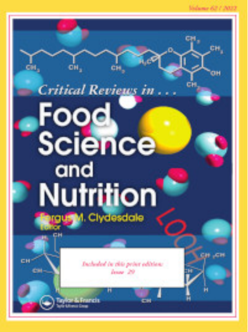

Critical Reviews in Food Science and Nutrition

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/bfsn20

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To cite this article: Jared Rivera, Kaliramesh Siliveru & Yonghui Li (2022): A comprehensive review on pulse protein fractionation and extraction: processes, functionality, and food applications, Critical Reviews in Food Science and Nutrition, DOI: 10.1080/10408398.2022.2139223

To link to this article: https://doi.org/10.1080/10408398.2022.2139223

CRITICAL REVIEWS IN FOOD SCIENCE AND NUTRITION
https://doi.org/10.1080/10408398.2022.2139223

REVIEW

A comprehensive review on pulse protein fractionation and extraction: processes, functionality, and food applications

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ABSTRACT

The increasing world population requires the production of nutrient-rich foods. Protein is an essential macronutrient for healthy individuals. Interest in using plant proteins in foods has increased in recent years due to their sustainability and nutritional benefits. Dry and wet protein fractionation methods have been developed to increase protein yield, purity, and functional and nutritional qualities. This review explores the recent developments in pretreatments and fractionation processes used for producing pulse protein concentrates and isolates. Functionality differences between pulse proteins obtained from different fractionation methods and the use of fractionated pulse proteins in different food applications are also critically reviewed. Pretreatment methods improve the de-hulling efficiency of seeds prior to fractionation. Research on wet fractionation methods focuses on improving sustainability and functionality of proteins while studies on dry methods focus on increasing protein yield and purity. Hybrid methods produced fractionated proteins with higher yield and purity while also improving protein functionality and process sustainability. Dry and hybrid fractionated proteins have comparable or superior functionalities relative to wet fractionated proteins. Pulse protein ingredients are successfully incorporated into various food formulations with notable changes in their sensory properties. Future studies could focus on optimizing the fractionation process, improving protein concentrate palatability, and optimizing formulations using pulse proteins.

KEYWORDS

Dry fractionation; meat analogs; plant proteins; protein extraction; protein functionality; pulse proteins

Introduction

The global population is projected to grow to 10 billion by the year 2050 and 11.2 billion by 2100 (UNFPA 2020). With the continuous increase in the global population, a major challenge is to ensure that the population has an adequate food supply and access to nutritious foods (Fernando 2021). Animal proteins obtained from sources such as meat, milk, egg, and fish have been the main protein source for the global population. However, the process of producing animal proteins is regarded to be inefficient and less sustainable in many previous studies (Li 2020). For example, Sranacharoenpong et al. (2015) compared the production of animal and plant protein sources, and they reported that producing 1 kg of beef would require 18 times more land, 12 times more fertilizer, 10 times more pesticide, 10 times more water, and 9 times more fuel relative to producing 1 kg of kidney beans. The increased interest in high-protein ingredients/food due to consumer health awareness has also led to an increase in demand for plant protein. Thus, the market for plant protein ingredients is projected to further increase in the future (Pam Ismail et al. 2020). Therefore, the development of sustainable and effective processes for plant protein production is essential to satisfy the increasing demand for plant proteins to produce high-quality plant protein ingredients for food manufacturing.

Among plant crops, pulse crops are a suitable alternative protein source as these crops are cheaper to produce while still having significant nutritional benefits (Schutyser et al. 2015). The term pulses refer to the nutritionally dense edible seeds from the legume family (USA Pulses 2022). The U.N. Food and Agriculture Organization (FAO) recognizes 11 groups of pulses which include dry beans, lupines, Bambara beans, broad beans, lentils, dry peas, chickpeas, pigeon peas, vetches, cowpeas, winged beans, and sword beans (Calles 2016). In the United States, the production of pulses has increased in recent years with dry beans, lentils, dry peas, and chickpeas having the highest production among pulse crops in terms of volume and acreage (Bond 2017). Dry beans are pulse seeds that are oval or kidney shaped while lentils are usually flat in shape. Dry peas are rounder in shape while chickpeas can have various shapes ranging from spherical, oval to elongate shaped. Compared with cereals such as wheat and corn, pulse crops have higher initial protein content (>20 g/100 g dry matter), better functional properties, and less allergenicity (e.g. comparing to wheat gluten) (Bresciani and Marti 2019; Boye, Zare, and Pletch 2010; Messina 1999). Pulse crops can also fix atmospheric

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nitrogen in the soil which increases total soil nitrogen (N) fertility (Schwenke et al. 1998). This capability makes pulse crops a good component in crop rotation regimens as they can improve soil conditions prior to planting of other major crops. Their use in crop rotations has been reported to increase yield and quality of subsequent crops as well as improve fertilizer use efficiency (Fan et al. 2020).

Like cereal crops, pulses also contain substantial amounts of non-protein components such as starch, dietary fiber, and lipids. Pulse protein fractionation methods, which could be broadly classified as wet or dry methods, have been developed to separate the pulse components. Wet methods involve the use of solvents and can produce fractions with higher protein content and better purity. Dry methods, which produce lower yields, and less purity products, use energy and resources more efficiently as they rely on milling and dry separation processes (Berghout, Boom, and van der Goot 2014; Schutyser et al. 2015). These fractionation methods separate the protein, starch, and dietary fiber components of the grain into individual fractions. Pulse proteins are commonly used as a nutrition source, or enrichment ingredient in a variety of food products. In contrast, pulse starches are less used in the food industry due to inferior quality (e.g., restricted swelling and solubility and faster retrogradation) and storage stability compared to conventional food starches such as wheat, potato, and corn starch (Ratnayake and Naguleswaran 2022). Pulse fibers, after further purification, can be used as ingredients in food, beverage, and pet-food applications due to their desirable functional properties (e.g. water binding, oil binding, swelling capacity, and viscosity) (Novak, Yang, and Chandak 2019).

Previous review articles such as those of Fernando (2021) and Zhu et al. (2021b) have focused on dry classification processes for producing pulse protein ingredients, as dry fractionation processes have been more efficient and less resource-intensive. The lower-purity pulse protein ingredients have been reported to impart some desirable properties to food products (e.g. texture and viscosity). However, the need for purer protein ingredients still exists for many food applications (supplements, plant-based meat, beverages, and functional foods) and nutritional needs, which show the need for developing more efficient wet fractionation methods or hybrid methods. Purer protein ingredients would also contain fewer amounts of bioactive components present in pulses such as trypsin inhibitors, phenolic compounds, and phytates. These compounds could be toxic, unpalatable, or anti-nutritive to humans as they could inhibit metabolism and reduce digestibility of essential nutrients (Kumar, Chakraborty, et al. 2022). Therefore, the objectives of this review are to systematically explore developments in wet, dry, and hybrid fractionation methods applicable for producing protein concentrates and isolates from pulses, review the effects of fractionation methods on protein functional properties and quality, and explore the uses of plant protein ingredients in various food applications.

Methods

In selecting the most relevant publications to be included in this review, literature search was carried out using the "Web of Science" and "Scopus" databases. The search engine "Google Scholar" was also used for the literature search phase. The keywords included in the search were: legume/pulses/seeds type (e.g. beans/lentils/peas/chickpea), protein, pretreatment/fractionation/isolation/application. The year of publication was set from "all years" to "present" with the type of documents included in the search being narrowed to either "paper" or "review." Duplicate search results from the databases used were merged, and suitable research papers or reviews were then selected as those that investigated fractionation methods for pulse proteins, pretreatment of pulse seeds, functionality of fractionated protein, and food applications of pulse protein ingredients. A flow chart for the literature search can be found in Figure 1.

Pulse grain pretreatment methods

Pretreatment of pulses is commonly used to ease the separation of the seed coat from the cotyledon (Fernando 2021). Other purposes of pretreatment methods include reducing anti-nutritional factors in pulse seeds and improving the quality of the end-products. These methods used can be broadly classified into dry or wet methods.

Dry pretreatment

Dry pretreatment methods include pitting or scratching the seed surface by abrasion (Narasimha, Ramakrishnaiah, and Prate 2003). The efficiency of this method was reported to be affected by seed characteristics (e.g. hardness and shape), dehulling parameters (pitting speed, roller gaps, and duration), and seed moisture content. Goyal, Vishwakarma, and Wanjari (2010) explored the effect of moisture content (6 to 14 g moisture/100 g) on the pitting and milling efficiency of pigeon pea. They reported that optimum pitting and milling efficiency for pigeon pea was achieved at 10% moisture content. Microwave treatment of the seeds improved the dehulling efficiency of pulses such as black gram seeds (Joyner and Yadav 2015a). Microwave treatment was also applied to chickpea to improve dehulling efficiency by Solanki, Gupta, and Alam (2021) wherein they reported improved yields at microwave power of 90 PL and exposure time of 2.5 min using their microwave system.

Aside from microwave treatment, Kumar, Chakraborty, et al. (2022) optimized the use of infrared (IR) heating on hull adherence and cotyledon integrity of pigeon pea by manipulating the voltage (203 to 237 V), time (1.3 to 4.7 min), and grain moisture (8.6 to 15.4 g moisture/100g). They reported that hull adherence was decreased in pigeon pea by IR treatment, with its effectiveness significantly affected by the parameters tested. The effects of IR heating on the quality of milled chickpea and navy bean flour were also evaluated in a separate study by Guldiken et al. (2022) wherein they manipulated the seed moisture (20 and 30 g moisture/100 g) and heating temperature (120 and 140 °C). The authors reported a decrease in the solubility, oil emulsification, and foaming ability of flours with IR treatment

