Plant Protein Ingredients

Subjects: Plant Sciences

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Many kinds of plant proteins are available as functional ingredients in foods, including those derived from cereals, legumes, oilseeds, and algae. Among these, pulse proteins are some of the most frequently used because they can be economically isolated from common natural resources (e.g., peas, chickpeas, lentils, and beans) that contain relatively high protein levels (>20 g protein/100 g dry matter), thereby enhancing their economic viability. Extraction and purification methods can convert pulses into functional ingredients with protein contents ranging from relatively low (<50%) to relatively high (>90%), including flour, concentrates, and isolates.

pulse proteins | legume protein | plant proteins | meat analogs | functional properties | protein isolates

1. Cultivars and Genotypes

Most plant proteins are composed of albumin and globulin fractions [1]. Different cultivars and genotypes naturally have different ratios of these protein components, which influence the functional properties of the extracted plant protein flours, concentrates, and isolates [1]. Indeed, many studies have shown that cultivars and genotypes have a significant impact on the functional performance of plant proteins.

For lentil proteins, the water-soluble fraction reported significantly varied results among cultivars, with red lentil Fırat and green lentil Pul II having the highest contents of around 70 g/100 g [2]. Moreover, past research reports that the proteins in a red lentil concentrate have a higher water-solubility than those in a green lentil one [3]. Lentil proteins have gelling properties that functionally vary among cultivars. For example, the proteins isolated from Ciftci and Kafkas red lentils only form weak gels, even when used at a relatively high concentration (14%). In contrast, those from Ali dayı and Fırat cultivars form hard gels under the same conditions [2]. The oil absorption capacity, foaming capacity, and foaming stability of these proteins were dependent on the cultivar type. Those from Fırat red lentil exhibited the best functional performance. Indeed, these proteins had a higher foaming capacity than soy protein isolate. In another study, Common Blaze red lentil concentrate produced by ultrafiltration had a higher fat absorption capacity than Grandora green lentil concentrate [3].

For chickpea proteins, many researchers have compared the functional performance of those derived from the Kabuli and Desi cultivars. Within the Kabuli type, different cultivars exhibit differences in their water absorption capacity and foaming properties. For instance, Sarı-98 chickpea protein reported a higher water absorption capacity (23%) and foaming capacity (18%) than other cultivars [4]. Another cultivar, Cevdetbey-98, also reported a

high water-soluble protein content, good gelation properties, and high oil absorption capacity compared to other cultivars (Canıtez, Gökçe). However, desi and Xena kabuli chickpea cultivars reported similar functional attributes, including water-solubility, water holding capacity, gelation properties, and emulsification properties [3]. In a related study, the functional performance of protein isolates derived from five genotypes of desi chickpea and one genotype of kabuli chickpea were compared [5]. The kabuli chickpea protein isolate had a lower water absorption capacity but higher oil absorption capacity than the desi chickpea protein isolate. These studies suggest that the observed differences may be due to more non-polar amino acids in kabuli chickpea protein, which can help it bind fats. The kabuli chickpea also exhibited the highest foaming stability after 120 min of storage, which may be necessary for some food applications.

Isoelectric points of isolates from different pea protein report similar results being in the range of pH 4.6 to 4.9 [6]. Their water and oil holding capacities were also similar among cultivars. However, the CDC Dundurn isolate had a significantly higher water-solubility (76%) than the other isolates studied (66%), probably due to a lower surface hydrophobicity. Cooper and CDC Dundurn isolates showed significantly lower emulsifying capacity than the other five cultivar isolates, although no significant difference was found among their emulsifying properties. The poor emulsifying performance of CDC Dundurn isolates may be associated with their low surface activity. As a result, this cultivar may not be suitable as an emulsifier in emulsion-based foods.

Moreover, this study suggests a synergistic effect of extraction method and cultivar type on the functional performance of pea proteins, including their water holding capacity, foaming capacity, foaming stability, and emulsifying properties. For example, CDC Meadow isolates had a relatively high-water holding capacity when extracted by salt extraction, but a low one when extracted by micellar precipitation. This demonstrates the importance of optimizing both cultivar-type and extraction method to obtain good functional performance in plant protein ingredients.

