Chapter 3

Pulse Proteins: From Processing to Structure-Function Relationships

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Abstract

Interest in alternative protein sources to those derived from animal, soy and wheat is on the rise, as consumers are searching for lower cost, healthier alternatives without compromising product quality and safety. Pulses are rich in protein, carbohydrates, vitamins and minerals and are low in fat. Although pea proteins experience greater integration into the plant protein ingredient market than others, lentil, chickpea, bean and faba beans are not far behind. This review discusses approaches used for extracting pulse proteins used to produce protein products (concentrates/isolates), mechanism driving structure-function relationships as well as potential applications.

Keywords: Pulse proteins, extraction, structure-function and applications, Legumin: Vicilin

1. Introduction

Pulses such as beans, peas and lentils have been consumed for thousands of years and represent one of the most extensively consumed food in the world [1]. Pulses play crucial roles in fulfilling the nutritional requirements of the growing population in a cost effective manner, especially for developing or underdeveloped countries where animal protein consumption is either limited or expensive [2]. Pulses are widely used for food purposes because of their high protein content, high nutritional and health beneficial properties, appropriate functional attributes, and associated low production cost and abundance [3]. The health benefits associated with pulse consumption include lowering of cholesterol levels, reducing the risks of various cardiovascular

diseases and cancers, and decreasing the risk of type-2 diabetes [4]. Along with protein, pulses provides dietary fiber and vitamins and minerals such as iron, zinc, folate, and magnesium [1]. Pulses also have an antioxidant and anti-carcinogenic effect because of the presence of phytochemicals, saponins and tannins in them [1].

For many years, pulses have been used in the preparation of wholesome nutritional meals in combination with other food sources or ingredients. Pulse crops such as pea, chickpea and common bean (*Phaseolus vulgaris* L.), when blended with regionally grown cereal grains, could be of immense value in helping to fulfill the nutritional requirements of people relying just on mono-carbohydrate diets [5]. However, the nutritional quality of pulses is limited because of the presence of heat labile and heat stable anti-nutritional factors (ANFs) [2]. The ANFs include proteins such as lectins and protease inhibitors, and other compounds such as phytate, tannins, saponins, and alkaloids [2]. The negative impact of these ANFs on consumption of pulses in human and animal diets has been extensively reported [6]. However, the processed forms of legumes (flours, concentrates or isolates) are reported to have lower levels of ANFs than their corresponding raw material (seeds) [7]. For instance, during the germination process, legumes were found to have a higher digestibility, soluble protein [8] and dietary fiber [9, 10], and reduced levels of ANFs [11]. Furthermore, protein isolates prepared by extraction or precipitation methods were also found to have reduced anti-nutritional factors such as trypsin inhibitors, glycosides (such as convicine and vicine) and hemagglutinins which would otherwise impair protein digestion and could be toxic for human consumption [5, 12–14]. The exploitation of protein isolates or concentrates in new food formulations is of great importance because of their high nutrition and functionality [15]. The utilization of right individual functional properties might be useful in producing different food products such as cakes, biscuits, beverages and breads.

2. Protein structure and legumin/vicilin (L/V) ratio

The majority of pulse proteins are albumin and globulin fractions, where globulins represent ~70% and albumins constitute 10–20% of the total pulse protein [5, 16]. In addition, other proteins are present in minor proportions such as prolamins and glutelins [17, 18]. These four proteins can be classified according to their solubility in various solvents based on the Osborne classification scheme [19]. For example, globulin proteins are soluble in dilute salt solution, albumins in water, prolamins in 70% ethanol solution, and glutelins are solubilized in dilute alkali solutions [19, 20].

Albumins encompass structural and enzymatic proteins, lectins and protease inhibitors, with their overall molecular mass (MM) ranging between 5 and 80 kDa [5]. In contrast, the salt soluble globulins include legumin (11S, S = Svedberg Unit) and vicilin (7S) proteins. The 11S fraction is a hexamer (MM of ~340–360 kDa) comprised of six subunits (MM of ~60 kDa) linked by non-covalent interactions. Each subunit pair is comprised of an acidic (MM of ~40 kDa) and basic (MM of ~20 kDa) chain joined by a disulfide bond [16, 21]. In contrast, the 7S fraction is a trimer with a MM of ~175–180 kDa, and lacks disulfide bridging [5]. Vicilin protein

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molecules also have been reported to have various subunits of 75, 43, 33, 56, 12 and 25 kDa [16, 21]. A third type of globulin is also present, although in lesser amounts as compared to other globulins, and is known as convicilin [22]. It is a 7S globulin, and a single convicilin molecule has an overall MM of 220–290 kDa, and consists of 3 or 4 subunits each with a MW of 70 kDa. This protein has a different amino acid profile than vicilin as it contains sulfur-containing amino acids, is immunologically similar to 7S vicilin, and contains very little carbohydrate [5]. Various pulse species have been reported to contain convicilin-type proteins. For example, Saenz de Miera et al. [23] investigated 29 different legume species from 4 genera (*Pisum, Lens, Vicia* and *Lathyrus spp.*), and reported the presence of 34 new convicilin gene sequences. All of the above studies considered convicilin as a third class of globulin molecules. However, O'Kane et al. [24] deny the consideration of convicilin as a third pea globulin based on their findings and reported that convicilin (a polypeptide) should be denoted as the R-subunit of pea vicilin molecules (salt extracted).