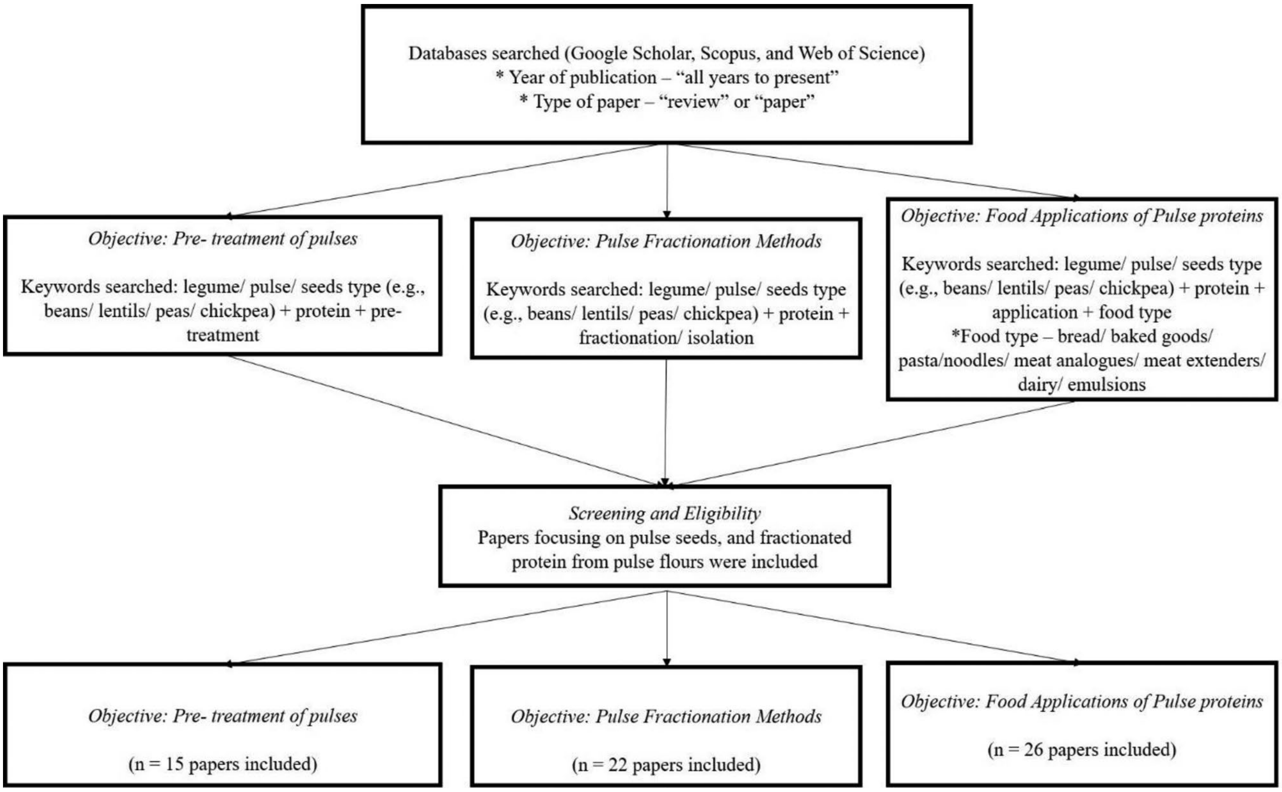

Figure 1. Flow diagram used for searching and selecting papers included in the review.

alone while increased moisture in addition to IR heating improved the functionalities mentioned. Sunil et al. (2018) optimized the ultrasound treatment of black gram seeds to improve dehulling efficiency using response surface methodology (RSM). They reported an increased dehulling yield and lower loss at the optimized conditions (513.4 W and 2.12 h) used. The improved yield was explained through the cavitation of the structure of the seed after treatment which loosened the seed coat. Stone et al. (2021) evaluated the effect of roasting (160 °C) in conjunction with seed moisture (20 or 30 g moisture/100 g) on the functional properties and nutritional quality of chickpea, green lentil, navy bean, and yellow pea flours. In their study, wet roasting the pulse seeds resulted in reduced solubility, improved water and oil holding capacity, and better digestibility of the protein and starch components.

Wet pretreatment

Wet methods have also been used for pre-treating pulse seeds, which mostly involve soaking and tempering pulse seeds in water or other chemical solutions for a set time followed by drying. This step allows the easier separation of the cotyledon from the seed coat while also softening the seeds to help control the particle size reduction of the seed endosperm. Zamindar et al. (2016) reported that using acid/alkaline tempering solutions lowered the shear strength of Iranian red kidney beans (Akhtar and Derakshan varieties), resulting in softer kernels. In another study, Fernando (2017) reported that tempering black beans (10 to 50 g moisture/100 g) resulted in better seed coat yield during milling compared to boiling followed by drying. The improved seed coat yield resulted in better separation of the seed coat from the cotyledon of black bean. The tempering process was also used in conjunction with heating processes as mentioned in the previously cited studies. The higher moisture content of the pulse seeds resulted in better dehulling and functional properties of milled pulse seeds.

Bellido et al. (2006) explored the use of tempering pretreatments (water, salt, and acid solutions) in the micronization of navy and black beans and reported lower bean hardness and lower protein solubility after treatment. They have also observed that using water alone had similar effect no the micronization of black and navy beans. These results were consistent with a subsequent study by Pathiratne et al. (2015), wherein the effects of micronization temperature (115 to 165 °C), and moisture level (8%, 16%, or 23%) on the functional properties of milled lentil flour were investigated. The authors reported inhibited endogenous enzyme activity (lipoxidase and peroxidase); changes in protein characteristics including decreased protein dispersibility index (PDI) were also observed which indicated lower solubility.

Steam pretreatment of black gram seeds was also studied by Joyner and Yadav (2015a, 2015b) wherein they reported increased dehulling efficiency of steam-treated seeds. This was attributed to the loosening of the attachment of seed coat to the endosperm due to disruption of chemical bonds of gums and mucilages (Joyner and Yadav 2015a, 2015b). Pretreatment methods also involve the use of edible oils to improve the dehulling process which is attributed to the penetration of oil through the seed, loosening the seed coat by dissolving pulse gums (Fernando 2021; Tiwari, Jaganmohan, and Vasan 2007). However, this treatment is mostly confined to pre-treating pigeon pea due to the stronger attachment of the seed coat to the cotyledon (Ghermezgoli, Ghassemzadeh, and Moghaddam 2017).

Enzyme pretreatments were used in a study by Verma et al. (1993) for improving the dehulling process of pigeon pea seeds which increased efficiency by 87%. Sreerama, Sashikala, and Pratape (2009) reported that pretreatment with protease enzyme was more efficient to increase dehulling efficiency compared to xylanase in green, black, red, and horse gram seeds. Dabhi, Sangani, and Rathod (2019) reported an increase in dehulling efficiency upon using different enzyme pretreatments on pigeon pea prior to dehulling with the enzyme solution containing xylanase, pectinase, and cellulose having the highest increase in dehulling efficiency in addition to lower energy requirements for the process and better nutritional quality. Wood et al. (2022) studied the effectiveness of eight enzyme pretreatment methods on chickpea by evaluating quality attributes such as visual quality, texture, dehulling, and milling quality of the treated seeds. Wood et al. (2022) showed that enzyme pretreatment of chickpeas improved mill yield and dehulling efficiency. Negative effects such as tougher seed coat, darker color, texture changes, and increased visual damage were also observed in this study. Wood et al. (2022) also reported that among the treatments used, the most viable enzymes for commercial scale use were *endo*-polygalacturonase, α-galactosidase, and cellulase.

Dehulling is also important as it removes anti-nutritional compounds which improves the flour quality and nutritional benefits of pulse products (Rahate, Madhumita, and Prabhakar 2021). The dehulling process is often coupled with aspiration to remove lighter impurities and minimize seed size reduction (Fernando 2021). Milling involves the use of impact mills such as hammer or pin mills to reduce the particle size of grains. Ideally, milling should be able to reduce the particle size adequately to separate the protein bodies from the starch granules with minimal starch damage (Boye, Zare, and Pletch 2010). Pulses are preferred for separation of proteins from starch bodies as they have larger and more uniform starch granules (25–40 μm) compared to cereal grains (Vose 1978).

Overall, these previous studies (summarized in Table 1) have demonstrated the improvement in dehulling efficiency and changes in the functional properties of various pulse seeds. The studies also indicated that the pulse seed characteristics play a role in the suitability of pretreatment methods. Defatting is often used to separate the lipid component of legumes or pulses prior to fractionation. According to Almeida Costa et al. (2006), the protein (18.5 to 21.9%), ash (3.0 to 3.8%), crude fiber (6.8 to 10.4%), and carbohydrate (52.5 to 56.4%) compositions of lentils, peas, beans, and chickpeas are comparable to each other. This indicates that the mentioned pretreatments could be applied to the pulse seeds using similar methods. The only difference is that chickpeas (6.69%) have a higher lipid content than the other pulse crops (2.15 – 2.65%). This lipid level is still much lower than oilseed legumes such as soybeans (28.2%) (Etiosa, Chika, and Benedicta 2018). This means that defatting may be still not necessary for chickpeas during pretreatment. However, the higher lipid content of chickpeas could cause negative effects on the handling of chickpea once it is milled into flour. Higher lipid contents would increase powder cohesiveness which leads to lower mill yield poorer separation during the

Table 1. Summary of pulse pre-treatments applied prior to milling or dehulling.

Pulse type	Pre- treatment	Effect	References
Pigeon pea	Infrared heating, grain moisture, time	Infrared heating decreased hull adherence; increased dehulling efficiency	Kumar, Chakraborty, et al. (2022)
Chickpea and navy bean	Infrared heating, grain moisture, temperature	Lower α-amylase, higher gelatinization, and increased hydrophobicity	Guldiken et al. (2022)
Chickpea, green lentil, navy bean, yellow pea	Roasting, and seed moisture	Roasting (160°C), and seed moisture affected functional properties, and amino acid profile of pulse seeds	Stone et al. (2021)
Chickpea	Enzyme treatment	Improved mill yield, dehulling, darker color, higher visual damage	Wood et al. (2022)
Chickpea	Microwave	Highest dehulling was achieved at 90 PL at 2.5 min	Solanki, Gupta, and Alam (2021)
Black gram	Ultrasound, exposure time	Optimum conditions of 513.4 W, and 2.14 h gave highest dehulling yield	Sunil et al. (2018)
Black beans	Water tempering	Tempering (10–50% moisture) increased seed coat yield more compared to boiling	Fernando (2017)
Red kidney beans	Acid/ Alkaline tempering	Acid tempering had the most reduction in seed hardness; alkaline tempering increased splitting	Zamindar et al. (2016)
Lentil	Micronization temperature, and moisture level	Lower enzyme activity, changes in functional properties depending on temperature, and moisture level	Pathiratne et al. (2015)
Black gram	Steam treatment	Increased dehulling efficiency	Joyner and Yadav (2015b)
Black gram	Microwave	Dehulling efficiency was highest at 972 J/g at power level of > 630 W	Joyner and Yadav (2015a)
Chickpea, lentil, pea, beans	Milling	Higher protein yield during dry fractionation of milled product	Pelgrom, Boom, and Schutyser (2015)
Pigeon pea	Moisture, Pitting	Maximum dehulling efficiency at 10% moisture	Goyal, Vishwakarma, and Wanjari (2010)
Black, green, red, and horse gram	Enzyme treatment	Protease enzyme was effective in increasing dehulling efficiency compared to the control.	Sreerama, Sashikala, and Pratape (2009)
Navy and black beans	Water, saline, and acid solution tempering with micronization	Lower bean hardness, and protein solubility	Bellido et al. (2006)

fractionation part. Hence, the milling method used should be optimized so as not to cause unnecessary particle size reductions to the pulse seeds during pretreatment. The optimization of the dehulling method should also be considered as each pulse seed type would have different seed coat adhesion strength which would affect dehulling efficiency.

