, better encapsulation efficiency, decreased cell-wall material for efficient release, improved powder granulation, masking the unpleasant taste and/or odor, extended shelf life, and enhancing compatibility in food matrix to increase the utilization of PPs for developing functional food formulations. More studies are also needed to establish which drying methods are suitable for the proteins from different sources having varied molecular structures to obtain the targeted powder product. Allergenicity, toxicology, kinetics, and other hazards in the novel protein-rich ingredients must be examined *in silico*, *in vitro*, and *in vivo* techniques. Furthermore, protein digestibility should be further assessed by studying protein digestibility to understand the effect of processing better.

AUTHOR CONTRIBUTIONS

Ertan Ermis: Conceptualization, methodology; project administration, data curation; formal analysis; investigation; writing-original draft; writing-review and editing. **Ismail Hakki Tekiner:** Formal analysis; investigation; writing-original draft, writing-review and editing. **Chi Ching Lee:** Formal analysis; investigation, writing-original draft, writing-review and editing. **Sumeyye Ucak:** investigation; writing-original draft. **Hasan Yetim:** writing-review and editing.

CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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