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Pulse proteins are novel ingredients to develop functional foods and beverages

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Abstract
Pulses are dried edible seeds found in the Leguminosae family plants, including the common bean, chickpea, dry pea, lentil, cowpea, mung bean, *faba* bean, lupins, among others. Pulses have been identified as a nutritious and inexpensive alternative source of protein. Pulses are also considered the most suitable for preparing protein ingredients (concentrates and isolates) because of their high protein content, wide acceptability and low cost. In addition, pulse proteins exhibit functional properties (foaming and emulsification, water and fat absorption and gelation) as well as nutraceutical/health benefiting-properties which makes them healthier and low cost alternative to conventional protein sources like soy, wheat and animals. The functional properties of pulse proteins have been exploited in the preparation and development of products such as bakery products, soups, extruded products and ready to eat snacks. This review provides an overview of the characteristics of pulse proteins, current and emerging techniques for their fractionation, their major functional properties and opportunities for their use in various applications.

Keywords: Pulses pulse protein, concentrates, isolates, application, functional properties

1. Introduction
Pulses are dried edible seeds found in the Leguminosae family plants, including the common bean, chickpea, dry pea, lentil, cowpea, mung bean, *faba* bean, lupins, among others. They are cultivated worldwide under a wide variety of growing conditions. Pulses constitute an important source of dietary protein for large segments of the world's population particularly in those countries in which the consumption of animal protein is limited by no availability or is self-imposed because of religious or cultural habits (Liener, 1962) [17]. Pulses provide energy, dietary fibre, protein, minerals and vitamins required for human health. Recent research studies suggest that consumption of pulses may have potential health benefits including reduced risk of cardiovascular disease, cancer, diabetes, osteoporosis, hypertension, gastrointestinal disorders, adrenal disease and reduction of LDL cholesterol (Hu, 2003 [15]; Jacobs & Gallaher, 2004; Philanto & Korhonen, 2003 [23]; Tharanathan & Mahadevamma, 2003) [36].
These pulses contain 20-30% proteins which are stored in the seed cotyledons as small spherical protein bodies. Protein content of different pulses varies with genotypes, germination, environmental conditions and application of fertilizers during growth and development (Singh 2017) [29]. The pulse proteins have essential amino acids composition complementary to cereals and are naturally gluten-free, thus, safe for the patients suffering from gluten intolerance/allergies.
Pulse protein ingredients must possess good functional properties, such as solubility and emulsifying properties, the utilization of right individual functional properties might be useful in producing different food products such as cakes, biscuits, beverages and breads. Proteins from different pulses vary in composition and structure and have different functional properties. Keeping the above in view, the present article attempts to review the composition, structure, functional properties and current applications of pulse proteins.

2. Composition of pulse proteins
Pulse proteins chiefly comprise of globulins which are soluble in salt solutions and albumins (soluble in water) while protamine (soluble in alcohol) and glutelins (soluble in dilute acid/base) are minor proteins and constitute a small portion, generally less than 5%. The ratio of albumins to globulins vary between 1:3 and 1:6.3 amongst different pulses (beans, lentils, black gram and chickpea) (Gupta and Dhillon 1993) [14].

Albumins primarily comprise metabolic proteins and include enzymatic and non-enzymatic proteins. They constitute only 10–20% of the total seed proteins. Pulse albumins are generally low in molecular weight (MW; 5-80 kDa) and higher in cysteine and methionine content than pulse globulins (Boulter and Croy 1997 [4]; Boye *et al.* 2010) [5]. Although, albumins are the most nutritive proteins in pulse seeds in terms of amino acid composition (Boulter and Croy 1997) [4], they may also contain some anti-nutritional/bioactive constituents e.g. trypsin and/or chymotrypsin inhibitors, hemagglutinins/lectins, and amylase inhibitors (Boye *et al.* 2010) [5]. Globulins, in general, account for 70-80% of seed proteins and function primarily as storage proteins. Legumins and vicilins are major globulins/storage proteins in pulse seeds. Based on their different sedimentation coefficients, they are also referred as 7S globulins and 11-12S globulins, respectively. They were present in ratios of 10.5:1, 1:6-9, 1:9, 1-3:1 and 4-6:1, respectively in lentils, French beans, cowpeas, peas and chickpeas (Gupta and Dhillon 1993) [14]. Legumins, generally, have higher amount of sulfur-containing amino acids (methionine and cysteine) than vicilins (Boulter and Croy 1997) [4].

3. Importance of pulse's proteins

Proteins have many functions. They are used in building tissues for growth and are also out or damaged tissue, muscles, skin, hair etc. contain large proportions of proteins. Cellular structures contain proteins. As children grow new cells and tissues are formed that need proteins, so proteins are so important for children. Many hormones such as insulin have proteins and they regulate body composition. Antibodies are proteins are important in immunity. Proteins are also needed for transporting O_2, fat etc. Proteins are involved in clotting of blood and finally proteins are also source of energy. Thus proteins are important for all especially for growing children.

4. Techniques for processing pulse protein concentrates and isolates

Generally, if high-protein pulse ingredients are required for food formulations, a protein enrichment or isolation process beyond dehulling/milling will be required. Typically, high protein fractions from pulses are termed concentrates or isolates, depending on the protein content.