Dry fractionation methods

Dry fractionation methods are viewed as a more sustainable alternative for producing protein-enriched fractions from pulses. This method only involves milling and dry separation of the milled products to produce protein- and starch-enriched fractions. The typical process flow for dry fractionation is shown in Figure 2. The increased interest in using dry fractionation methods stems from the need for an established process which is both energy and resource-efficient while preserving the protein quality (Schutyser et al. 2015). Dry fractionated proteins are also approved for organic food production and do not require E-numbers, as no chemicals are used in the production process, satisfying the demand for "clean label foods" (Schutyser et al. 2015). The drawback of dry fractionation is its lower degree of protein enrichment and lower protein purity relative to wet fractionation. Hence, research studies have aimed to optimize the dry fractionation process to improve protein content and purity of the separated protein fraction.

The dry fractionation process mainly relies on the milling step to produce high-quality protein concentrates. The process relies on the assumption that milling can mechanically separate the protein bodies from the other components of the pulse seed. Morphology studies of pulse seeds show that pulses have a more uniform starch granule size (approx. 20 µm) wherein the granules are embedded in protein bodies (1–3 µm) and matrices surrounded by a fiber-rich cell wall (Tyler and Panchuk 1982). Due to this difference in size, milling is expected to disentangle the protein bodies from the starch granules into smaller particle sizes compared to the starch granules. Ideally, the milling process selected should reduce the particle size of proteins to sizes smaller than the starch granules while causing minimal starch damage.

Pelgrom et al. (2013) were able to optimize a milling method that gives consistent protein enrichment of yellow pea proteins by setting the classifier wheel speed of an impact jet mill to 4000 rpm, which resulted in optimum disentanglement of starch and protein bodies after air classification without increasing damaged starch in the flour. The use of the optimized milling method resulted in a protein content of 55% (dry weight basis) in the pea protein concentrate obtained after air classification. In a subsequent study, Pelgrom, Boom, and Schutyser (2015) hypothesized that optimal detachment happens when the particle size distribution (PSD) of the pulse flour has maximum overlap with the PSD of the starch granules obtained. They were able to achieve maximal detachment among the starch, protein, and fiber-rich fractions from dry bean, chickpea, lentils, and pea by optimizing impact mill settings prior to the air classification step. The identification of mill settings that give optimal PSD is needed as too coarse grinding can result in all components (starch, proteins, fiber) having similar particle sizes while too fine grinding would result in starch and protein particles having the same sizes, lowering protein purity and protein separation efficiency (Pelgrom et al. 2013).

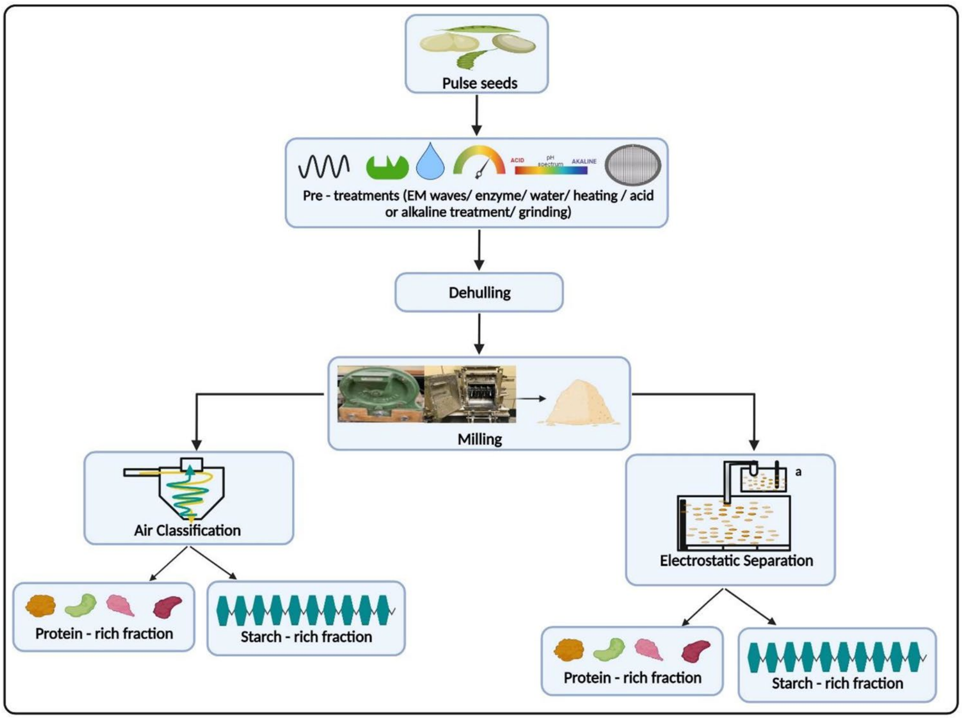

Figure 2. Typical dry fractionation process (adapted from Tabtabaei et al., 2019); Figure made with BioRender.com.

Seed composition, including lipid and fiber content, also plays a role in the efficiency of fractionation as Tyler (1984) reported that seeds with high fiber content typically resulted in lower protein separation efficiency after air classification and that fat inhibits starch and protein separation (Dijkink et al. 2007). Sieving is a separation step that mainly relies on the particle size differences of individual components to achieve separation. Maaroufi et al. (2000) reported that an increased protein (21.8 to 25.7%) and lower starch content in pea proteins are produced using pilot scale sieving. It should be noticed that sieving is generally unsuitable for pulse flour fractionation due to powder cohesiveness and high fat content in some pulses such as lupine and chickpea. These characteristics would result in a phenomenon called sieve blinding (clogging of sieves) reducing the separation efficiency during sieving.

Air classification

The milled products undergo protein separation processes to increase protein concentration of the fractions. Air classification is the most common dry method used to produce protein and starch-enriched fractions from pulse flours. The air classification process relies on the principle that the flour particles could be separated based on their aerodynamic properties determined by their particle size and density characteristics (Fernando 2021). In the protein enrichment of pulse flours, this corresponds to the difference in the particle size of protein (approx. 5 μm) and starch particles (approx. 15 to 40 μm) (Pelgrom, Boom, and Schutyser 2015). Furthermore, the separation is influenced by the cut point for particle size of the material where 20 μm is commonly used for pulse flours (Schutyser and van Der Goot 2011). This particle size is slightly below the common starch granule size. The process is often carried out using centrifugal air classifiers with a classifier wheel or rotors (Dijkink et al. 2007). Once the ground material is fed into the air classifier, centrifugal and gravitational forces separate the flour into fine (protein) and coarser (starch, and dietary fiber) particles. Legume flours typically give higher protein concentration (49 to 70 g protein/100 g dry matter) than cereal crops due to their higher initial protein content and larger starch granules (Vose 1978).

The separation efficiency of air classification is also affected by the process. Higher protein content in pea protein concentrates was observed by Pelgrom et al. (2013) by increasing the milling speed of yellow pea, resulting in maximal protein content of 55 g protein/100 g dry matter. Silventoinen et al. (2021) reported that protein separation efficiency in rye and wheat bran by air classification was influenced by the milling method used, wherein pin disk milling had a positive effect. However, the milling speed should be optimized, as a very high milling speed leads to the production of extremely fine particles. These fine particles have higher surface areas leading to higher cohesive forces, which result in poorer flow and reduced yield.

Process parameters are also important as Pelgrom et al. (2013) reported that pea protein separation efficiency was higher at lower classifier wheel speed during air classification. This effect was attributed to the observation that faster classifier wheel speeds increased the rejection of protein-rich fine particles leading to lower protein yield. The decrease in the protein yield was also worsened by the poor flow properties, and high cohesion of the particles caused fine particles to stick to the classifier walls leading to fouling and higher losses (Pelgrom et al. 2013). These results were also consistent in a follow-up study by Pelgrom, Boom, and Schutyser (2015) where the slower classifier wheel speeds resulted in higher protein separation for yellow pea, beans, chickpea, and lentil seeds. A study by Wang and Maximiuk (2019) reported that increasing classifier wheel speed during air classification of field pea flours resulted in increased protein yield, content, and decreased starch concentration in the protein-rich fraction. Furthermore, they also reported that increasing air flow rate resulted in a lower protein content in the protein-rich fraction.

Rempel, Geng, and Zhang (2019) studied the preparation of pea protein and starch in an industrial scale setting using a cut point of 22 μm for pea flours. In their study, a 43.5% yield of fine fraction was reported, and the protein content of the fine fractions doubled (42–50%) after processing. Funke, Boom, et al. (2022) studied the dry fractionation process for lentils using impact milling, and two successive air fractionation process and reported protein contents from 11% to 54% for the fine fractions produced with particle sizes from 6 to 78 μm. Zhu et al. (2020) studied the optimization of both milling and air classification processes to improve the protein enrichment of mung bean fractions. They reported that coarse milling (at 40 Hz) with an impact mill provided sufficient separation of protein bodies without excessively damaged starch. Furthermore, higher classifier wheel speeds, higher induced draft fan frequency, and lower feed rate conditions reduced cut point and fish-hook effects which improved protein separation efficiency (Zhu et al. 2020). Fish-hook effect is a phenomenon in hydrocyclone operations where the recovery of ultrafine particle sizes in the underflow increases with particle sizes smaller than the critical particle size (Majumder, Yerriswamy, and Barnwal 2003). Optimized conditions based on the response surface methodology (RSM) results show a protein separation efficiency of 84 ± 4%. Muller et al. (2008) reported the use of flow aids, such as fused silica gels, during air classification to be effective in increasing the yield of the protein-rich fractions after air classification.