The ratio of legumin:vicilin (L/V) is not fixed and may vary among different pulse varieties and species. The ratio of L/V for pea, soybean and faba bean varies in the range of 0.2–8.0, 1.3–3.4 and 1.7–3.7, respectively [25–35]. Various studies reported that L/V ratio for wrinkled pea seeds (0.2–0.6) represents a smaller ratio compared to the smooth pea seeds (0.3–2.0) [28, 30, 35, 36]. Various factors including the methods used in the preparation of protein materials (concentrates or isolates), processing parameters like pH and temperature and environmental or agronomic factors may account for the variation in these ratios, which in turn could also have influential effects on the physiochemical properties of pulse protein materials [16, 21, 37, 38]. As a part of their studies, Barac et al. [38] extracted the proteins from six varieties (genotypes) of pea (Calvedon, L1, L2, L3, Maja and M.A) and indicated that genotypes with high 7S protein levels or low 11S protein levels yielded higher amounts of protein (protein extractability) compared to the other genotypes. Moreover, pure vicilin solutions were observed to have better functional properties (such as emulsification and gelation) than the pure legumin solutions [38]. It was indicated that a low L/V ratio for preparation of protein isolates could be desirable. In the Mertens et al. [35] study on smooth pea seeds, it was reported that agronomic factors, including variety, cultivar type and location, affected the protein content and L/V ratio with high significance. However, some varieties were less sensitive to the prevailing climatic conditions than others. This approach could be beneficial from an industrial point of view as it could manifest in picking stable and less sensitive L/V ratio lines for specific product quality characteristics [35].

Various groups have researched relationships between L/V ratios and their functional attributes. A number of studies noted that pea vicilin showed higher emulsifying properties than corresponding pea legumin [39–41], which was attributed due to higher solubility [42] and surface hydrophobicity [5] of vicilin proteins. Furthermore, Shen and Tang [43] reported that emulsifying properties of vicilins were found to be dependent on both the legume source (Kidney bean, red bean and mung bean) and their protein concentration (0.25–2.5% w/v). The differences in the emulsion properties of vicilins at different concentrations were majorly related to the variation in zeta potential and interfacial characteristics, and were also found to be dependent on other factors such as protein folding, penetration and structural

rearrangement at the interface [43]. Bora et al. [44] studied the heat induced gelation of mixed pea globulins and found that 7S globulin had the capacity to undergo heat gelation while 11S globulin did not although used the same optimal conditions of gelation with 15% globulin solutions, pH 7.1 and heating at 87°C for 20 min. However, Nakamura et al. [45] observed that the gels formed by 7S globulins of soybean are less strong and transparent as compared to those formed by 11S globulins, which were much harder and turbid in nature. The study suggested that the extent of interaction in gel formation of a mixed system of 7S and 11S globulins is affected by factors such as the 11S/7S ratio and the composition of their subunits. Cserhalmi et al. [39] reported that mixed globulins and 7S fractions of pea proteins had increased surface hydrophobicity and emulsifying properties compared to the albumins and 11S fractions. Moreover, for all the pea varieties tested, the emulsifying and surface hydrophobicity properties were different from each other. Thus, varying the L/V ratio could be used in obtaining the desired functional attribute in new food formulations.

The quantification of 7S and 11S fractions present in isolates or concentrates is an essential step for calculation of L/V ratio which can be achieved using various methods described in literature. Methods include ammonium sulfate salt extraction [46], isoelectric precipitation [47], sodium dodecyl sulfate-polyacrylamide-gel electrophoresis (SDS-PAGE), gel chromatography [48], selective thermal denaturation [49], sucrose gradient centrifugation [50] and zonal isoelectric precipitation [51, 52]. The effective separation and the choice of technique should be dependent on factors such as nature of sample (isolates, concentrates, seed), extraction technique employed and the level of purification required. For testing of functional and physicochemical properties of 7S and 11S fractions, it is required that enough quantity of these samples is obtained whichever technique is used without compromising the purity.