4.1 Air classification

Air classification has been used with a variety of different pulses, including pea, *faba* bean and lupin (Pelgrom, Berghout, van der Goot, Boom, & Schutyser, 2014 [21]. Dehulling of seeds may be carried out prior to further processing. Advantages of this include reduction of antinutritional factors (ANFs), removal of bitter/astringent components, and improved colour. Dehulling may also result in a slight increase in protein content of the seeds (Saldanha do Carmo *et al.*, 2020) [24].

The principle behind air classification is based on the separation of particles in an air stream based on their size and density (Sozer, Holopainen-Mantila, & Poutanen, 2017) [30]. Pulses must be milled finely enough that cells are disrupted, allowing separation of starch granules from protein bodies. Starch granules should be liberated with minimal damage, while the protein matrix is ground to smaller particles (Pelgrom, van der Goot, & Boom, 2015) [22]. Impact milling or jet milling may be used to achieve this (Pelgrom, Vissers, Boom, & Schutyser, 2013).

An overview of the milling and air classification process is shown in Fig. 1. Rotor-type classifiers are generally used for air classification of finely milled flours. The flour is dispersed in an air stream, and is then passed to a rotating classifier wheel, where small and large particles are Air classification is considered more sustainable than aqueous fractionation, due to far lower energy and water demands, and also does not require a drying process, or the addition of chemicals which are necessary in some aqueous processes (Pelgrom *et al.*, 2013; Vogelsang-O'Dwyer *et al.*, 2020) [38]. In addition, air classified protein concentrates may retain more native conformation, and consequently better functionality compared to some protein isolates due to the milder processing conditions involved (Pelgrom *et al.*, 2013; Vogelsang-O'Dwyer *et al.*, 2020) [38]. A potential disadvantage of air classification is the lower achievable protein content compared to aqueous processing. Air classified protein concentrates from various pulses have been reported with protein contents in the range of 49-70% of dry matter (Schutyser *et al.*, 2015) [22].

4.2 Aqueous fractionation

Aqueous fractionation involves the extraction of protein from either flaked or milled pulses in an aqueous solvent, followed by recovery/isolation of proteins. A de-fatting step may be carried out before extraction, depending on the type of pulse used (Boye, Aksay, *et al.*, 2010) [6]. Sometimes an air classified high protein fraction is used as the starting material (Sumner, Nielsen, & Youngs, 1981) [32]. The protein extract is usually dried to facilitate storage and transport. While for dry fractionated concentrates, the protein content is generally less than 70% of dry matter, aqueous processes generally result in higher protein content compared to dry fractionation, often 80-90%. Although the protein content may be lower in some cases, for simplicity all ingredients described in this section will be referred to as isolates. An outline of the processes mainly used to produce pulse protein isolates is shown in Fig. 2.

4.3 Isoelectric precipitation

Isoelectric precipitation (IEP) is the most commonly used method for production of pulse protein isolates and has been used with a wide variety of different pulses (Karaca, Low, & Nickerson, 2011) [16]. This method takes advantage of the different solubility of pulse proteins depending on the pH environment. The lowest solubility is observed near the isoelectric point, pH ~4-5. At higher or lower pH values, away from the isoelectric point, the protein solubility is higher, where the proteins gain a net negative or positive surface charge (Karaca *et al.*, 2011) [16]. Most commonly, pulse proteins are extracted in mild alkaline solution, and subsequently recovered by IEP. The extraction pH, usually achieved with addition of NaOH, is typically in the range of 8-11, but may also be higher (Boye, Aksay, *et al.*, 2010 [6]; Liu, Damodaran, & Heinonen, 2019) [19]. Extraction pH, temperature and time as well as flour/solvent ratio may be optimised to deliver maximum yield and/or protein content. As well as the extracted protein, this mixture contains insoluble seed material including starch and insoluble fibres, which must be removed using filtration/sieving or centrifugation. The protein is then precipitated with the

addition of acid such as HCl, typically around pH 4–5 where the solubility of the majority of the proteins is minimal (Boye, Zare, & Pletch, 2010) [5]. The precipitated protein must then be separated from the supernatant, typically using centrifugation. The purity of the protein sediment may be increased by washing steps with water or acid solution. The recovered protein is then resuspended, most often neutralized with alkali addition, and a heat treatment step may be carried out to improve microbial quality (D'Agostina *et al.*, 2006) [10]. The liquid protein isolate is typically dried, to give a product which can be stored for later use. At laboratory scale, freeze-drying followed by milling may be used, whereas at pilot or industrial scale, spray-drying is typically used (Burger & Zhang, 2019; Chen *et al.*, 2019). The smaller fraction of acid soluble proteins remaining in the supernatant after IEP (rich in albumins), may be processed separately, isolated using ultrafiltration/diafiltration (UF/DF), to give an acid soluble protein isolate. This has been carried out in a lupin protein isolation process, and was referred to as 'type F' lupin protein isolate due to its excellent foaming properties (D'Agostina *et al.*, 2006) [10].