Pulse seed composition, type, and morphology were also reported to affect the efficiency of air classification. The moisture content of the ground seeds influenced the air classification process, and the separation was better for flours with lower moisture content (Tyler and Panchuk 1982). De Angelis et al. (2021) reported that the type of pulse seeds (green pea, red lentil, and chickpea) all had significant differences in the separation of the ground material components, which resulted from the difference in chemical compositions. The lipid content of the pulse seed was also reported to be critical for air classification efficiency, as fat inhibits proper separation of protein and starch components by reducing the powder dispersibility (Xing, Utami, et al. 2020; Dijkink et al. 2007). Thus, the lipid content of ground material negatively affects the separation efficiency of the protein and starch materials (Schutyser et al. 2015). Furthermore, Pelgrom, Boom, and Schutyser (2015) reported a 27% increase in the protein content of fine fractions by defatting lupine flour prior to the air classification.

Electrostatic separation

Electrostatic separation is another dry fractionation method which separates particles by electrical forces acting on charged materials (Fernando 2021). Electrostatic separation has been mainly used in mineral processing, but it has been increasingly used for fractionation of food materials (Wang et al. 2014). The most common method used for charging food materials for dry fractionation is through triboelectric charging which charges materials by colliding materials with one another in a sealed chamber causing electron transfer, and generating charged materials (Landauer and Foerst 2018). The application of this method on protein/starch mixtures (e.g. pulse flours) relies on the principle that the protein particles could be charged to a higher extent compared to the carbohydrate/starch component due to structural differences. Protein particles have more ionizable structures due to the presence of the N- and C-terminus and amino acid residue components allowing the protein to be charged more readily, while carbohydrates lack ionizable groups in their structures (Tabtabaei et al. 2017). The efficiency of separation is also dependent on the protein source (e.g. pulse type), as the charging behavior of mixture components is dictated by their structure, particle size and shape, and surface properties (Pelgrom, Boom, and Schutyser 2015).

The efficiency of electrostatic separation of protein-starch mixtures is also affected by the process parameters. Selection of appropriate mill settings that will produce the optimum particle size is important as too intense milling will increase the occurrence of particle agglomeration which inhibits separation. Vitelli et al. (2020) reported that using pin milling increased the protein content of the protein-rich fraction from navy bean flour obtained by electrostatic separation, compared to hammer milling. Wang et al. (2014) reported that smaller particle sizes are more likely to acquire higher charge density due to the greater surface area. Landauer and Foerst (2018) reported that increasing the strength of the electric field used for separation greatly increased the separation efficiency of protein particles (cathode). Starch extraction (anode) remained the same, while gas flow rate had negligible effects on separation of protein-starch mixtures (whey and barley starch).

Tabtabaei et al. (2016) optimized the triboelectrification for navy bean flours, and reported that among the process parameters tested, air flow rate (laminar), plate voltage (high voltage), and tribo-charger length (long length) had significant effects on the protein content (50.4%) of navy bean protein fractions. In a subsequent study by Tabtabaei et al. (2017), they reported that a two stage triboelectric separation can improve the protein separation efficiency without affecting protein content, and that plate fouling had negligible effect on separation. Xing, Utami, et al. (2020) explored the effects of tube material and tube diameter on the separation of lupine flour and gluten-starch mixture. In their study, they reported that for both samples, protein materials obtained a positive charge while starch molecules had a negative charge. Furthermore, only the diameter of the tube used had a significant effect on the protein enrichment (37 to 65 g protein per 100 g flour) of lupine flour, while tube material (stainless steel, aluminum, polytetrafluoroethylene, and nylon) was not significant. Xing et al. (2018) have demonstrated that tribopipe materials such as nylon, aluminum, and stainless steel are more effective as tube materials for triboelectric charging. The residence time also needs to be optimized for the process as excessive residence time can cause overcharging of the powder particles, leading to an increased chance of oppositely charged particles coming into contact and forming particle agglomerates (Tabtabaei et al. 2016; Tilmatine et al. 2010). Lastly, Landauer and Foerst (2019) also reported that increasing the contact number of particles resulted in an increase in the protein content in the cathode side while the anode protein content was only slightly affected.

Powder composition also had an effect as higher protein content in the material increased selectivity, and improved triboelectric separation. The gas flow rate during the separation process also needs to be optimized, as unsuitable rates can increase particle agglomeration. Cangialosi et al. (2008) reported that high gas flow rates lead to shorter residence time, and greater inertial forces in the tribopipe and separation chamber, which can break down agglomerated particles. Ideally, a turbulent gas flow rate (Reynolds number > 2400) would be preferred for tribocharging particles for dry separation (Landauer and Foerst 2018). The risk of powder explosion is also present during electrostatic separation due to the contact of combustible dry powder plant materials with electrical charges which is controlled by using inert gases (Wang et al. 2015). The feed rate of the materials was also reported to be important as too dense material flow could lower the charging efficiency and increase particle agglomeration, reducing separation (Traore Ndama et al. 2021). Zhu et al. (2021c) reported that the use of a magnetic field increased the protein content by 3.6%, and purity by 1.8% compared to using electric fields alone in electromagnetic separation for producing pea protein concentrate.

In summary, the published studies (summarized in Table 2) indicate that the effectiveness of the air classification and electrostatic separation processes in protein enrichment of pulse flour fractions is dependent on achieving sufficient separation of starch from protein bodies with minimal damaged starch. This was achieved by optimizing the milling process to produce effective particle size ranges before classification. The process of air classification was also optimized including changing the fan speed, and classifier wheel speeds. These parameters need to be optimized for each

Table 2. Summary of dry fractionation methods used to prepare pulse flour fractions.

Pulse type	Method	Findings	References
Green lentil	Impact milling, two successive air classification	Protein content – 11% to 54%; differences in rheology properties with less refined fractions exhibiting more solid like behavior	Funke, Boom, et al. (2022)
Navy bean	Hammer, and pin milling + electrostatic separation	Pin milling resulted in better electrostatic separation over hammer milling; protein content – 32.6–39.3% (g/ dw)	Vitelli et al. (2020)
Yellow pea, green pea, and split yellow pea	Industrial scale milling, and air classification	Fine fraction yield – 44%; protein content – ≤ 50%; protein yield was acceptable for industrial scale processes	Rempel, Geng, and Zhang (2019)
Field peas	Milling and air classification optimization - classifier and wheel speed	Optimal condition – 4350 rpm (classifier wheel speed); 50 m^3/ h (air flow rate); increased protein content in the finer flour fractions.	Wang and Maximiuk 2019
Navy bean	Two – stage triboelectric separation	Two stage process improved protein separation; protein content – 36 to 38%; higher functional properties compared to the wet fractionated protein	Tabtabaei et al. (2017)
Yellow pea, lentil, chickpeas, and beans	Milling, and air classification optimization	Protein content – 45.3 to 58.5% (g/dw); Higher protein content was present in finer fractions obtained	Pelgrom, Boom, and Schutyser (2015)
Yellow pea	Optimized milling and air classification through air flow and classifier wheel speed	Fractionation at 4000 rpm was optimal; protein content was ≥ 51% (w/ dw); protein recovery 76.8% (w/ dw). High protein water holding capacity for protein, and gel (after heating)	Pelgrom et al. (2013)
Yellow pea	Pilot scale sieving	Protein content increased from 21.8% to 25.7% after sieving	Maaroufi et al. (2000)

pulse crop to be processed as their different kernel characteristics (e.g. shape and size) would influence how the seeds are milled/ground to specific particle sizes. Furthermore, the difference in composition of the seeds would also influence separation which leads to the need to optimize air classification and electrostatic separation parameters. Pretreatments could be used to reduce the bran content of pulse crops prior to milling and dry classification. This would improve the purity and separation efficiency of the process.

Wet fractionation methods

Wet fractionation methods have long been used to commercially produce protein concentrates from pulse crops (Berghout, Boom, and van der Goot 2014). Various studies have explored the use of wet extraction processes to isolate proteins from legumes such as peas, lentils, and beans (Tang et al. 2021; Ruiz-Ruiz et al. 2012; Dalgetty and Byung-Kee 2003; Sosulski and Sosulski 1986). Common methods usually produce concentrates with protein content ranging from 60% to 80% (protein concentrates) or >80% (protein isolates). Wet fractionation methods are the only method capable to produce protein isolates (Ratnayake and Naguleswaran 2022). The typical wet fractionation method is shown in Figure 3. For starch-rich pulse seeds, the process starts with milling pulse seeds into pulse flour, usually using a pin mill. The flour produced is then dispersed in water followed by hydrocyclone treatment to produce protein and starch fractions. In the case of pulses with high oil content, the resulting flour is first defatted to separate the oil before the hydrocyclone treatment (Schutyser et al. 2015). Proteins are then solubilized in acid or alkaline conditions to separate the proteins. The solubilized proteins are precipitated by adjusting the pH to the isoelectric point of the target protein. This is followed by neutralization to the neutral pH, followed by a drying process to produce powdered protein concentrates or isolates.

A common issue with wet fractionation method is its energy and resource-intensiveness, as the process uses substantial amounts of water and chemicals to produce protein fractions. It was reported that producing 1 kg of lupine protein isolate would use >80 kg water, 22.4 kg hexane, 40 g NaOH, and 40 g HCl (Berghout, Boom, and van der Goot 2014). Hence, improving the sustainability and efficiency of the extraction process has been among the main goals of research involving wet fractionation of pulse flours. Berghout, Boom, and van der Goot (2014) explored the use of an aqueous extraction method to produce producing protein isolates from lupine. They used an extraction process without defatting while altering the pH and temperature parameters, wherein protein concentrates were produced after ultrafiltration and freeze drying. Their results showed that ultrafiltration of full fat lupine flour yielded fractions with comparable protein content (0.72 to 0.85 g protein per g dry matter) to the conventional wet extraction method (0.85 g protein per g dry matter) while having a protein recovery of 0.6 g/g dry weight for the aqueous extraction process.