Not all pulse proteins show large variations in functionality among their genotypes. For example, several faba bean *Vicia faba* L. genotypes were reported to have similar functional properties [7]. These authors compared the physicochemical properties and functional performance of protein isolates obtained from seven different genotypes of faba bean. The zeta-potential, surface hydrophobicity, protein solubility, oil holding capacity, emulsion capacity, creaming stability, emulsification activity, and stability indices of all genotypes were reported to be quite similar. As the differences in the molecular properties of proteins obtained from different genotypes are relatively small compared to those from different cultivars, there is less concern on which genotype to choose for different food applications. Moreover, the effects of environmental conditions on pea genotype performance have been difficult to isolate [8]. It has also been demonstrated that areas with low rainfall and high temperatures were associated with higher protein content in the same pea genotypes [9].

2. Different Forms of Plant Proteins

Plant protein ingredients typically come in the form of flours, concentrates, or isolates, depending on their total protein concentrations, typically 50–70%, over 80%, and over 90%, respectively. The more extensive (and

expensive) the extraction process used, the higher the protein content in the final ingredient. Protein ingredients also contain different types of proteins, which may have different molecular conformations (native or denatured) and aggregation states (e.g., monomers, dimers, trimers, etc.) depending on their biological origin and the extraction and drying methods used. The concentration, type, conformation, and aggregation state of the proteins in an ingredient play a major role in determining its functionality. In addition, protein ingredients also contain other components that can impact their functional performance, including starches, fibers, lipids, and minerals. Many plant proteins are used as texturized proteins as meat extenders or a meat analog by providing an economical, functional, and high-protein food ingredient [10]. Texturized vegetable proteins are characterized by having a structural integrity and identifiable structure. Obtaining reliable sources of ingredients with the required functional attributes is one of the major challenges in the plant-based food area.

Researchers have shown that protein isolates typically have a higher water holding capacity than the corresponding flour form. The higher protein content of isolates attribute to the increase in water holding capacity, while some of the non-protein components in flours may be a barrier to water penetration, such as starch granules, fibers, or lipids. For instance, it was reported that lentil protein isolates had a higher water holding capacity than lentil flours, which was attributed to their lower lipid content and smaller particle size [11]. The oil holding capacity of chickpea protein isolates (2.1 to 4.0 g/g) was reported to be significantly higher than their corresponding flours (1.1 to 1.2 g/g) [5]. Conversely, the gelation properties of great northern bean and chickpea protein isolates were reported to be significantly lower than their corresponding flours [5]. In this case, the gelling properties of the ingredients may depend not only on their total protein content but also on the type, denaturation, and aggregation state of the proteins, and the presence of any non-protein components [12]. The foaming capacity of chickpea protein isolates (30 to 44%) has been reported to be significantly higher than that of their corresponding flours (15 to 20%) [5]. These results suggest that the functional performance of plant proteins is strongly dependent on the protein concentration and form of the ingredients used. Consequently, it is important to identify a protein ingredient that has the molecular, physicochemical, and functional attributes required for the specific application.

3. Commercial or Laboratory Processed Plant Proteins

Many of the published studies on the functional properties of plant proteins have used isolates or concentrates prepared in a laboratory using small-scale extraction and drying procedures. These studies have provided valuable information about the functional performance of distinct kinds of plant proteins. Nevertheless, there are usually major differences in the functional performance of proteins produced in the laboratory and using commercially viable large-scale processing operations. Commercial processing operations often reduce the functional performance of plant protein ingredients because of protein denaturation, protein aggregation, or the presence of non-protein components that interfere with protein performance.

Similarly, it was reported that the water-solubility of pea protein isolates prepared in the laboratory (66%) was considerably higher than commercial ones (5%) [6]. Several studies have also shown that there are appreciable differences in the water-solubility of commercial soy protein isolates purchased from different sources, which may be due to differences in their biological origin, or the methods used to extract, dry, and store them [2][13]. For

instance, although a commercial soy protein isolate and a soy protein extract had similar total protein contents (0.90–0.92 g/g), the water-soluble protein content of the soy protein extract (0.57 g/g) was much higher than that of the commercial protein isolate (0.21 g/g) [2]. This effect might be because the soy proteins were denatured under the highly acidic conditions used in acid precipitation methods, or the higher and longer temperature exposures experienced during drying, which occurs more often in large-scale industrial production [13]. Moreover, other commo practices used in the commercial production of proteins can decrease their solubility and functionality. In addition, plant proteins are often a side product generated during the isolation of edible oils (such as soybean oil), which often involves the use of organic solvents that can denature proteins. In some cases, the functional performance of protein ingredients can be improved by utilizing additional processing operations, such as homogenization or ultrasonic treatment to dissociate aggregates [13].