While alkaline extraction is most commonly used in IEP processes, it is also possible to carry out the extraction at neutral or acid pH. While alkaline extraction is used to increase the amount of protein solubilized by increasing the negative charge on the proteins, depending on the type of pulse used, it may be possible to extract a high proportion of protein at pH 7. This allows for milder extraction conditions and less chemical addition. The usefulness of this is apparent as increasing extraction pH may have a negative effect on functionality (Arntfield & Maskus, 2011).

Neutral extraction has been used in the preparation of various protein isolates including lupin (D'Agostina *et al.*, 2006) [10], and *faba* bean protein isolate while extraction at pH 7.5 has been used for lentil protein isolate (Alonso-Miravalles *et al.*, 2019) [2]. Acid extraction of proteins has also been employed, i.e. where the pH is lowered to below the isoelectric point. In the lower pH range below the isoelectric point, e.g., pH 2-3, high solubility of pulse proteins may also be observed, as the proteins carry a net positive charge. The process is similar to alkaline extraction/IEP described above, except the initial extraction pH is in the lower range. It has been reported that this technique can be used to produce products with better sensory properties compared to alkaline extraction (Nickel, 1981), along with the deactivation of lipoxygenase at low pH (Swanson, 1990) [33]. While less common, this approach has been used for pulse proteins, including pea (Naczk, Rubin, & Shahidi, 1986) and *faba* bean protein isolate (Vogelsang-O'Dwyer *et al.*, 2020) [38].

4.4 Ultrafiltration

Ultrafiltration (UF) with diafiltration (DF) is another technique which is used for pulse protein isolation. The protein extraction and fibre/starch removal steps are similar to those described for IEP. The protein extract is passed through UF membranes which are designed with a pore size such that proteins are retained while smaller soluble components, such as oligosaccharides, are removed (Arntfield & Maskus, 2011). The isolate can then be spray-dried or freeze-dried similarly to IEP isolates. There are several potential advantages of UF compared to IEP processes. These include the retention of a more complete protein fraction of the extract, including albumins, whereas IEP preferentially recovers the globulins (Arntfield & Maskus, 2011; Boye, Zare, & Pletch, 2010) [5]. The proteins recovered using UF may also retain more native structure as extremes of pH are not necessary in the process. In addition, UF isolates tend to have lower ash and sodium content, as the neutralization step using alkali such as NaOH is not required (Alonso-Miravalles *et al.*, 2019) [2]. Ultrafiltration may also result in higher protein content depending on the process; Boye, Aksay, *et al.* (2010) [6] achieved consistently higher protein contents for UF protein isolates compared to IEP protein isolates, using various types of pea, lentil and chickpea as the input material.

4.5 Salt extraction/micellization

In this technique, proteins are extracted from seed material in a salt solution such as 0.5 M NaCl at neutral pH. Following removal of starch/insoluble fibre, the protein extract is diluted with cold water (Paredes-Lopez, Ordorica-Falomir, & Olivares-Vazquez, 1991). The dilution causes the proteins to precipitate due to the change in ionic strength. The term micellization is used as the proteins precipitate in the form of micelles (Muranyi, Otto, Pickardt, Koehler & Schweiggert-Weisz, 2013; Paredes-Lopez *et al.*, 1991). The precipitated protein may then be recovered by centrifugation, washed, resuspended and spray dried. The resulting isolates may differ from IEP isolates in terms of appearance and functionality (Muranyi *et al.*, 2013). Similarly to UF, the micellization process has the advantage of a milder process with less extreme pH changes, and therefore potentially less protein denaturation during the process (Muranyi *et al.*, 2016).

5. Functional Properties of Pulse Proteins

Pulses are good sources of protein and have a balanced amino acid profile and superior protein digestibility. In addition the protein and protein isolates from pulses have excellent functional properties that make them useful ingredients in innovative food product development. Examples of such functional properties include solubility, water and oil binding capacity, foaming and gelation and viscosity imparting properties. The functional properties of pulse proteins have been exploited in the preparation and development of products such as bakery products, soups, extruded products and ready to eat snacks (Boye and Pletch, 2010) [5].

The excellent emulsification properties of pulse proteins and protein isolates is attributed to the presence of hydrophilic amino acids that promotes formation of "oil in water" emulsions. Specifically, in pulse proteins, water-soluble albumins, and salt-soluble globulins predominate (Macrone *et al.*, 1998), with prolamins and glutelins present in smaller concentrations. Globulin proteins contribute most to the functionality in food products. Some of the functional characteristics of pulse proteins that needs to be considered for product development of different value added products are:

5.1 Protein solubility

Protein solubility is an important factor influencing product functionality and effective utilization. The solubility of most pulse proteins is highest at low acidic and high-alkaline pH values. Lowest solubility is observed at pH values close to the isoelectric point. This high solubility at acidic pH values could make pulse PIs very promising candidates for use in acidic and neutral beverages as well as in soup and salad dressing applications (Boye *et al.*, 2010a [6], 2010b).

Opportunities may exist to enhance the solubility of pulse protein flours, concentrates and isolates through hydrolysis and physicochemical modification. For example, interactions of *faba* bean (*Vicia faba* L.) legumin protein and hydrolyzed legumin with polysaccharides (chitosan) increases its solubility at the isoelectric point and at higher pH values (Braudo *et al.*, 2001). Thus, processing techniques that enhance pulse protein solubility could be explored to enhance application of pulse protein in foods requiring greater solubility.