Ruiz-Ruiz et al. (2012) explored the effects of process variables on the wet extraction process for hard-to-cook beans (*Phaseolus vulgaris L.*) and reported that only flour/water ratio and pH levels had significant effects on the protein and starch yields. Their optimized wet extraction process (flour/water ratio of 1:10 w/v, pH 8, 1 h soaking time) resulted in protein concentrations of 73.03% while meeting the amino acid profile (except for methionine and cysteine) requirements for children and adults. Kornet et al. (2022) optimized the pea protein fractionation process using isoelectric precipitation. In their study, the fractionation process involved dispersing pea flour in water (pH = 8.0)

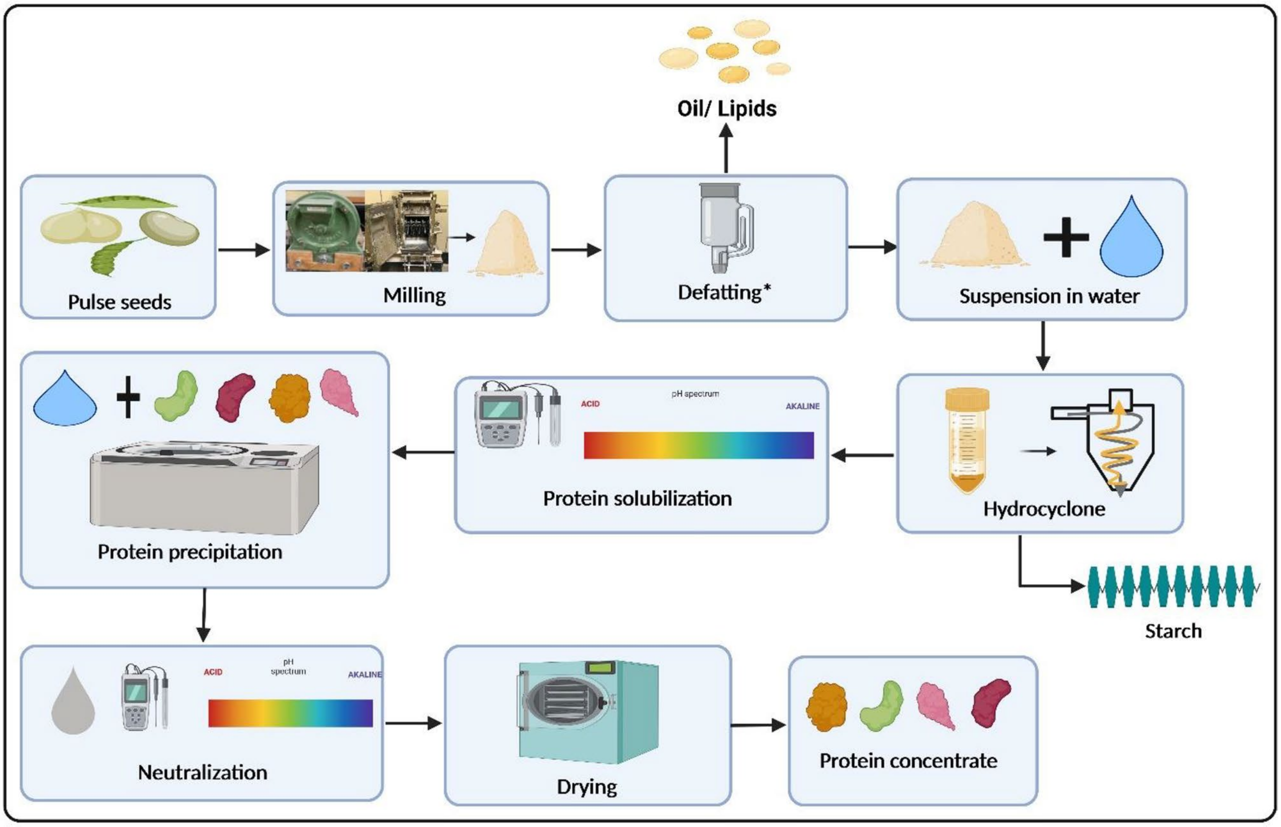

Figure 3. Typical wet fractionation process; * only done if pulse seed has substantial fat content (adapted from Schutyser et al. 2015); Figure made with BioRender.com.

followed by centrifugation. Pea proteins were further separated by isoelectric precipitation (pH = 4.5), resulting in a protein recovery of 74.6% with the isolated fraction having protein content of 54.8%. Their fractionation process also resulted in the separation of the globulin (86.3%) and albumin (52.0%) proteins of pea fractions. These fractions showed differing functionalities, as albumin fractions resulted in a stiff cohesive interfacial layer leading to higher foaming ability and stability, while globulins had poor foaming properties. The pea protein fraction (w/o isoelectric precipitation) had better stability against flocculation compared to each protein fraction.

Improvements in the protein functionality and quality of wet-fractionated proteins have been sought due to the expectation that protein denaturation could occur as the protein is exposed to harsh chemical conditions and elevated temperatures (Schutyser et al. 2015). Gonzalez-Quijada, Alanis-Guzman, and Serna-Saldivar (2003) reported that both pH and temperature had significant effects on ebony seed protein yield and purity. Their results indicated that extraction conditions at pH = 11 and 25 °C increased protein content and lowered tannins, phytates, and trypsin inhibitors content of the protein isolate. Alsohaimy (2007) explored the use of different protein precipitation methods (isoelectric point, ammonium sulfate, methanol, and ethanol) on chickpea, lentil, and bean protein functionality. Alcohol-precipitated proteins had the highest water absorption and foaming capacities, while isoelectric point precipitation gave the best emulsifying properties. Makri and Doxastakis (2006) explored the use of different wet extraction methods (isoelectric precipitation at 4000 and 9000 rpm, ultrafiltration, and 2% NaCl) on bean proteins. They reported that the proteins from ultrafiltration produced the most stable foam at pH = 5.5. Lqari et al. (2005) reported that hydrolyzing lupine protein isolates from wet extraction processes led to improved functional properties (solubility, oil absorption, foam capacity, stability, and emulsion capacity).

Lee et al. (2007) optimized the temperature and pH conditions of the wet extraction method for lentils and reported that a pH of 8.5–9.0 and temperature of 30–35 °C led to optimal protein extraction. Foaming capacities decreased with higher pH while foam stability increased. Papalamprou, Doxastakis, and Kiosseoglou (2010) explored the effects of extraction pH and protein recovery methods on protein quality and reported that different methods and pH caused changes in protein functionality and structure. Furthermore, the extraction method affected the ability of the proteins to adsorb at the oil -water interface resulting in a more stable emulsion. Jarpa-Parra et al. (2014) performed another optimization study for wet extraction of lentil protein, and they reported that the solid/solvent ratio and pH were significant factors. They also reported that increasing pH to alkaline levels (pH 10) caused partial hydrolysis which improved the solubility, foaming, and gelling property of the protein isolates recovered with the protein functional properties comparable to whey and egg proteins.

With the goal of preserving protein quality, Geerts et al. (2017) developed a mild fractionation process for yellow peas consisting of milling (pin and impact milling) followed by suspension of the pea flour in water (pH adjusted to 6.8) and centrifugation. This method relies on separating the flour components (starch, protein, dietary fiber) based on their density. This separation method produced a soluble fraction with a protein content of 60.9% ± 0.5%. Furthermore, Geerts et al. (2017) have also evaluated protein quality in forming emulsions and they reported that the soluble protein fraction has good emulsification properties. This was indicated by the monomodal particle size distribution of the emulsion; good stability against heating and freeze-thaw processes were also observed. Similar results were reported by Kornet et al. (2021) who used a fractionation process consisting of dispersing pea flour in water (pH = 6–8) with agitation, centrifugation (10,000×*g*, 30 min), and isoelectric precipitation steps. This process produced fractions with protein contents ranging from 18.8 to 87.3 g per 100 g dry matter. They also reported that the separated protein fraction had higher gel firmness per mass of protein while also showing a transition from elastic to viscous behavior upon deformation. Betancur-Ancona, Gallegos-Tintore, and Chel-Guerrero (2004) optimized a wet fractionation process for Lima beans and reported that a 1:6 (w/v) flour: water ratio, pH 11, and 1 h extraction time are optimum conditions for alkaline extraction. Their extraction process was able to produce a protein fraction with 71.13% protein content with an in vitro digestibility of 79%.

Based on the previous studies published (summarized in Table 3), wet fractionation methods for pulses can produce fractions with much higher protein content than the dry fractionation methods. The trend in the recent studies has been to utilize or optimize mild wet fractionation processes for pulse protein production. Most of the studies have focused on fractionating pea proteins and dry bean proteins. Hence, future studies could be done for wet fractionation methods on other important pulses such as lentils and chickpea.

Hybrid fractionation methods

Previous studies have explored the effectiveness of combining various fractionation methods to improve the protein separation and quality while also increasing process

Table 3. Summary of wet fractionation methods used to prepare pulse protein fractions.

Pulse type	Method	Findings	References
Yellow pea	Mild fractionation (pH = 8.0), centrifugation, and drying	Protein recovery: 74.6%; Protein content: 54.8%; Further fractionation influenced functionality of protein concentrate	Kornet et al. (2022)
Yellow pea	Milling, mild fractionation, and centrifugation	Protein content – 60.25%, mildly fractionated pea protein produced stable emulsions, and good viscosity	Geerts et al. (2017)
Lentil	Alkaline extraction, optimized pH, extraction temperature, solid/ solvent ratio, and extraction time	Optimal condition at pH – 9.0, and solid/solvent ratio of 1:10; protein content of 82 g/100 g; protein yield of 14.5 g/100 g; pH influenced solubility, gelling, and foaming properties	Jarpa-Parra et al. (2014)
Lupine	Ultrafiltration, freeze drying, altering pH and temperature, without defatting	Protein yield of 0.72–0.85 g/ g dry matter); Protein recovery of 0.6 g/g dry matter; Functionality was comparable at temperatures <90˚C	Berghout, Boom, and van der Goot (2014)
Hard- to-cook beans	Alkaline protein extraction, optimized flour/ water ratio, pH, and extraction time	Concentrate crude protein content – 73.03%; amino acid requirements were satisfied (except methionine and cysteine) high purity for starch, high proportions of insoluble fiber;	Ruiz-Ruiz et al. 2012
Chickpea	Alkaline/ slightly acidic extraction + isoelectric precipitation (pI) or ultrafiltration (UF)	Protein content – 925.2 g/ kg (pI); 900.4–925.2 g/ kg; emulsion stability was dependent on processing condition used.	Papalamprou, Doxastakis, and Kiosseoglou (2010)
Chickpea, lentil, and lupine	Alkaline precipitation + isoelectric precipitation, ammonium sulfate, methanol, or ethanol	Protein recovery – Chickpea (55.80–73.60%), Lupine (79.90–81.10%), Lentil (62.30–80.0%); recovery was dependent on pH, and precipitation method used	Alsohaimy (2007)
Australian lentils (two varieties)	Alkaline extraction, optimized temperature, and pH levels for extraction	Optimal conditions – pH of 8.5 or 9.0 at 30 or 35 °C; protein yield – 53.4–63.4%; Functionality of proteins extracted were dependent on extraction pH	Lee et al. (2007)
Common beans, scarlet runner beans	Isoelectric precipitation, ultrafiltration, and 2% NaCl	Protein content (Common beans) – 66.0–82.5% Protein content (Runner beans) – 68.7–71.5%; Acceptable emulsion and foaming capacity and stability	Makri and Doxastakis (2006)
Lima beans	Alkaline extraction, optimized flour: water ratio, pH, and extraction time	Optimal conditions – pH –11; flour: water ratio – 1:6; extraction time – 1 h; protein content – 711.3 g/ kg	Betancur-Ancona, Gallegos-Tintore, and Chel-Guerrero (2004)

sustainability. The combination of dry fractionation with wet fractionation is expected to produce milder process conditions that could help preserve native protein quality and functionality. Pelgrom, Boom, and Schutyser (2015) explored the use of a hybrid dry-wet fractionation involving air classification followed by aqueous separation and ultrafiltration of the protein suspension to produce refined pea protein concentrates. The modified process was able to produce a protein fraction with a yield and purity of 63%, and 67 g/100 g dry matter, respectively. Avila Ruiz et al. (2016) reported that air classification followed by aqueous separation and ultrafiltration was able to produce a quinoa protein yield of 61.2% with a purity level of 59.4%. Furthermore, the proposed method also used 98% less water compared to conventional wet fractionation methods. This was acceptable as high purity is not highly essential in some food applications since starches and dietary fibers also impart desirable nutritional and functional benefits (Schutyser et al. 2015).