The nature of the processing operations used to create plant protein isolates has been shown to impact their denaturation state using differential scanning calorimetry (DSC). The thermal denaturation temperature of a laboratory-produced pea protein isolate was reported to be considerably higher (T_d = 82.6–94.3 °C) than that of a commercial pea protein isolate (T_d = 72.8–72.9 °C) [14]. Moreover, the laboratory-produced pea protein isolate had a much higher transition enthalpy (ΔH_d = 15.8–17.8 J/g protein) than the commercial pea protein isolate (ΔH_d = 0.033–0.036 J/g protein), which indicates that the commercial ingredient was much more denatured. Furthermore, when the commercial pea protein isolates were heated to about 86 °C, there was a lack of a thermal transition peak, which means that most of the protein isolates were already denatured during processing. As a result, a much higher concentration of the commercial pea protein isolate (14.5%) was required to form a gel than the laboratory-produced version (5.5%). The laboratory-produced pea protein isolates also formed stronger gels than the commercial ones, which was attributed to the higher amount of native protein that could participate in network formation.

It has been reported that commercial soy protein isolates had a higher water holding capacity and lower water-solubility than laboratory-processed ones [15]. Interestingly, when the proteins in the laboratory version were intentionally thermally denatured, the water holding properties of the laboratory-produced and commercial soy protein isolates were similar. This study therefore strongly suggests that protein denaturation is responsible for the poor functional performance of the commercial soy protein isolates. In other studies, commercial soy protein isolates have been shown to have a higher water holding capacity than laboratory-produced ones (7.94 g water/g compared to 1.69 g water/g), but a lower oil holding capacity (1.16 g oil/g compared to 8.23 g oil/g) [2]. The apparent viscosity of commercial soy protein isolates has been reported to be higher than laboratory-produced ones, which may be because some of the proteins have become denatured and aggregated [15].

Overall, these studies demonstrate the importance of carefully characterizing the functional performance of commercial plant protein ingredients from different sources, because they can vary widely. Moreover, the functional performance of laboratory-produced plant proteins reported in the literature may be considerably different from that of commercial plant protein ingredients.

4. Structure of Plant Proteins

The molecular structure of plant proteins has a major impact on their functional performance. In this section, therefore briefly explain the impact of protein structure on the functional attributes of selected plant proteins. Most plant proteins are primarily comprised of salt-soluble globulins and water-soluble albumins, which occur in a ratio of approximately 70% to 20%, depending on the source of the plant proteins, with the remainder being other minor proteins [1][16]. Legumin (11S) and vicilin (7S) are the main types of globulins found in plant proteins [3]. The most common minor proteins present are convicilin, prolamins, and glutelins [3]. The ratio of legumin and vicilin in plant protein ingredients varies depending on their biological origin and can affect their functional properties. For instance, it was reported that the relatively low legumin-to-vicilin ratio found in pea proteins can increase their functional performance, including their emulsifying and gelling properties, because of their higher protein extractability [17]. Furthermore, it was demonstrated that the amino acid composition, water holding, and oil absorption capacities of red lentil proteins from three origins (USA, Nepal and Turkey) were significantly different [18]. The amino acid compositions of plant proteins also vary depending on the type of species and genotype of the plant used as a source [19]. For example, it was demonstrated differences in essential amino acid content between pea, soy, and lupin proteins: 30%, 27%, and 21%, respectively [20]. In contrast, researchers have demonstrated similar amino acid profiles between faba bean and pea flours, with leucine and lysine found in the highest amount [21][22]. The amino acid composition impacts the functional properties of proteins because it determines the ratio of polar to non-polar groups, as well as the balance of positive, negative, and neutral groups, which affects their surface hydrophobicity and electrostatic interactions. Therefore, the structure of plant proteins is another factor that must be considered when optimizing their functional performance.