5.2 Water-holding capacity

Water-binding capacity (WBC), water-absorption capacity (WAC) and water-holding capacity (WHC) are the terms often used to describe the hydration properties of pulse protein and refer to the amount of water that can be absorbed per gram of sample material. These terms are frequently used interchangeably, although the measurement methods used may differ. WBC is an important measurement in food processing applications. Materials that have a low WBC may not be able to hold water effectively, whereas materials that have a high WBC or WAC may render food products brittle and dry, especially during storage (Boye *et al.*, 2010a) [6].

The role of proteins in water absorption is equally important. WHC values for different pulse protein concentrates range between 0.6 and 2.7g^{-1} (Boye *et al.*, 2010a) [6]. Interestingly, pea PI, which is the most readily available commercial pulse protein isolate, was found to have higher swelling ability than whole seed FL our and fi ber products, suggesting that proteins also contribute to swelling in seed FL ours, especially when the product is heated (Torruco-Uco and Betancur-Ancona, 2007; Agboola *et al.*, 2010). Kaur and Singh (2007) found that protein isolates from different chickpea cultivars had higher WHC compared to the whole flours. WBC of whole pulse flour may, however, sometimes be higher than that of other derived flour products depending on processing and product composition. In another study, pea PI processed using dehulled seed had lower WHC values compared to whole seed FL our perhaps owing to its lower levels of starch and Fiber compared to fibre products or whole flour, where the FL our is milled from whole seeds containing hulls (Agboola *et al.*, 2010).

5.3 Oil-absorption capacity

Fat- or oil-absorption capacity (FAC or OAC), also sometimes referred to as the fat- or oil-binding capacity (FBC or OBC), or fator-oil-holding capacity (FHC or OHC), is calculated as the weight of oil absorbed per weight of sample. FAC varies for different pulse FL ours and fractions usually ranging from ≈ 0.4 to 5g^{-1}. Very few studies have systematically looked at the effect of processing on the FAC of pulse FL ours and fractions. The mechanism of fat absorption involves physical entrapment of oil. Thus, particle size, pulse Flour composition, moisture content, microstructure are some of the factors that can impact FAC. Additionally, different protein compositional profiles and amounts of non-polar amino acid residues as well as differences in conformational features, and starch–protein–lipid binding could contribute to differences in oil retention characteristics in pulse flours (Lazou and Krokida, 2010). FAC of pulse protein flours may also vary depending on the source and the conditions used for processing. Among pulse concentrates treated by ultrafiltration (UF), red lentil concentrate has the highest FAC, followed by yellow pea, green lentil, kabuli chickpea, and desi chickpea, however, no significant differences were observed between the FAC of concentrates processed using isoelectric precipitation (IEP) (Boye *et al.*, 2010a) [6]. FAC of chickpea protein isolate (PI) processed by micellization was 2g^{-1} but was only 1.7g^{-1} for PI prepared by IEP (Paredes-López *et al.*, 1991).

5.4 Gelling properties

Gelation occurs when proteins and starches form a three-dimensional network that is resistant to flow under pressure. This ability to form gels is an important functional property in food processing and food formulation for products such as puddings and jellies and in many dessert and meat applications. Gelling capacity is frequently measured by the least gelling concentration (LGC), which may be defined as the lowest concentration required for a self-supporting gel to form. A lower LGC indicates that the sample has a better capacity to form gels.

For pulses protein extracts as an example, protein extracts produced using ultrafiltration had better gelling properties (i.e. lower LGC) than extracts produced using isoelectric precipitation. Differences between pulse flours and fractions as a result of pulse type, variety, conditions used for processing and drying, product purity and composition of the extracts, as well as storage conditions can influence gelling properties (Boye *et al.*, 2010a) [6].

5.5 Emulsifying properties

Emulsifying properties of pulse flours can be attributed mostly to the protein components of pulses. Proteins act as emulsifiers by forming a fi lm or skin around oil droplets dispersed in an aqueous medium, thereby preventing structural changes such as coalescence, creaming, flocculation, or sedimentation. The emulsifying properties of proteins are therefore affected by the hydrophobicity/ hydrophobicity ratio of the proteins and by structural constraints that determine the ease with which the proteins can unfold to form a fi lm or skin around dispersed oil droplets. Emulsifying properties are generally evaluated by two indices, namely emulsifying activity (EA) and emulsifying stability (ES). In a simplified system, EA measures the amount of oil that can be emulsified per unit of protein, whereas ES measures the ability of the emulsion to resist changes to its structure over a defined time period (Boye *et al.*, 2010a) [6].