Dumoulin et al. (2021) explored the efficiency of a dry-wet process to produce faba bean protein concentrates. Their proposed method (air classification with alkali extraction) was able to recover 87% of the total seed protein (92% of dehulled seeds proteins), although the anti-nutritional factor contents were close to traditional one step dry or wet-fractionation methods. The method was also reported to consume less energy and 5.5 times less water per kg of protein obtained compared to traditional wet extraction methods. Yang et al. (2022) used a dry-wet hybrid process composed of air classification followed by aqueous extraction, and subsequent ultrafiltration to produce mung bean protein concentrates; this process was able to produce a protein yield of 80.9%. Furthermore, the protein concentrate produced had lower viscosity compared to the wet-extracted proteins at similar concentrations, while having similar heat-set gelation quality to commercial protein concentrates.

Different dry fractionation methods have also been combined in a study by Xing, Utami, et al. (2020) wherein they reported that separating pea flour by air classification followed by electrostatic separation improved protein purity (up to 63.4–67.6 g per 100 g dry matter). This was higher than the protein purity (up to 57.1 g/100 g) achieved through air classification alone. A study by Xing, Dekker, et al. (2020) explored the production of chickpea protein concentrates through the combined dry fractionation and solid-state fermentation. In their study, chickpea seeds were milled using a pin mill to produce grits, which were further milled with an impact mill to produce chickpea flours followed by air classification in producing starch, protein – rich fractions, and chickpea flour. These fractions were then fermented using *Pediococcus pentosaceus* and *Pediococcus acidilactici* cultures. This process resulted in a protein yield of 28.4% and protein content of 45.3 g per 100 g dry matter with improved nutritional characteristics (e.g. lower α-galactosides and phytic acid content) and modified functionality such as increased water-holding capacity and lower foam capacity due to proteolysis (Xing, Dekker, et al. 2020).

These studies (summary can be found in Table 4) show that mixed dry- wet fractionation methods produced fractions with higher protein content and purity than traditional dry fractionation processes while still having lower purity and yield compared to wet fractionation methods. However, superior functional properties of protein fractions from the dry-wet methods were noted when compared to the wet-fractionated protein isolates. The fractionation processes have mostly focused on yellow pea; future studies could focus on using these processes on other major pulse crops such as lentils, dry beans, and chickpeas. Lastly, optimized milling and air classification steps in hybrid processes should be achieved to have greater separation efficiency of the protein and starch components before further separation by wet methods.

Comparison of protein properties from different fractionation methods

Proteins obtained through dry fractionation methods are regarded to have superior functional properties compared to wet-fractionated proteins (Pelgrom et al. 2014). The studies conducted by Tabtabaei et al. (2019) and Jafari et al. (2016) have indicated that the protein functionality and quality of electrostatically separated proteins were similar to the proteins in the original flour. In another study, Opazo-Navarrete et al. (2018) reported that dry milling followed by sieving better preserved the native protein properties of quinoa proteins based on DSC analysis of wet and dry fractionated quinoa proteins. However, one of their main disadvantages is the lower purity of the fractionated protein.

Few studies have directly compared the protein functionality from dry and wet fractionation of similar pulse crop

Table 4. Summary of hybrid fractionation methods used to prepare pulse protein fractions.

Pulse type	Method	Findings	References
Mung beans	Air classification + wet extraction and centrifugation	Protein yield of 71.61 and 80.9% at pH = 6.5 and pH = 8; Protein content – 64.57% (pH = 6.5) and 64.22 (pH = 8); heat set gelation was not affected; lower viscosity compared to commercial mung bean protein concentrate	Yang et al. (2022)
Faba beans	Air classification (twice) + wet extraction and centrifugation	87% of the total seed protein was recovered in two protein fractions; protein content were 54% and 61%; water consumption was 5.5 times less per kg protein; nutritional quality was comparable although anti-nutritional factors were similar to one step dry and wet classified concentrates	Dumoulin et al. (2021)
Pea, lentil, and chickpea	Air classification + electrostatic separation	Protein content increased from 57.1 to 63.4 g/100 g after electrostatic separation. Yield of 15.8 g/ 100 g; protein purity of 63.4–67.6%	Xing, Utami, et al. (2020)
Chickpea	Air classification + spontaneous solid-state fermentation	Protein yield of 28.4% and content of 45.3 g/100 g; fermentation improved nutritional and functional properties of chickpea protein concentrate	Xing, Dekker, et al. (2020)

samples. Vogelsang-O'Dwyer et al. (2020) compared the faba bean protein concentrates obtained by dry and wet fractionation methods. They reported that faba bean protein produced from dry fractionation (milling with air classification) had better solubility (85% vs. 32%) at pH 7, foaming capacity, and similar foaming stability compared to wet-fractionated (isoelectric precipitation) faba bean protein. However, the wet-fractionated protein had better digestibility, and lower trypsin inhibitor activity compared to the dry fractionated faba bean protein. These results were attributed to the difference in the purity of the protein fractions as the carbohydrate content of the dry fractionated protein most likely increased the protein functionality while the better digestibility could be attributed to the higher purity of the wet fractionated proteins; this was attributed to the lower amounts of cell wall material in the wet fractionated protein. In a study by Opazo-Navarrete et al. (2017), the opposite trend was observed in terms of protein digestibility. The quinoa proteins produced by dry fractionation had faster digestibility compared to wet fractionated protein isolates which was attributed to lower agglomeration of particles. Funke, Loeffler, et al. (2022) reported that dry fractionated lentil protein could stabilize a 10% oil/water emulsion, while a wet fractionated protein concentrate did not produce an emulsion. This was explained by the differences in steric repulsion and mechanical strength of the interfacial layers of the protein concentrates.

Tabtabaei et al. (2019) studied electrostatic separation of navy bean flour and reported that the dry classified protein concentrates exhibited better solubility, emulsifying, and foaming properties while also better preserving native protein functionality compared to bean protein isolates obtained by wet fractionation. In contrast, Aryee and Boye (2017) reported that wet fractionated lentil protein isolates had better solubility, water-holding capacity, and fat absorption compared to milled lentil flour. Sridharan et al. (2020) compared the emulsifying capabilities of native pea flour and pea flour isolates. They reported that they had similar emulsifying capacities in terms of rheological evaluation suggesting that further purification may not be necessary for protein emulsions. Kornet et al. (2020) reported that aqueous wet fractionation improved the specific viscosity, specific volume, and lowered the solubility of yellow pea proteins; these improved functionalities were attributed to the formation of aggregates with rarefied structure.

In terms of the amino acid composition of the protein concentrates produced, Jafari et al. (2016) reported that dry classification (electrostatic separation) better preserved the amino acid composition of navy bean flour while wet fractionated protein isolates exhibited lower sulfur containing amino acids. The trend was explained by incomplete extraction of proteins and denaturation of the proteins during wet fractionation. High purity of the protein ingredients may not be necessary in food applications as the non-protein components (lipids, starch, and dietary fiber) can impart added beneficial nutritional and functional properties to the food (Tabtabaei et al. 2016). Dry fractionation process was also the most sustainable, followed by the hybrid fractionation, and wet fractionation based on exergetic analysis (Geerts et al. 2018). Although dry fractionated proteins have better functionality relative to wet fractionated proteins, their lower purity also causes disadvantages such as higher amounts of bioactive components including anti-nutritional factors, enzyme inhibitors, and lectins causing negative effects on protein digestibility (Do Carmo et al. 2021). Lower purity could also lead to off odors and flavors which affect the sensory profile of plant protein enriched foods.

The presence of anti-nutritional factors such as enzyme inhibitors (e.g., trypsin and chymotrypsin), phenolic acids, phytic acid, and flatulence factors is a potential problem in wet and dry fractionated protein concentrates. These anti-nutritional factors are undesirable as they affect the palatability and digestibility of the protein concentrate. For instance, high levels of dietary trypsin inhibitors in legumes and pulses result in substantial reductions in protein digestibility (<50%) and protein quality (Sarwar Gilani, Xiao, and Cockell 2012). De Angelis et al. (2021) reported that yellow peas have higher phytate content compared to green and red lentil, green pea, and Kabuli chickpeas. In dry classified pulse flours, the fine fractions of the pulses tested had higher phytate content (8.7 mg/g dry matter) compared to the coarse fraction. Among the fractions tested, coarse fractions of green peas (6.86 mg/g dry matter) and fine fractions of yellow lentil (14.06 mg/g dry matter) and green peas (13.95 mg/g dry matter) had the highest phytate content. Phytates bind minerals (Fe, Zn, and Ca) in the GI tract reducing mineral bioavailability.