5. Extraction Methods

There are two main categories of protein extraction methods used commercially: dry and wet extraction [23]. These methods are designed to isolate the proteins from the other major components in the original plant material, such as starches, fibers, lipids, and minerals. The most common wet extraction methods include isoelectric precipitation and salt extraction [24]. Isoelectric precipitation (IEP) involves dispersing plant flours in a strong alkaline or acid solution to increase the charge on the protein molecules and thereby solubilize them. The pH of the resulting solution is then adjusted close to the isoelectric point of the proteins to precipitate them. This method is therefore based on reducing the electrostatic repulsion between the protein molecules since they have no net charge around their isoelectric point. Salt extraction (SE) involves dispersing plant flours in a concentrated salt solution, such as ammonium sulfate or sodium chloride solution. At a sufficiently high salt concentration, the proteins that are associated with each other are due to a salting-out effect, which leads to the formation of a protein-rich precipitate phase. Another protein extraction method based on the use of salt is micellar precipitation (MP), but this approach is used to isolate proteins that have a high solubility in concentrated salt solutions but a low solubility in dilute salt solutions [25]. In the MP method, a protein flour is dispersed in a concentrated salt solution to solubilize the proteins (salting-in). This protein solution is then diluted with distilled water to reduce the salt concentration, which results in the association of the protein molecules and the formation of micelle-like protein aggregates that can be removed by centrifugation.

After the precipitated proteins have been collected, they can be re-dispersed within another aqueous solution with a pH and ionic strength that favors high protein solubility. If required, any residual acids, bases, and/or salts used to induce precipitation can be removed by dialysis or filtration [24]. Commercially, ultrafiltration (UF) is often used for this purpose [3]. UF is a type of membrane filtration method that involves applying high hydrostatic pressure to a solution that is in contact with a semipermeable membrane to separate the salts from the protein.

In general, wet processing is an efficient means of carrying out protein extraction, typically leading to samples with a minimum of 70% total protein content [7]. Nevertheless, there are considerable differences in the final protein contents obtained using different wet processing methods for different plant proteins. For instance, chickpea, faba bean, lentil, and pea protein isolates obtained using IEP were reported to have a higher protein content (82% to 88%) than those produced by salt extraction (73–82%) [24]. Soy protein isolates obtained using IEP have also been reported to have a protein content (82% to 86%) that is within this range [26].

The functional properties of plant proteins are highly dependent on the extraction methods used to produce them. For instance, chickpea, faba bean, and pea protein isolates produced by IEP have been reported to have a considerably higher water-solubility than those produced by salt extraction, although lentil and soy protein isolates gave similar values using both methods [24]. In addition, Langton and co-workers used an extraction method called soaked protein extract and IEP for faba beans [27]. The protein content was higher for an alkaline extract (82%) than for a soaked extract (67%). The researchers suggested that the lower protein content in the soaked extract could be due to the absence of a precipitation step resulting in more water-soluble non-protein components, e.g., oligosaccharides in the extract. Moreover, it has been reported that the water holding capacity of pea protein isolates depended on the extraction method used [6]: MP (3.2–3.6 g/g) > IEP (2.4–2.6 g/g) > SE (0.34–2.6 g/g). The authors suggested that the MP method may have exposed more polar groups on the plant protein surfaces, thereby leading to better hydrogen bonding with water. The emulsifying activity of pulse protein isolates produced by IEP has been reported to be significantly higher than those produced by SE [24]. The mean particle size of the oil droplets in emulsions produced by homogenization was reported to be appreciably smaller when the proteins were extracted by IEP rather than by SE [24]. This effect was attributed to the fact that the protein isolates produced by IEP had a slightly higher surface potential and surface hydrophobicity than those produced by SE [6][24]. The surface hydrophobicity of globulins is reported to be higher than that of albumins, which may account for the fact that a higher fraction of globulins is extracted than albumins due to the greater hydrophobic attraction between them [6]. The emulsifying properties, creaming stability, and foam expansion was also reported to be higher for protein isolates produced by IEP than by SE. These results suggest that protein isolates produced by IEP may be better for applications in food emulsions, where small stable oil droplets are often required.

Proteins with improved functional properties can be obtained by using UF alone rather than extraction methods that require pH adjustment or salt addition. For instance, it has been reported that pulse protein concentrates extracted using UF had a slightly higher protein content than those extracted using IEP [3]. The pulse protein concentrates produced by UF were also reported to have higher oil holding capacity and better gelling properties than those produced by IEP. But the water holding capacity, emulsifying properties, and foaming capacity of the protein concentrate were not found to depend on the extraction method. Notably, however, some pulse protein extracts

produced by IEP, including green lentil and chickpea concentrates, had higher foaming stability than those produced by UF. This result suggests that both protein type and extraction method should be carefully considered when developing an extraction method for producing plant protein ingredients for specific applications.