There is great variability in the emulsifying properties reported for different pulse flours and protein fractions. Pulse varieties and fractions having higher amounts of vicilin proteins are likely to have better emulsifying properties (Dagorn-Scaviner *et al.*, 1987). Albumin proteins of great Northern bean were found to be better emulsifiers than the globulins, and the total globulin and 7S fractions of pea varieties had better emulsifying properties and higher surface hydrophobicity (S0) than the albumin and 11S fractions (Cserhalmi *et al.*, 1998). Techniques such as hydrolysis as well as processing conditions can impact the emulsifying properties of foods. Hydrolysis improved the EA of pea PI by approximately twofold with a maximum occurrence at a degree of hydrolysis (DH) of 3.7. Similarly, hydrolysis plus interaction with chitosan improved the ES of *faba* bean (Braudo *et al.*, 2001).

5.6 Foaming properties

Another characteristic of proteins is that they exhibit foaming properties. The most frequently used indices for measuring foaming properties are foam expansion (FE), foaming capacity (FC), and foam stability (FS). Foams are formed when proteins unfold to form an interfacial skin that keeps air bubbles in suspension and prevents their collapse. Foam formation is important in food applications such as beverages, mousses, meringue cakes, and whipped toppings (Boye *et al.*, 2010b). There is great variation in the FE and FS values for different pulse proteins. Desi and kabuli chickpea concentrates processed by UF had higher FE values compared to yellow pea, green pea, and red lentil protein concentrates processed by both IEP and UF (Boye *et al.*, 2010a) [6]. Processing treatments such as boiling, fermenting, roasting, and malting decrease FC. Hydrolysis, on the other hand, improved the FE of pea PI nearly threefold at a DH value of 2.5. In the acid and alkaline regions, FC and FS are improved, as evidenced by the higher solubility of pigeon pea (Cajanus Cajan L.) proteins, and FC and FS also improved in both regions than in the isoelectric region. The FS of pigeon pea concentrate also improved with an increase in protein concentration and ionic strength (Akintayo *et al.*, 1999).

5.7 Nutraceutical/bioactive properties

Traditionally, pulses have been of interest particularly because of high protein content. However, recently, pulse proteins have also been of interest owing to their bioactive properties such as those considered to be related with the management of type-2 diabetes and obesity and lowering of serum cholesterol and low-density lipoprotein-cholesterol levels (Boye *et al.* 2010 [5]; Li *et al.* 2017). Pulse protein ingredients may contain bioactive constituents like lectins/ hemagglutinins and enzyme inhibitors, possessing health benefits like they may help in lowering serum glucose levels and alleviating obesity (Campos-Vega *et al.* 2010; Tiwari and Singh 2012; Shevkani *et al.* 2015b). In addition, the substitution of animal proteins with pulse proteins has also been found to decrease the levels of low-density lipoprotein cholesterol, non-high-density lipoprotein cholesterol, and apolipoprotein responsible for cardiovascular diseases (Li *et al.* 2017).

6. Applications

Pulse proteins as concentrates and isolates find applications in a number of food systems. The pulse protein isolates are often prepared from dehulled/defatted pulse flours by alkaline extraction and isoelectric precipitation method involving stirring in aqueous alkali (NaOH) solution at pH between 8 and 11, followed by stirring for periods varying from 30 to 180 min to maximize solubilisation of the proteins, filtration/centrifugation to remove insoluble material/residue and precipitation of proteins at isoelectric pH (Boye *et al.* 2010 [5]; Tiwari and Singh 2012). The inclusion of pulses and pulse proteins in diet is generally recommended because of nutritional as well as nutraceutical properties. Recently, pulse proteins have been explored for nutritional and functional improvement of a number of food products including cakes, muffins, crackers, pasta, meat products, beverages, etc.

6.1 Cereal-based (bakery and pasta) foods

Pulse proteins, as concentrates or isolates, are primarily used in novel and traditional foods as nutritional additives and ingredients to improve nutritional value and to impart necessary sensory characteristics (structure, texture, flavour, and colour). The essential amino acid content complimentary to cereals makes them particularly suitable for improving the protein quality of cereal-based foods. Pulse proteins find applications mainly due to their low cost, comparative functionality, acceptability and high nutritional and nutraceutical properties. In the cereal-based foods, pulse protein ingredients not only improved protein quality but also contributed to improved texture of finished products by increasing water absorption of dough/batters. The addition of thermally modified cowpea proteins improved water absorption and acceptability of wheat bread and sponge cake (Campbell *et al.* 2016) [9].

It was also reported that glycated and denatured cowpea proteins can be used to replace whole egg by 20% in sponge cake (Campbell *et al.* 2016) [9]. Chickpea proteins partially substituted soy (one third of soy) in breads without negatively affecting the texture of finished product (Serventi *et al.* 2018) [26].

Pulse proteins also improved technological quality attributes of gluten-free foods by increasing viscoelasticity of batters or by forming protein network in the dough systems. Protein isolates from kidney beans, peas and cowpeas were explored for possible application in gluten free muffins (Shevkani and Singh 2014 [27]; Shevkani *et al.* 2015a) [28].

Kidney bean and pea protein isolates at incorporation level of 10% enhanced viscoelasticity (dynamic rheological moduli) of corn starch-based batters and resulted in muffins with quality attributes (crust colour, specific volume, springiness, cohesiveness, appearance and porosity) comparable to the muffins prepared with wheat gluten (Shevkani and Singh 2014) [27].