For wet fractionation, Gomezulu and Mongi (2022) investigated the anti-nutritional factors in pigeon pea protein isolates. They reported that the anti-nutritional factors including alkaloid (<20 mg/100g), hemagglutinin (<50 mg/100g), tannin (<1.05/100 g), and cyanogenic glycosides (<12.42 mg/100g) in the protein isolates were present at levels lower than maximum. Wet fractionation relies on alkaline or acid extraction to isolate the proteins in pulse crops. Sęczyk et al. (2019) reported that the solubility of white bean (albumin and globulin) proteins incubated with phenolic compounds were influenced by pH and ionic strength. Significant increase in solubility of protein-phenolic compounds was achieved at neutral pH (6–8) while lowered solubility was achieved in strong acid (pH = 3) and alkaline conditions (pH = 11). Thus, the pH levels used in wet extraction processes is vital as it could also increase the solubility of phenolic compounds, causing it to be extracted along with the protein concentrate potentially affecting its digestibility and palatability. For instance, Sęczyk, Gawlik-Dziki, and Świeca (2021) reported that protein digestibility in white bean paste was lowered when mixed with phenolic compounds (gallic acid, ferulic acid, chlorogenic acid, quercetin, apigenin, and catechin). In contrast, Rivera Del Rio et al. (2022) reported that mild fractionated pea proteins had comparable digestibility to a conventional wet fractionated pea protein isolate. However, they also noted that non- protein materials (e.g. starch) can inhibit digestion, and denaturation (via heating) of the protein increased digestibility of both wet and dry protein concentrates. Vogelsang-O'Dwyer et al. (2020) reported that wet fractionated faba bean proteins had better digestibility than dry fractionated faba bean proteins. The presence of these anti-nutritional factors explains this trend as they observed lower trypsin inhibitor activity in the wet fractionated protein sample. Trypsin inhibitors are one of the most common anti-nutritional factors and they reduce digestion and absorption of dietary proteins. Overall, wet fractionated proteins could exhibit better digestibility than dry fractionated proteins due to their higher purity. However, studies are needed to further investigate this as interactions of components, differences in phytochemical, and fractionation processes used could impact digestibility.

The sensory attributes of protein concentrates are also affected by the levels of phytochemical compounds present. In general, pulse protein concentrates have sensory profiles associated with bitterness and astringency which were found to be associated with anti-nutritional factors. Cosson et al. (2022) identified a total of 48 phytochemicals in pea proteins responsible for their sensory attributes; phenolic compounds (caffeic acid), flavonoids (quercetin-3-O-glucoside), and six saponin compounds highly correlated to bitterness and astringency. Lastly, they also suggested that conditions such as high temperatures and acidity used in producing protein concentrates resulted in different phytochemical profiles. Phytochemical compounds are known to be unstable and easily destroyed at non-optimal pH and temperature conditions. By this, dry fractionated proteins could be more prone to higher levels of anti-nutritional factors as this process does not involve high temperatures or pH changes. Dry and wet fractionated protein concentrates have limited applications in foods due to their sensory profiles. Low substitution levels were often preferred for pulse protein-containing food formulations. Modification of the protein properties could offset the negative effects on the sensory properties of fractionated pulse proteins.

A study by Xu et al. (2020) explored the role of sprouting on the flavor profile of chickpea, lentil, and yellow pea isolated by alkaline extraction with isoelectric precipitation method. They reported that germination plays a role in the beany odor and flavor perceived in protein concentrates due to the lipoxygenase, and free radicals produced through sprouting. Vatansever et al. (2021) developed the use of a supercritical carbon dioxide + ethanol extraction to improve the functionalities of a pea protein isolate. The treatment caused higher moisture, starch, and protein content than the untreated sample; higher denaturation temperature, protein solubility, emulsion, and foaming properties for the treated pea protein isolate were also observed. In another study, Shi, Arntfield, and Nickerson (2018) reported that cooking Canadian pulses (peas, lentils, chickpeas, faba beans, and common beans) reduced anti-nutritional factors (lectins, total, and soluble oxalates) except phytic acid. Lastly, varieties of the pulse crop used significantly influences the sensory characteristics of protein concentrates.

Arteaga et al. (2021) reported that under the same fractionation process, different varieties of peas had different sensory profiles. Some cultivars had protein isolates with stronger "pea-like" aroma, while some had a stronger "green" aroma. These differences were attributed to the different levels of phytochemicals in pea cultivars. Currently, studies on investigating the effects of fractionation processes on the phytochemical content of protein concentrates and their impact on sensory attributes are limited. Research on this could greatly help in identifying which phytochemicals should be removed or retained to improve sensory attributes of protein concentrates. Future studies on developing processes that remove or destroy phytochemicals in pulse protein concentrates are also needed as limiting these compounds can help improve pulse protein palatability and nutritional value.

Overall, the fractionation process has an influence on the levels of anti-nutritional factors present in pulse protein concentrates. The levels of these factors can affect the sensory and digestibility profiles. Hence, consideration should be given on selecting process parameters that would inhibit the transfer of these anti-nutritional factors. Furthermore, future studies could be done on directly investigating the impacts of anti-nutritional factors on the sensory and digestibility of pulse protein concentrates; these could help in determining which anti-nutritional factors negatively impact pulse protein acceptability.

Functional properties and food applications of fractionated pulse proteins

Functional properties

The applications of the fractionated pulse protein ingredients are mostly dependent on their functional properties (Goldstein and Reifen 2022; Shen, Hong, and Li 2022; Shen and Li 2021). These include solubility, water binding capacity, fat binding capacity, emulsifying properties, foaming, gelation, and so on. Pulse protein solubility is mostly dependent on the pH level of the medium with solubility being greatest at pH levels away from the protein's isoelectric point (low acidic or high alkaline pH) value (Boye, Zare, and Pletch 2010). Water binding capacity (WBC) refers to the quantity of water that can be absorbed per gram of protein. Fat binding capacity (FBC) defines the amount of fat/oil that can be absorbed per gram of protein material. These functional properties influence the texture, sensory quality (visual and organoleptic), and process quality (cook yield, water loss) of foods with pulse protein ingredients. Emulsifying activity refers to the amount of oil emulsified per unit of protein, while emulsifying stability refers to the ability of the emulsion to remain stable over a certain time (Boye, Zare, and Pletch 2010). Proteins can stabilize emulsions by coating oil droplets dispersed in an aqueous medium; the protein coating inhibits creaming, coalescence, and flocculation of lipid droplets in the medium.

Foaming capacity and foam stability are other functional property of proteins where capacity refers to the amount of interfacial area created by whipping protein while stability refers to the time required for the foam to lose certain amount of its volume (Mauer et al. 2003). Proteins can form foams once their structure is denatured (e.g. through whipping). Denaturation exposes the hydrophilic and hydrophobic ends which are attracted to water and air respectively. Foams are then eventually formed once the protein molecules are linked with each other, entrapping air in suspension preventing their collapse (Boye, Zare, and Pletch 2010; Zayas 1997). Thus, the foaming functionality of proteins depends on factors such as hydrophobic and hydrophilic residues, protein source, and process parameters such as temperature, pH, and mixing time (Zayas 1997). Gelation property of proteins are typically measured as the least gelling concentration (LGC), which is the smallest amount of protein in a slurry required to form a stable gel (Chandra, Singh, and Kumari 2015). With this, when proteins can create stable gels at low amounts, the proteins are regarded to have better gelation functionality. These functionalities determine the suitability of protein ingredients in various food applications including fortification and gluten-free formulations. Pulse proteins when used as food ingredients, can impart nutritional benefits including better amino acid profiles, low energy density, and higher protein content (Gangola et al. 2022). The next section reviews studies that used fractionated pulse protein ingredients in various food applications. A summary of these applications can be found in Table 5.

Applications in bakery goods

As pulses are high in protein, adding them into food products will result in higher protein content and improve amino acid profile (Des Marchais et al. 2011). The consumption of pulse protein – enriched foods is important as it helps provide all the essential amino acids needed by humans while improving protein content. In bakery products, pulse protein ingredients have been used as an enrichment ingredient to compensate for essential amino acid deficiencies (lysine and threonine) in wheat flour ingredients. Pulse flours were reported to contain high amounts of phytate, which reduce protein digestibility and mineral bioavailability (Des Marchais et al. 2011). Furthermore, substitution levels above 10% were reported to be harmful as it negatively affected bread quality by reducing dough stability and loaf volume while increasing crumb hardness (Dhingra and Jood 2004; Sadowska et al. 2003; Pollard et al. 2002).

Des Marchais et al. (2011) used pea protein isolates (10% flour basis) produced by wet fractionation (ultra-filtration and diafiltration) in bread making. The use of the protein isolate resulted in higher flour water absorption, without affecting dough stability and development time while also yielding higher bread protein content (20%). In a subsequent study, Aider, Sirois-Gosselin, and Boye (2012) explored the use of wet fractionated chickpea, lentil, and pea protein concentrates in bread making, and reported lower bread volume (at >3% level substitution), darker crumb color (due to lentil), and increased crumb hardness. In another study, Ugwuona and Suwaba (2013) reported that the use of jack bean protein concentrates (at 20%) in bread making resulted in similar changes in bread volume with increased protein content (9.45 to 11.16%), but with decreased carbohydrate content (72.12 to 50.39%). Furthermore, the bread quality parameters (volume, color, texture, and flavor) and consumer acceptability scores of the substituted bread formulations were statistically similar. The reported changes were also verified in more recent studies on reformulated breads using fractionated legume proteins. Belc et al. (2021) studied the effects of enriching (5%, 10%, and 15%) bread with pea, or soy protein concentrate, and reported an increase in bread protein content (1.1 to 1.7 times higher than control) with varying effects on bread quality based on the ingredient used. They reported that the use of soy protein led to lower bread volume and softer crumb hardness. The use of pea protein led to a greater decrease in bread volume and darker crumb color. Consumer acceptability tests also indicated that breads enriched with pea protein were less liked compared to soy protein enriched breads. Shivaani (2020) reported that the use of soy protein isolates (at 20% substitution) resulted in similar effects on bread quality.

Bojnanska, Musilova, and Vollmannova (2021) studied the effect of using legume (chickpea, broad bean, common bean, and red lentil) ingredients (5%, 10%, and 15%) in wheat–rye flour bread formulation. They reported similar findings where higher enrichment levels resulted in lower bread volume, increased resistance to starch retrogradation, and changes to aroma and flavor. Lastly, only the 5% enrichment level had similar acceptability to the control formulation. Xing et al. (2021) explored the use of dry fractionated chickpea protein as a sourdough ingredient for protein enrichment of wheat bread. The results of their study showed that there was a 38.5% increase in bread protein content (at 30% level), and increased mixing time with larger levels of substitution. This also resulted in a lower specific bread volume, and denser crumb structure. The cited effects to bread quality (lower bread volume) were attributed to the dilution of the gluten network of the bread.