3. Protein extraction

Protein extraction is dependent on many factors such as pH, temperature, particle size, ionic strength, type of salt used, and solvent to flour ratio [53, 54]. Various extraction methods are being studied so as to maximize the protein yield without compromising the protein functionality of the concentrate or isolate product. The protein extraction processes which are being exploited in the preparation of protein-rich materials (such as isolates and concentrates) can be classified into dry and wet methods [55–57].

3.1. Dry processing

Dry processing of pulses is typically done by air classification, which involves the separation of flours on the basis of particle size and density using an air stream into protein and starch rich fractions [21, 58]. Air classification has been found to be suitable for legume crops low in fat, such as field pea and common bean. Flours are first fractionated into starch (SI) and protein (PI) rich concentrates using an air classification method. SI is then remilled and fractionated to give SII and PII concentrates [55]. Protein separation efficiency (PSE) is defined as the percentage of total flour protein recovered in the PI and PII fractions, and measured as the

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subtraction of % total flour protein recovered in SII fraction from 100% [55]. For legume crops high in fat such as soybean and chickpea, particle agglomeration is detected which interferes with PSE [59–61]. Dry processing has major advantage over wet extraction methods as the native functionality of proteins is retained and a lower amount of energy and no water is required [62]. Moreover, in contrast to wet extraction methods where both protein concentrates and isolates can be produced, dry processes are suitable only for preparing protein concentrates with protein content from 40–75% [63] probably because of the presence of higher amount of other compounds such as oil and fibers, and protein loss in coarse fractions [64].

Tyler et al. [55] studied the fractionation of eight legumes (cowpea, great northern bean, lima bean, mung bean, navy bean, lentil, faba bean and field pea) using flours produced by pin milling followed by air classification and found faba bean (63.8–75.1%) and lima bean (43.4–49.6%) to have the highest and lowest protein concentrations in the protein-rich fractions. According to the authors, the suitability of pin milling followed by air classification is strongly correlated with the PSE of the legumes. Mung bean, lentil and great northern bean were found to have the highest mean PSE values of 88.9, 87.2 and 87.0%, respectively, whereas lima bean, cowpea and navy bean showed the lowest at 80.2, 78.2 and 80.3%, respectively. The other two legumes, faba bean and field pea, had PSE values of 84.1 and 82.8%, respectively. Overall, the authors indicated that except for lima bean and cowpea, the legumes were found to be suitable for separation of protein and starch fractions by the pin milling and air classification method.

3.2. Wet processing

In general, wet extraction methods can be exploited for preparing both protein concentrates and isolates at levels of 70% and 90% protein (or higher), respectively. However, it should be noted that currently there is no universal classification scheme which separates concentrate from an isolate for all the legumes. The various wet extraction processes include acid/alkaline extraction-isoelectric precipitation, ultrafiltration and salt extraction. Legume flours dispersed in aqueous solutions typically show high solubility when subjected to alkaline or acidic extraction conditions at pH 8–10 and below 4 respectively [63].

3.2.1. Acid/alkaline extraction-isoelectric precipitation (IEP)

Briefly, proteins are first dissolved under alkaline (alkaline extraction) or acidic (acid extraction) conditions, followed by a clarification step and then precipitation by adjusting the pH to the isoelectric point (pI) of the protein [65]. In solutions with the pH < pI, proteins assume a net positive charge, whereas at pHs > pI proteins assume a net negative charge. Under solvent conditions where proteins carry a net positive or negative charge, repulsive forces between proteins repel neighboring molecules, and also promote protein-water interactions for improved dispersion and solubility. Near the pI value, proteins tend to carry a neutral net charge, allowing neighboring proteins to aggregate via attractive van der Waals forces and hydrophobic interactions. Under these conditions, protein-protein interactions are favored over protein-water interactions, and thus protein is precipitated out of the solution.

According to Han and Hamaker [65], alkaline extraction followed by isoelectric precipitation is the most widely used method for obtaining extracts with protein purity greater than 70%. During alkaline extraction, legume proteins become solubilized at high pH values. The solution can then be clarified by centrifugation to remove insoluble material such as insoluble fiber, carbohydrates and insoluble proteins (e.g., prolamins). Protein concentrates or isolates can be formed by reducing the pH of the supernatant to near the pI of the protein using an acid such as HCl [63, 66]. The study of Can Karaca et al. [16] showed that isolates prepared from legumes (faba bean, chickpea, lentil, pea and soybean) by an alkaline extraction/IEP method had higher overall protein content (85.6%) as compared to those prepared by a salt extraction method (78.4%). Moreover, it was reported that both legume source and protein extraction method along with their interaction had significant effects on protein levels of the isolates, and also on physicochemical and emulsifying properties. The overall surface charge, solubility, hydrophobicity and creaming stability for IEP produced isolates was higher as compared to isolates produced by salt extraction [16]. The effect of processing or extraction conditions on the protein content of isolates can also be well observed from the studies of Flink and Christiansen [67] and McCurdy and Knipfel [68]. In the former study, faba bean isolates with protein contents of 80.0–90.0% were obtained when the bean:solvent ratio was 1:5 (w/v) with pH 8 to 10 at 23°C for 10 min, and the precipitation of protein was carried out at pH 3–5. While in the latter study, the protein content of faba bean isolates was 76.4–94.0% using a bean:solvent ratio of 1:5 w/v with pH 7–10, for 30 min, temperatures of 10°C and 20°C, and precipitation at pH 4–5.3.