Cowpea proteins also improved technological quality attributes of rice muffins depending on level of incorporation and protein characteristics (Shevkani *et al.* 2015a) [28]. It was demonstrated that emulsification and foaming were desirable characteristics of protein isolates for quality improvement of rice muffins. Shaabani *et al.* (2018) [25] recently studied the effect of addition of varying levels of chickpea protein isolate, transglutaminase and xanthan gum on the rheological properties and quality attributes of millet muffins and showed that chickpea proteins with transglutaminase contribute to the formation of protein network in the muffins.

6.2 Imitation milk

Soy proteins have found tremendous success in imitation milk applications. Very few reports can be found in the literature, however, on the use of pulse protein flours in the development of imitation milk products. This may probably be due to the high starch and fibre contents of pulses which make them less suitable for beverage applications where low viscosities are desired. Swanson (1990 [33], and references therein) reported on the use of pea and lentil protein isolates for the preparation of imitation milks. The use of lentil protein isolate resulted in a milk of intermediate quality equivalent to milk prepared from soy protein isolates whereas the use of pea protein isolate gave milk of poorer quality. Successful application of pulse ingredients in imitation milk products will require detailed studies on their rheological and flavour properties under different conditions of processing and the development of effective technologies to remove residual starch.

6.3 Bean curd

One of the most consumed soy foods is tofu, a curd made from extracted soymilk with the appearance of cheese or firm yogurt. Tofu-making begins with the extraction of soymilk from soybeans followed by heating to denature the soy proteins and inactivate trypsin inhibitors. A coagulant (e.g., magnesium chloride, calcium sulphate, glucono-delta-lactone) is subsequently added to induce curd formation. The coagulated soymilk is then separated into whey (the uncoagulated soymilk) and curds (the coagulated soymilk), and the curds are subsequently pressed to form tofu. Cai *et al.* (2001) [8] conducted studies on bean curd formation using protein extracts of several pulses (chickpea, *faba* bean, lentil, mung bean, smooth pea, pea, and winged bean) using CaSO4 as coagulant with or without heat denaturation. For all pulse fractions, a protein concentration of 2.3–3% and the use of 1.5% CaSO4 as coagulant yielded the best result for curd formulation. Chickpea and *faba* beans had comparable textural properties ranking second to soybean. For lentil, mung bean and smooth pea flour, the moisture content of curds was significantly higher than for soy curds. Curds from lentil, mung bean and smooth pea had the lowest springiness and cohesiveness. A laboratory-scale technique was developed by Sri Kantha, Hettiarachchy, and Erdman (1983) [31] for the production of winged bean curd. Preparation of high protein curd from other pulses such as field pea has been reported (Gebre-Egziabher & Summer, 1983) [12]. Further studies on the aggregation and gelling properties of pulse proteins will be useful to establish their potential application in the formation of curd-like products such as imitation tofu and cheese.

6.4 Meat products

Pulse proteins in comminuted cooked meat products improved textural properties and yield. Abdel-Aal *et al.* (1987) [1] used chickpea and *faba* bean protein and flours (levels of 20–40%) as extender in sausages. They reported that proteins were more acceptable than flours for all substitution levels. Ghribi *et al.* (2018) [13] recently investigated the effect of incorporation of lower levels of (1.5, 2.5 and 5%) chickpea protein concentrates on the properties of cooked sausage and reported that chickpea proteins not only reduced cooking yield and lipid oxidation but also increased colour stability, total antioxidant capacities, protein content and acceptability by consumers.

6.5 Edible/biodegradable

films Among various biodegradable polymers, pulse proteins have been regarded as a viable alternative for petroleum based polymeric products and attracted increasing attention in both academic research and industries because of their excellent accessibility and low cost (Tang *et al.* 2009 [35]; Shevkani and Singh 2015). A flexible composite film was fabricated using kidney bean protein isolate and chitosan as antimicrobial food-packaging by Fan *et al.* (2014) [11]. They showed that the films produced at pH 3.0 and loaded with nisin had antimicrobial activity against Bacillus subtilis and Staphylococcus aureus (Fan *et al.* 2014) [11]. Shevkani and Singh (2015) investigated film-forming properties of kidney.

6.6 Encapsulation material

Pulse proteins have also been used as encapsulating material in the food industry to protect bioactive, volatiles and other components from deterioration as well as to mask the undesirable flavours in foods. Bioactive substances such as omega-3 fatty acids, phytosterols and carotenoids, can be encapsulated using the emulsions of vegetable proteins (Nesterenko *et al.* 2013). Pea protein isolate stabilized emulsions effectively reduced oxidation of conjugated linoleic acid during storage (Lin *et al.* 2017). Chickpea protein encapsulation improved the stability of folate in processed foods (Ariyarathna and Karunaratne 2015) [3].