Dry processing, also known as air classification, can also be used to produce protein concentrates, but the total protein content is typically relatively low (<50%). In this method, the raw material is ground into a powder and then an air stream is blown through it, which separates the protein and starch fractions based on differences in their particle sizes and densities. For some applications, the dry processing method has advantages over wet processing methods for protein extraction, even though the final protein content is lower. For instance, it has been reported that the final protein content of a faba bean extract was around 64% for air classification but around 90% for IEP [28]. However, the water solubility of the proteins under neutral pH conditions was considerably higher for the air-classified protein (85%) than for the IEP protein (32%). The authors postulated that this difference was due to an increase in the surface hydrophobicity of the faba bean proteins caused by denaturation during the drying process required after wet extraction. Moreover, the air-classified faba bean proteins had a higher foaming capacity and gave a higher gel strength than the IEP ones. The authors suggested that differences in protein denaturation and carbohydrate content in the faba bean protein extracts may have contributed to the observed differences in their functional properties.

Furthermore, other processing methods combined with extraction could change the protein structure and thus influence the functional properties, e.g., heat treatments and extrusion. For instance, black bean protein concentrates treated with high temperature-pressure cooking, showed higher emulsion capacity compared to the raw treatment [29]. Finally, extraction methods can be used individually or in combination to produce a range of protein isolates with different functional attributes from a single flour. Consequently, functional ingredients with properties tailored to specific food applications can be created, e.g., solubility, emulsifying, foaming, or gelling.

6. Drying Methods

After wet protein extraction, proteins are usually dried to improve their handling and storage stability. Commercially, the most common method used to convert protein solutions into powders is spray drying (SD). This process rapidly converts a liquid into a powder by spraying the liquid into a chamber containing a hot gas, which causes the water to rapidly evaporate. The temperature-time profiles experienced by the proteins must be controlled to avoid excessive protein denaturation. Freeze drying (FD) is more commonly used in research studies to create protein powders from protein solutions on a small scale. The protein solution is frozen and then placed under a vacuum, which converts the ice into a vapor by sublimation, thereby leading to a dried powder. Commercially, spray drying is much more common, as freeze drying is a relatively expensive, time-consuming, low-throughput, and laborious method. Other drying methods have also been developed that use lower processing temperatures to avoid protein denaturation, including vacuum drying (VD) and refractance window drying (RWD). Compared to the other two methods, VD has a faster drying rate, lower drying temperature, and uses an oxygen-deficient processing environment. The drying process used to create a protein powder is known to impact the functional properties of the protein ingredient due to differences in the heating temperatures and times used.

As an example, the functional attributes of lentil protein isolates produced by SD, FD, and VD have been compared [30]. Initially, it was hypothesized that the isolates produced by SD would have the worst functional performance because the temperatures involved in spray drying can reach 80 °C or above. However, the protein isolates produced by SD had a comparable (high) solubility to those produced by FD, which was attributed to the cooling effect associated with water evaporation during spray drying that prevents the temperature of the proteins from becoming too high [31]. In addition, the proteins are only held at high temperatures for a relatively short time in SD. The high solubility of the protein powders produced by SD may also have been due to their smaller and more uniform particle size distribution. Joshi and co-workers showed that lentil protein isolates produced by FD had different functional attributes than those produced by SD. Specifically, the protein isolates produced by VD had significantly lower water solubilities and formed weaker gels, which may have been due to a greater extent of protein denaturation caused by the relatively long drying period (up to 48 h) used. Soy protein isolate produced by VD has also been reported to be more denatured than that produced using other drying methods [32]. VD may also promote protein denaturation when gas bubbles are formed when a vacuum is pulled since proteins are known to adsorb to air-water interfaces and partially unfold, which is known as surface denaturation.

The water-solubility of chickpea protein isolates was found to be appreciably lower when RWD (74.5%) was used to extract them than when FD (94.2%) was used [33]. This effect may have occurred because RWD employs a higher temperature than FD, which may promote more thermal denaturation of the proteins. However, the protein isolates produced by RWD were reported to have a higher water holding capacity than those produced by FD. The RWD protein isolate also had better emulsifying activity than the FD protein isolate, which was attributed to a higher surface hydrophobicity caused by partial protein unfolding. On the other hand, the foaming and gelling properties of the FD protein isolate were better than those of the RWD protein isolate. These results highlight that the drying method used alters the functional performance of protein isolates and should be optimized for specific applications. For instance, RWD protein isolates may be more suitable for application in emulsified foods due to their high surface hydrophobicity and emulsifying activity. In contrast, FD protein isolates may be better in food applications that require good foaming and gelling properties.

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