Acid extraction (in principle similar to alkaline extraction) involves the preliminary extraction of proteins under acidic conditions. This process could result in high solubilization of proteins prior to protein recovery (IEP, Ultrafiltration (UF)), as proteins tend to be more soluble under acidic conditions (pH below 4) [5]. In a study by Vose [69] for preparation of faba bean (*Vicia faba equina* L. cv. Diana) and pea (*Pisum sativum* L. cv. Trapper) IEP isolates, the cyclone discharge obtained from pin milling these two legumes was acidified directly using 2 N HCl to a isoelectric point of 4.4–4.6. This process resulted in pea and faba bean protein isolates with 91.9% and 91.2% protein, respectively [5].

3.2.2. Ultrafiltration/diafiltration

In the literature, membrane separation methods were shown to produce protein isolates with higher functionality [70, 71] and were effective in reducing levels of anti-nutritional components which include protease and amylase inhibitors, lectins and polyphenols [72–74]. UF and microfiltration are membrane-based fractionation methods using pressure as the driving force for separation. Microfiltration can be used to separate particles or macromolecules larger than 0.1 μm, whereas ultrafiltration removes similar particles in the range of 0.001–0.02 μm [75]. For preparation of protein materials using ultrafiltration, the supernatant after alkaline or acidic extraction is processed using either UF or diafiltration (DF) together to isolate the protein material. UF is often combined with DF to improve protein recovery, where water is added to the retentate for dilution purposes, followed by re-ultrafiltration.

Vose [69] used the UF procedure to produce faba bean and pea protein isolates which protein levels of 94.1% and 89.5%, respectively. Boye et al. [66] evaluated the protein content of isolates obtained from different pulses (pea, chickpea and lentil) using alkaline extraction-IEP and UF/DF extraction methods. The protein content in concentrates obtained by the UF/DF method was found to be higher than in those obtained by IEP. For instance, for yellow pea, green lentil, red lentil, and desi and kabuli chickpea, UF/DF gave protein levels of 83.9%, 88.6%, 82.7%, 76.5% and 68.5%, respectively. In contrast, for IEP extraction, protein levels were 81.7%, 79.1%, 78.2%, 73.6% and 63.9% respectively for the same legume crops. Moreover, it was reported that UF was different from IEP in terms of protein composition as the isolates prepared by UF comprised both globulins and albumins, whereas the isolates prepared by IEP were observed to contain only globulins [63, 76, 77].

3.2.3. Salt extraction

Salt extraction is a process where globulin proteins are separated from albumins on the basis of solubility [5], as described previously in the Osborne classification scheme [19]. Proteins contain both hydrophobic and hydrophilic amino acids. The majority of hydrophobic moieties are buried inside the quaternary or tertiary structure due to a hydrophobic effect, and the majority of hydrophilic moieties are on the surface, free to participate in protein-water interactions. 'Salting-in' of proteins typically occurs at low salt levels, where the ions act to increase order of the protein's hydration layers and promote protein-water interactions [78–83]. However, at high levels of salt, hydration layers can be disrupted as ion-water interactions become favored over protein-water interactions in a 'salting-out' process [78–83]. As the ions attract water molecules away from the surface of the proteins, protein-protein aggregation is favored due to hydrophobic interactions. Aggregates continue to grow in size and number until they fall out of solution as a precipitate. The ability of ions to 'salt-in' or 'salt-out' proteins depends on both the ionic strength and type of cations and/or anions present, as described according to the Hofmeister series [Anions: SO_4^{2-} > HPO_4^{2-}> acetate$^-$ > Cl^- > NO_3^-; Cations: $N(CH_3)_4^+$> NH_4^+> Na^+ = K^+ > Li^+ > Mg^{2+}] [84].