7. Allergenicity of pulse proteins

Although pulse proteins have been recognized to be important food ingredients because of their nutraceutical properties, at the same time, they represent potential food allergens. Indeed, many pulse proteins (storage globulins, 2S albumins, a-amylase inhibitors) have been recognized to have allergenic properties and high stability towards severe treatments aimed at their inactivation (Cuadrado *et al.*, 2009). Structure and properties common to plant allergens facilitate their grouping into four classes:

- Bet v 1 homologues responsible for cross-reacting pollen-fruit allergy syndromes
- Class I chitinases of fruit and vegetables responsible for cross reacting latex-fruit allergy syndromes
- A-class proteins: 2S albumins, a-amylase inhibitors, lipid transfer proteins
- Seed storage globulins. Most of these proteins are glycoproteins of low molecular weight (10-20 kDa), with a compact structure stabilized by disulphide bonds. They are thermo stable, resistant to proteolysis and show biological activities conferring resistance to pests and pathogens.

8. Conclusions

Pulses are a rich source of energy, carbohydrates, protein, fats, vitamins, minerals and many bioactive compounds, which can be used to fortify food formulations and functional foods or be used in nutraceutical and/or pharmaceutical applications. A wide variety of food products can be envisaged in this regard which could provide many health benefits to consumers. Moreover, incorporation of pulses into various products builds on the growing trend toward more environmentally friendly food production and a better balance between the provisions of foods derived from animal and plant sources. However, there continues to be a need for further research to improve the knowledge base of the properties of pulses and pulse fractions in order to identify the appropriate conditions to optimize their functionality when used in foods. Additionally, cheaper technologies for processing pulses into flours and for fractionating them into value added ingredients such as starches, fibers and protein powders are needed and will go a long way to stimulate the utilization of these ingredients in novel food and industrial food applications.

9. References

1. Abdel-Aal EM, Youssef MM, Shehata AA, El-Mahdy AR. Some legume proteins as bread fortifier and meat extender. Alex J Agric Res. 1987;32:179-189.
2. Alonso-Miravalles L, Jeske S, Bez J, Detzel A, Busch M, Krueger M, *et al.* Membrane filtration and isoelectric precipitation technological approaches for the preparation of novel, functional and sustainable protein isolate from