Salts formed between cations and anions with higher precipitation ability in the series decrease the solubility of non-polar amino acids, favoring hydrophobic interactions to 'salt-out' proteins. On the contrary, salts formed between cations and anions with lower precipitation ability in the series weaken the hydrophobic interactions and result in increasing solubility of non-polar amino acids, thus favoring the 'salting-in' process [85]. Broadly speaking, ammonium sulfate $(NH_4)_2SO_4$ and sodium chloride (NaCl) are the most commonly used salts for research purposes [16, 86–88]. Typically in the salt extraction procedure, proteins are initially dissolved in an aqueous NaCl solution (0.3–0.5 M) [86, 88] at neutral pH, followed by a clarification procedure to remove insoluble material. Precipitation of the protein can be triggered by either diluting the supernatant with water to lower the ionic strength or by dialysis to remove the salts, resulting in the formation of protein micelles which grow in size and number until precipitation ensues. Alsohaimy et al. [87] prepared protein isolates from chickpea, lupin and lentil using IEP and ammonium sulfate precipitation. For all of these legumes, the latter method resulted in higher protein content (chickpea – 90.6%, lupin – 92.6%

and lentil − 93.0%) in comparison to the former method (chickpea − 81.4%, lupin − 87.3% and lentil − 80.0%). On the contrary, Can Karaca et al. [16] produced isolates from chickpea, faba bean, pea and lentil using IEP and a salt extraction method and found that the protein levels obtained using the IEP method (chickpea − 85.4%, faba − 84.1%, pea − 88.8%, and lentil − 81.9%) were found to be higher than the ones produced by the salt extraction method (chickpea − 81.6%, faba − 82.0%, pea − 81.1%, and lentil- − 74.7%) [16].

4. Functional properties of pulse proteins

Protein flours, concentrates and isolates can be incorporated into various foods to increase their nutritional value and/or to provide specific and desirable functional attributes [5]. These functional attributes may include solubility, gelation, emulsifying ability, oil and water absorption capacity, and foaming. Moreover, functional properties of legume proteins contribute an important aspect in determining the competitiveness of the protein ingredient or the product in the market, as they can impact the sensory, physical and chemical properties of a food, which includes texture and organoleptic characteristics. In the literature, the functional attributes of legume proteins vary considerably due to differences in the raw material, processing, extraction methods and environmental conditions used during testing.

4.1. Solubility

Protein solubility plays a major role in various food applications as a number of functional properties such as foaming, gelation or thickening, and emulsification are closely related and often dependent on protein solubility. High protein solubility may be helpful in producing food products such as beverages, infant milk powder, imitation milk and other products which require instant solubility with no residues left. For instance, imitation milk produced using lentil protein isolate was reported to have the same quality as compared to milk prepared from soy protein isolate, however had a lower quality than when pea protein isolate was used [21]. The solubility of protein depends on various attributes including hydrophobic/hydrophilic balance of the protein molecule (mainly the surface composition: polar/non polar amino acids), pI, pH, temperature, ionic strength and the type of ions present in the solution [63]. Proteins exhibit minimum solubility at their pI because of a zero net surface charge, resulting in aggregation of protein molecules into larger structures, followed by precipitation. On the contrary, when the pH values are greater or less than the protein's pI, proteins exert a positive or negative net charge into solution, repelling one another to maximize solubility.

The solubility profiles of concentrates and isolates from various pulses obtained by IEP or UF were found to be lowest between pH 4 and 6, and significantly increased with pH shifting to either more acidic or alkaline conditions [63]. Boye et al. [66] reported that the solubility of pea, chickpea and lentil protein concentrates, which were processed using IEP and UF/DF techniques, were highest at pHs 1–3 and pHs 7–10. Moreover, the solubility profile varied with different varieties where, UF-yellow pea and UF-red lentil concentrates had the highest solubility at neutral pH, while at pH 3 and 8–10 solubility was highest for only UF-red lentil.

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In both cases, the lowest solubility was found for UF-chickpea (desi). The study by Can Karaca et al. [16] on five different legumes (pea, chickpea, faba bean, lentil and soybean) showed higher overall solubility (determined at neutral pH) of these legume isolates prepared by the IEP method (85.9%) as compared to ones prepared by a salt extraction method (61.5%). For the IEP method, the pea protein isolate had the lowest solubility (61.4%); soybean isolates had the highest solubility (96.5%); and pea, lentil and chickpea isolates exhibited intermediate solubility (>90.0%). However, highly variable results were obtained for the solubility of salt-extracted isolates with values of 30.1% and 96.6% for chickpea and soybean respectively, while intermediate solubility was observed for lentil (89.8%), pea (38.1%), and faba bean (52.5%). Solubility profile of isolates produced from kabuli (PBG-1, PDG-4, PDG-3, GL769 and GPF-2) and desi chickpea cultivars (L550) were found to be non-significant as a function of genotype ($p>0.05$) [89]. However, in the study of Barac et al. [38], the solubility profile of six pea genotypes (Maja, Calvedon, Miracle, L1, L2 and L3) were found to be significantly different from each other except L2 and Maja ($p<0.05$).

4.2. Oil holding and water hydration capacities (OHC, WHC)

OHC and WHC refer to the extent to which oil and water, respectively, can be bound per gram of the protein material or legume flour [5, 63]. These properties are essential with respect to maintaining the quality of a product, its shelf life and consumer acceptability (texture and mouth feel). The ability of a protein to bind oil and water is important in preventing cook loss or leakage from the product during processing or storage [63]. Failure of a protein to bind water could lead to brittle and dry characteristics of the product [5]. WHC values for pulse protein concentrates, such as pea, faba bean, lentil and chickpea, have been determined by various groups [66, 89, 90] and fall in the range of 0.6–4.9 g/g, suggesting that both pulse genotype and manner of processing could impact values. For instance, Kaur and Singh [89] found that protein isolates prepared by kabuli chickpea cultivars (PBG-1, PDG-4, PDG-3, GL769 and GPF-2) produced significantly lower WHC than desi chickpea (L550) ($p<0.05$) which clearly indicates the impact of different cultivars in assessing functionality. Boye et al. [66] reported that for all the legumes studied (red and green lentil, desi and kabuli chickpea, yellow pea), IEP protein concentrates had higher WHCs than did ones prepared by UF (with the exception of red lentil protein concentrates) although no substantial differences were observed between WHC values between the processing treatments. The yellow pea concentrate (IEP) had the highest WHC value which was much higher than those of the kabuli and desi chickpea concentrates (IEP and UF) indicating the more significant effect of pulse type compared to extraction method on WHC.

OHC values reported by various authors [86, 89, 90] for different pulses range from 1.0–3.96 g/g, and seem to depend again on the type and variety of pulse used, and the method of preparation of the protein product. Boye et al. [66] studied the UF and IEP concentrates produced from red and green lentil, yellow pea and kabuli and desi chickpea. They reported that pulse variety and processing conditions had a larger impact on the OHC of yellow pea, kabuli chickpea and red lentil concentrates as compared to those made from desi chickpea and green lentil. Moreover, UF concentrates made from yellow pea, red lentil and kabuli chickpea

had significantly higher OHC than their corresponding IEP concentrates. Red lentil and yellow pea concentrates produced by UF had the highest OHC of 2.26 g/g and 1.17 g/g respectively. However, no significant differences in OHC were observed between the IEP produced concentrates ($p>0.05$) [66]. In the study of Kaur and Singh [89], chickpea protein isolates were reported to have higher OHC than the corresponding flour samples. Moreover, in contrast to WHC, the OHC of kabuli chickpea was reported to be significantly higher than desi cultivars ($p<0.05$).

The water and oil holding properties of legume proteins may be essential in formulation of food products such as meat, pasta, cookies, etc. In producing low fat meat products, water is added to substitute the fat loss. And, water holding compounds are added to prevent cooking losses and meat shrinkage which includes proteins (whey, soy and collagen), lipids (soy lecithin) and carbohydrates (flours, starches and gums) [91]. For instance, soy proteins added to ground beef improves the tenderness, moisture retention, decreases cooking losses, and inhibits rancidity [92]. Deliza et al. [93] replaced meat in ground beef mixture with hydrated textured soybean protein (15 or 30%) and found that beef patties were more tender as compared to controls, although the overall flavor quality was reduced with having less beefy flavor. However, legumes (navy beans, chickpeas, mung beans and, red kidney beans) when substituted at a level of 15% in beef mince resulted in acceptable products, with chickpea preferred over other legumes [94].

4.3. Emulsification

An emulsion is a mixture of two or more immiscible liquids (usually oil and water), where one of the liquids (the dispersed phase) is mixed in to the other (the continuous phase) in the form of small spherical droplets [95]. Emulsions are generally classified into two types: oil-in-water (O/W), in which oil droplets are dispersed within an aqueous phase (e.g., milk, mayonnaise, cream and soups); or water-in-oil (W/O), in which water droplets are dispersed within an oil phase (e.g., butter and margarine). Emulsions are thermodynamically unstable and with time separate into oil and liquid layers due to collision and coalescence of droplets [95]. Stabilizers such as emulsifiers can be used to produce stable emulsions. For instance, protein as an emulsifier acts by adsorbing onto the oil-water interface to form a viscoelastic film surrounding the oil droplets. Stability is enhanced through electrostatic charge repulsion (depending on the pH), steric hindrance or increases to the continuous phase viscosity [95].