lentils. European Food Research and Technology, 2019. https://doi.org/10.1007/s00217-019-03296-y
3. Ariyarathna IR, Karunaratne DN. Use of chickpea protein for encapsulation of folate to enhance nutritional potency and stability. Food Bioprod Process. 2015;95:76-82.
4. Boulter D, Croy RRD. Legume seed storage proteins. Adv Bot Res. 1997;27:2-70.
5. Boye J, Zare F, Pletch A. Pulse proteins: processing, characterization, functional properties and applications in food and feed. Food Res Int. 2010;43:414-431.
6. Boye JI, Aksay S, Roufik S, Rib´ereau S, Mondor M, Farnworth E, *et al.* Comparison of the functional properties of pea, chickpea and lentil protein concentrates processed using ultrafiltration and isoelectric precipitation techniques. Food Research International. 2010a;43(2):537-546.
DOI: https://doi.org/10.1016/j. foodres.2009.07.021
7. Burger TG, Zhang Y. Recent progress in the utilization of pea protein as an emulsifier for food applications. Trends in Food Science & Technology. 2019;86:25-33.
DOI: https://doi.org/10.1016/j.tifs.2019.02.007
8. Cai R, Klamczynska B, Baik BK. Preparation of bean curds from protein fractions of six legumes. Journal of Agricultural and Food Chemistry. 2001;49:3068-3073.
9. Campbell L, Euston SR, Ahmed MA. Effect of addition of thermally modified cowpea protein on sensory acceptability and textural properties of wheat bread and sponge cake. Food Chem. 2016;194:1230-1237.
10. D'Agostina A, Antonioni C, Resta D, Arnoldi A, Bez J, Knauf U, *et al.* Optimization of a Pilot-scale process for producing Lupin protein isolates with valuable technological properties and minimum thermal damage. Journal of Agricultural and Food Chemistry. 2006;54(1):92-98.
DOI: https://doi.org/10.1021/jf0518094
11. Fan JM, Ma W, Liu GQ, Yin SW, Tang CH, Yang XQ. Preparation and characterization of kidney bean protein isolate (KPI)–chitosan (CH) composite films prepared by ultrasonic pretreatment. Food Hydrocoll. 2014;36:60-69.
12. Gebre-Egziabher A, Summer AK. Preparation of high protein curd from field peas. Journal of Food Science. 1983;48:375-377.
13. Ghribi AM, Amira AB, Gafsi IM, Lahiani M, Bejar M, Triki M, *et al.* Toward the enhancement of sensory profile of sausage ''Merguez'' with chickpea protein concentrate. Meat Sci. 2018;143:74-80.
14. Gupta R, Dhillon S. Characterization of seed storage proteins of lentil (*Lens culinaris* M). Ann Biol. 1993;9:71-78.
15. Hu FB. Plant-based foods and prevention of cardiovascular disease: An overview. American Journal of Clinical Nutrition. 2003;78:544-551.
16. Karaca AC, Low N, Nickerson M. Emulsifying properties of chickpea, *faba* bean, lentil and pea proteins produced by isoelectric precipitation and salt extraction. Food Research International. 2011;44(9):2742-2750.
DOI: https://doi.org/10.1016/ j.foodres.2011.06.012
17. Liener IE. Toxic factors in edible legumes and their elimination. American Journal of Clinical Nutrition. 1962;11:281-298.
18. Lin D, Lu W, Kelly AL, Zhang L, Zheng B, Miao S. Interactions of vegetable proteins with other polymers: structure function relationships and applications in the food industry. Trend Food Sci. Technol. 2017;68:130-144.
19. Liu C, Damodaran S, Heinonen M. Effects of microbial transglutaminase treatment on physiochemical properties and emulsifying functionality of *faba* bean protein isolate. Lebensmittel-Wissenschaft und-Technologie-Food Science and Technology. 2019b;99:396-403.
DOI: https://doi.org/10.1016/j.lwt.2018.10.003 made with luvede. (2020). Retrieved from https://madewithluve.de/
20. Nesterenko A, Alrica I, Silvestrea F, Durrieu V. Vegetable proteins in microencapsulation: a review of recent interventions and their effectiveness. Ind Crop Prod. 2013;42:469-479.
21. Pelgrom PJM, Berghout JAM, Van der Goot AJ, Boom RM, Schutyser MAI. Preparation of functional lupine protein fractions by dry separation. Lebensmittel-Wissenschaft und-Technologie - Food Science and Technology. 2014;59(2):680-688.
DOI: https://doi.org/10.1016/j.lwt.2014.06.007
22. Pelgrom PJM, Wang J, Boom RM, Schutyser MAI. Pre and post treatment enhance the protein enrichment from milling and air classification of legumes. Journal of Food Engineering. 2015b;155:53-61.
DOI: https://doi.org/10.1016/j. jfoodeng.2015.01.005
23. Philanto A, Korhonen H. Bioactive peptides and proteins. Advances in Food and Nutrition Research. 2003;47:175-181.
24. Saldanha do Carmo C, Silventoinen P, Nordgård CT, Poudroux C, Dessev T, Zobel H, Sahlstrøm S. Is dehulling of peas and *faba* beans necessary prior to dry fractionation for the production of protein - and starch-rich fractions? Impact on physical properties, chemical composition and techno-functional properties. Journal of Food Engineering. 2020, 278.
DOI: https://doi.org/10.1016/j. jfoodeng.2020.109937
25. Shaabani S, Yarmand MS, Kiani H, Emam-Djomeh Z. The effect of chickpea protein isolate in combination with transglutaminase and xanthan on the physical and rheological characteristics of gluten free muffins and batter based on millet flour. LWT-Food Sci Technol. 2018;90:362-372.
26. Serventi L, Vittadini E, Vodovotz Y. Effect of chickpea protein concentrate on the loaf quality of composite soy-wheat bread. LWT-Food Sci Technol. 2018;89:400-402.
27. Shevkani K, Singh N. Influence of kidney bean, field pea and amaranth protein isolates on the characteristics of starch-based gluten-free muffins. Int. J Food Sci. Technol. 2014;49:2237-2244.
28. Shevkani K, Kaur A, Kumar S, Singh N. Cowpea protein isolates: functional properties and application in gluten-free rice muffins. LWT-Food Sci. Technol. 2015a;63:927-933.
29. Singh N. Pulses: An overview. J Food Sci Technol. 2017;54:853-857.
30. Sozer N, Holopainen-Mantila U, Poutanen K. Traditional and new food uses of pulses. Cereal Chemistry. 2017;94(1):66-73.
DOI: https://doi.org/10.1094/cchem-04-16- 0082-f
31. Sri Kantha S, Hettiarachchy NS, Erdman JW Jr.. Laboratory scale production of winged bean curd. Journal of Food Science. 1983;48:441-444.
32. Sumner AK, Nielsen MA, Youngs CG. Production and

evaluation of pea protein isolate. Journal of Food Science. 1981;46(2):364-366.
DOI: https://doi.org/10.1111/j.1365-2621.1981.tb04862.x
33. Swanson BG. Pea and lentil protein extraction and functionality. Journal of the American Oil Chemists Society. 1990;67:276-280.
34. Tabtabaei S, Vitelli M, Rajabzadeh AR, Legge RL. Analysis of protein enrichment during single- and multi-stage tribo-electrostatic bio separation processes for dry fractionation of legume flour. Separation and Purification Technology. 2017;176:48-58.
DOI: https://doi.org/10.1016/j.seppur.2016.11.050
35. Tang CH, Xiao ML, Chen Z, Yang XQ, Yin SW. Properties of cast films of vicilin-rich protein isolates from Phaseolus legumes: influence of heat curing. LWT-Food Sci. Technol. 2009;42:1659-1666.
36. Tharanathan RN, Mahadevamma S. Grain legumes – A boon to human nutrition. Trends in Food and Science Technology. 2003;14:507-518.
37. Yepes OO, Di Giogio L, Goyanes S, Mauri A, Fama´ L. Influence of process (extrusion/thermo-compression, casting) and lentil protein content on physicochemical properties of starch films. Carbohydr Polym. 2019;208:221-231.
38. Vogelsang-O'Dwyer M, Petersen IL, Joehnke MS, Sorensen JC, Bez J, Detzel A, Zannini E. Comparison of *faba* bean protein ingredients produced using dry fractionation and isoelectric precipitation: Techno-functional, nutritional and environmental performance. Foods. 2020;9:3. https://doi.org/10.3390/ foods9030322