Protein emulsifiers are used worldwide because of their ability to adsorb at the droplet surface in an O/W emulsion during the process of homogenization, thereby reducing interfacial tension. The adsorbed protein molecules present at the surface act as a separating membrane preventing coalescence with the neighboring droplets [63]. To be an effective emulsifier, protein must exhibit the following properties: fast adsorption at the oil-water interface, ability to form a protective and cohesive layer around the oil droplets, and ability to unfold at the interface [96]. Various studies reported that the emulsifying ability of legume protein concentrates or isolates are dependent on the type of legume or the method (IEP/UF/salt extraction) used in their preparation. For instance, Fuhrmeister and Meuser [71] reported that a pea

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protein isolate prepared by an IEP method was found to have lower emulsifying ability as compared to one prepared using UF.

Emulsion activity index (EAI) refers to the area of emulsion stabilized per gram of emulsifier or protein material and expressed as m^2/g whereas emulsion stability index (ESI) refers to the measure of stability of this emulsion as a function of the time. Emulsion capacity (EC) is the amount of oil homogenized per gram of protein material and expressed as g oil/g protein whereas creaming stability (CS) is the ability of an emulsion to resist creaming and the formation of a serum layer as time passes, and measured as %. The study conducted by Can Karaca et al. [16] on different legumes (pea, chickpea, faba bean, soybean and lentil) showed that both legume source and extraction method (IEP or UF) had significant effects on emulsifying and physicochemical properties. Both EAI and ESI were significantly affected by legume source and extraction method, whereas EC was dependent on the legume source only. However, Boye et al. [66], studying the functional properties of chickpea, lentil and pea protein concentrates, concluded that IEP and UF preparation methods had little impact on emulsifying properties. Barac et al. [38] studying functional properties of six pea genotypes reported significant differences in emulsifying properties (EAI and ESI) as a function of Genotype and pH. The EAI of pea genotypes tested in this study was significantly higher than the commercial pea protein isolates tested.

Emulsifying and other functional properties of proteins can also be improved with protein modifications such as limited enzymatic hydrolysis using proteases (e.g. trypsin). The hydrolysis reaction results in partial unraveling of protein molecules thus exposing more ionic and hydrophobic groups for interaction with oil droplets [97]. For instance, trypsin treated oat bran protein with a ~4–8% degree of hydrolysis (DH) had improved solubility, water holding, foaming and emulsifying properties as compared to those of native proteins [98]. On the contrary, Avramenko et al. [99] reported detrimental effects of trypsin mediated hydrolysis (DH~4–20%) of lentil protein isolates. Here, except zeta potential, all the physicochemical properties (surface hydrophobicity and interfacial tension) and emulsifying properties (emulsion activity and stability indices) were found to have lower values as compared to the unhydrolyzed lentil protein isolate. This suggests that processing conditions might have specific effects dependent on protein source.

Legume proteins play a vital role in the formulation of a number of novel foods (such as sausages, bologna, meat analogues, cakes and soups) by formation and stabilization of emulsions. Meat analogues are foods which are made from nonmeat ingredients, structurally similar to meat and may have the same texture, flavor, appearance, and chemical characteristics [100]. Some of the traditional foods such as wheat gluten, rice, mushrooms, tofu and legumes when added with flavors mimic the finished a meat products such as chicken, beef, sausage etc. [100]. Soybean protein is an important meat analogue since it has meat like texture and provides a similar amino acid profile to meat proteins [100]. Tofu is a widely consumed meat analogue made from soy, which provides a good source of protein, calcium and, iron. In general, the market for meat analogues is large and includes vegetarians, vegans, and people who do not eat meat products because of religious or cultural practices.

4.4. Foaming

Similar to emulsions, foams also have two immiscible phases (aqueous and gas), and require an energy input to facilitate their formation. Foams are comprised of a dispersed gas phase within a continuous aqueous phase [96]. Proteins in solution adsorb to the gas-liquid interface in a similar manner as in emulsions to form a viscoelastic film surrounding the gas bubbles that helps resist rupturing and bubble fusion [63]. In contrast to emulsions, the major driving mechanism associated with foam instability is associated with Oswald ripening, which involves the diffusion of small gas bubbles through the continuous phase in order to become absorbed into a larger gas bubble [96]. Rupture of the viscoelastic film leads to drainage of the continuous liquid phase through the film matrix. Various food products are available which use protein as a stabilizer including meringues, whipped desserts, mousses and leavened bakery products [101]. Vose [69] reported that the foaming properties of faba bean and yellow pea isolates, prepared using UF, were higher than that of skim milk powder, wheat flour and soy protein isolates. A faba bean isolate was observed to have better foaming properties than pea protein isolate.

Foaming capacity (FC) refers to the volume of foam generated after homogenization of a certain amount of protein solution whereas foam stability (FS) refers to the ability to retain foam structure and resistance in the formation of serum layer as a function of time. In the study of Sathe and Salunkhe [102] on great northern bean (*Phaseolus vulgaris* L.) protein materials, the FCs were in the following decreasing order: albumins (180%) > protein concentrate (164%) > globulins (140%) ~ egg albumin (140%) > flour (132%) > isolate (106%), where egg albumin was the standard for measuring foaming capacity. These results indicated that all great northern bean protein materials except the isolate, had FCs that were comparable to or higher than that of egg albumin. However, the foaming stabilities were as good as egg albumin, and hence the overall foaming ability was given only a fair mark [5, 102]. Boye et al. [66] studied and compared the functional properties of yellow pea, green and red lentil, and kabuli and desi chickpea protein concentrates prepared using IEP and UF techniques. In their studies, they found that foaming capacity (which ranged from 98% to 106%) was similar for pea and lentil protein concentrates irrespective of extraction method used. However, the desi and kabuli chickpea concentrates prepared by the IEP method showed higher foaming capacity than the others. In general, it was observed that chickpea showed higher foaming capacity and expansion but lower foam stability as compared to the other sources. Furthermore, variability was observed in foaming stability with kabuli and desi chickpea and green lentil concentrates prepared by the IEP method having higher foam stability values compared to concentrates prepared by the UF method. Barac et al. [38], studying the functional properties of isolates produced from six pea genotypes using the IEP method, reported significant differences in their foaming properties as a function of genotype and regardless of changes in pH. Generally, a low foam stability was observed probably because of the low concentration of protein used in the formation of the protein solution. However, foaming capacity was highest for Maja cultivar, which was significantly higher than the commercial pea protein isolate.

5. Applications

Nowadays, there has been a growing interest by the food industry towards utilizing pulse proteins in novel products due to their nutritional value, availability, low cost, desired functional properties and beneficial health effects [3]. Pulse protein concentrates and isolates are being applied in many food products such as beverages, imitation milk, baby foods, bakery products, meat analogs, cereals, snack foods, bars, and nutrition supplements. Examples of some of the food applications of pulse proteins from literature offering opportunities for novel product development are presented in **Table 1**. Pulse proteins are also used in non-food applications such as microencapsulation of bioactive ingredients. Pulse proteins can serve as good encapsulating agents due to their amphiphilic nature, ability to stabilize oil-in-water emulsions and film forming abilities. Some of the current examples of pulse protein-based microcapsules include: alpha-tocopherol [103], polyunsaturated fatty acids-rich oil [104] and conjugated linoleic acid [105] encapsulated with pea protein, flaxseed oil encapsulated with chickpea or lentil protein [106], *Bifidobacterium adolescentis* [107] and folate [108] encapsulated with chickpea protein.

Pulse protein	Application	Protein Conc'n (%)	Outcome	References
Chickpea	Pasta	5–15	Quality characteristics of the cooked pasta were not affected by increasing protein content.	[109]
Chickpea, faba bean, lentil, mung bean, smooth pea, pea, and winged bean	Bean curd	2.3–3	Chickpea and faba beans had comparable textural properties to soybean.	[110]
Lentil and white bean	Cake	3	Lentil and white bean protein extracts tested were found to be suitable to replace soy and pea in bakery products.	[111]
Pea protein	Gluten-free bread	1–6	Pea protein addition improved rheological and structural properties of the dough.	[112]
Lupin	Bread	5–10	Lupin protein addition increased the dough development time, stability and the resistance to deformation and the extensibility of the dough.	[113]
Lupin	Fermented sausage	2	Products containing lupin protein showed no difference in firmness, appearance and color compared to control.	[114]
Pea and sweet lupin (cross-linked)	Sausage-like vegetarian substitute	9	Sensory profile and textural properties were overall accepted.	[115]

Table 1. Some examples of food applications of pulse proteins.

6. Challenges for pulse protein ingredients

Application of pulse protein ingredients in food products is limited due to the formation of a green or beany off-flavor during storage [116]. The most potent odor-active volatiles have been identified in soy protein. One of the key off-flavors in soy protein is reported to be *n*-hexanal,

which is a degradation product of linoleic acid. Fermentation with *Lactobacillus* or *Streptococci* strains was suggested to overcome this hurdle [117]. In the case of pulse proteins, Murat et al. [118] showed that the flavor profile is evolving during the extraction process from pea flour to pea protein extract. The odor active compounds were found to be different between pea flour and pea protein powder. Schindler et al. [116] identified 23 highly odor-active compounds in pea protein extracts including *n*-hexanal, 1-pyrroline, dimethyl trisulfide, 1-octen-3-one, 2,5-dimethyl pyrazine, 3-octen-2-one, *β*-damascenone, and guaiacol. The authors suggested that lactic acid fermentation improved the aroma of pea protein extracts by decreasing the *n*-hexanal content and reducing or masking off-flavors.

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