The use of plant protein ingredients at high levels was more successful in non-bread products such as cookies and cakes wherein the formation of a gluten network is not required (Bresciani and Marti 2019; Zucco, Borsuk, and Arntfield 2011). Sarabhai et al. (2015) reported that the use (5%, 7.5%, and 10% flour basis) of soy protein concentrate, along with whey protein improved the quality of rice flour cookie and helped maintain gluten within allowable limits for consumers with celiac disease. Mancebo, Rodriguez, and Gomez (2016) studied the inclusion of pea protein concentrate in sugar snap cookie formulation and reported that its addition increased dough hydration and dough consistency while also reducing cookie spread and increasing darker cookie color. The sensory acceptability of the cookies made was not affected by the protein addition. Ostermann-Porcel et al. (2017) used soy protein (0%, 15%, 30%, and 50%) with manioc flour for a gluten-free cookie formulation. They reported increased protein and dietary fiber content, increased cookie hardness, lower whiteness, and similar sensory acceptability for the cookies enriched with soy protein relative to the control formulation.

Table 5. Food applications of fractionated pulse protein ingredients (DF: dry fractionated; WF: wet fractionated).

Product	Protein ingredient used	Findings	References
Bakery products			
Crackers	Faba bean protein (60%), and isolate (90%)	Improved nutritional and functional properties (lower starch and fat content)	Gangola et al. (2022)
Wheat bread	Pea protein, or soy protein concentrate	Higher decrease in bread volume, crumb color for pea protein fortified breads; breads made with pea protein was less liked in sensory evaluations	Belc et al. (2021)
Wheat bread	Chickpea protein (DF)	38.5% increase in bread protein content; lower bread volume, and denser crumb structure	Xing et al. (2021)
Wheat-rye bread	Chickpea, broad bean, red bean, and red lentil	Lower bread volume, increased resistance to starch retrogradation, and increased flavor; 5% substitution had similar acceptability to control	Bojnanska, Musilova, and Vollmannova (2021)
Sugar snap cookies	Pea protein concentrates	Increased hydration properties, reduced cookie dimensions, increased hardness, and darker color	Mancebo, Rodriguez, and Gomez (2016)
Wheat bread	Chickpea, lentil, and yellow Pea (WF)	Lower mass volume, darker color, and higher bread hardness	Aider, Sirois-Gosselin, and Boye (2012)
Wheat bread	Yellow pea protein (WF)	Bread protein content increased above 20%; higher dough consistency and decrease in loaf specific volume	Des Marchais et al. (2011)
Pasta			
High protein pasta	Pea protein isolate	Use of pea protein improved chemical score, amino acid digestibility, color, and cooking quality	Messia et al. (2021)
Pasta	Chickpea protein isolate	Increased swelling index, texture properties, and higher cook loss	El-Sohaimy et al. (2020)
Gluten – free noodles	Chickpea protein isolate	Higher protein content, dough elasticity, and viscous moduli; darker color, and longer cook time	Ahmad Sofi et al. (2020)
High protein pasta	Pea protein isolate	Protein content increased by 2.9 times, increased hardness, cook time, and lower cook loss	Rousta, Yazdi, and Amini (2020)
Pasta	Faba bean protein concentrate or isolate	Reduced postprandial glycemia and appetite of consumers, increased protein content, and nutritional quality	Chan et al. (2019)
Pasta	Pea protein isolate	Did not alter pasta structure, improved mechanical strength, extensibility, and cooking quality	Muneer et al. (2018); Mercier et al. (2011)
Pasta	Faba bean protein isolate, and common bean protein isolates	Increased cook loss, lower resilience, and increased *in-vivo* protein digestion	Laleg et al. (2017); Segura-Campos et al. (2014)
Meat and meat analogue applications			
Beef patties	Modified (deamidation and conjugation) pea protein (as meat extender)	Reduced cook loss, and hardness; increased yield, and tenderness	Shen, Hong, Du, et al. (2022)
Vegetable hamburger patties	Pea, lentil, faba bean concentrate, and pea protein isolate	Darker color, lower textural integrity, high moisture, and water absorption compared to soy-based formulation	Kim et al. (2021)
Meat analogs	Pea protein (DF and WF)	Lower hardness, high oil absorption, neutral sensory profiles	De Angelis et al. (2020)
Low – fat pork burger	Lentil, chickpea, peas, and bean protein (as meat extenders)	Improved cook yields, lower diameter reduction, and cook loss	Argel et al. (2020)
Beef and pork patties	Navy, red, kidney, and small red beans (as meat extender)	At 50:50 ratio, optimal weight loss, diameter loss, color, and texture	Holliday et al. (2011)
Dairy alternatives and emulsified foods			
Mayonnaise	Modified pea protein (conjugated with guar gum)	Use of modified pea protein (6 and 8%) exhibited better emulsification and viscoelasticity compared to pea protein isolate and egg yolk powder in mayonnaise application	Shen, Babu, et al. (2022)
Vegan mayonnaise	Faba bean, white bean, and cowpea protein isolate	Use of faba bean protein resulted in stable emulsion (< 6 months), and acceptable economic return	Abdel-Haleem, Omran, and Hassan (2022)
Plant-based milk	Lentil protein isolate with sunflower oil	Comparable sensory profile to commercial plant-based milk alternatives	Jeske et al. (2019)
Plant-based milk	Lupine protein	Acceptable sensory profile due to reduced lipoxygenase activity	Jacobs et al. (2016)
Salad dressing	Lentil, pea, or chickpea protein isolate	Optimized protein levels led to improved rheological, and textural properties of pulse-based salad dressing	Ma, Boye, and Simpson 2016

Malcolmson et al. (2013) reported that supplementation of crackers with lentil flours resulted in a crisp texture, with improved flavor, and a darker color compared to the control formulation. Han, Janz, and Gerlat (2010) also explored the use of pea protein isolates along with normal chickpea, red and green lentil, yellow pea, and navy bean flours in cracker making and reported comparable consumer acceptability to the control. Gomez, Doyague, and de la Hera (2012) used air classified pea protein in cake formulation, and reported lower specific volume, increased firmness, and decreased acceptability scores. Gangola et al. (2022) used faba been protein isolates (90%) and concentrate (60%) in cracker formulations and reported improved nutritional profiles for crackers including higher protein, dietary fiber, and resistant starch content than wheat-based cracker formulations.

Applications in pasta formulations

Pasta products play an important role in human nutrition as they are among the most consumed foods in the world. Improving the nutritional profile of pasta can be achieved by increasing their protein content and balancing their amino acid profile. In general cereal-based pastas have low lysine, which is an essential amino acid to humans. Pulse crops have high lysine contents; hence, the inclusion of pulse proteins in noodles and pasta formulations can increase the lysine and protein content of these products. Messia et al. (2021) incorporated pea and soy protein isolates in the formulations for high protein pasta and reported that pasta formulations with pea and soy protein isolates showed better chemical score, digestible indispensable amino acid scores, improved color and cooking quality (firmness). The effects of pea protein concentrate fortification (0–15%) on pasta structural characteristics (porosity, shrinkage, and density) during drying was investigated by Mercier et al. (2011). In their study, they reported that pea protein (isolate) fortification did not significantly alter pasta structure (porosity, density, and shrinkage) with fortified samples having higher effective moisture diffusivity coefficients (5% and 10% fortification) than non-fortified samples. Muneer et al. (2018) reported similar findings with pea protein fortification of pasta sheets wherein the addition of pea protein (isolate) in the formulation improved the mechanical properties (strength, and extensibility) and cooking quality of fortified pasta samples. The findings from these studies indicated that the use of pea proteins did not affect pasta structure; this is important as one of the hurdles for legume fortification of breads is the loss of bread structure.

Rousta, Yazdi, and Amini (2020) published a study on the optimized formulation for pea protein isolate (24%) on pasta formulations and reported an increase in protein content, hardness, cooking time, and cook loss with higher protein fortification. Faba bean protein concentrates and isolates were also used in pasta formulations from a study by Chan et al. (2019). The addition of faba bean protein concentrate (25%) and isolates (25%) resulted in health benefits to consumers including reduced postprandial glycemia, increased satiety, and higher nutritional quality. Similar nutritional benefits were also reported by Greffeuille et al. (2015) wherein the inclusion of faba bean proteins (35%) in pasta increased satiety and digestive comfort of consumers although there was no impact on glycemic or insulin response. Laleg et al. (2017) incorporated faba bean protein isolate in pasta formulations and reported similar findings including higher cook loss, lower resilience, and higher in vitro protein digestion. The drying process also influenced protein aggregation as increasing temperatures encouraged aggregation which resulted in lower pasta resilience. The sensory profiles from the faba protein fortified pasta were comparable to commercial gluten-free pasta samples. In contrast, Segura-Campos et al. (2014) incorporated common bean protein hydrolysates (enzyme hydrolyzed) in semolina pasta (5% and 10%) and reported similar physical characteristics for fortified (5 and 10%) and non-fortified pasta samples. Furthermore, improved sensory profiles and higher antioxidant activity (TEAC) were also observed for fortified pasta samples demonstrating potential health benefits. El-Sohaimy et al. (2020) explored the effects of semolina pasta fortification with chickpea protein isolate (2.5%, 5%, 7.5%, and 10%) and reported increased swelling index, texture (hardness, cohesiveness, springiness, gumminess, chewiness). However, several adverse effects were also reported including increased cook loss and lower protein digestibility.

Fractionated proteins from pulse crops have also been used in gluten-free formulations. Ahmad Sofi et al. (2020) explored the development of high protein gluten-free noodles made from rice flour by incorporating chickpea protein isolates (2%, 4%, 6%, 8%, and 10%). Positive effects including higher protein content, antioxidant activity, decreased glycemic index (GI), and increased dough elastic and viscous moduli. Negative effects such as darker noodle color and longer cooking time was also observed; among treatments, the 6% fortification had the most acceptable characteristics. Shukla et al. (2021) conducted a study on optimizing gluten-free pasta formulation using faba and pea protein isolates and reported that using protein ratios of 30:70 and 43:57 (pea: faba) resulted in acceptable extrusion, cook time, cook loss, swelling index, color, and hardness.

Based on the published studies, the inclusion of fractionated protein ingredients is a feasible way to improve the nutritional benefits of pasta foods. Briefly, the use of pulse protein ingredients resulted in improved protein content, in vitro protein digestibility, cooking quality, and increased antioxidant activity. The drawbacks include reduced texture (hardness and resilience), darker noodle color, and cook loss. Among these, the reduction in texture quality is the most important detriment. However, the published studies have reported that increasing drying temperature can help increase protein aggregation which can mitigate the loss of structure brought by using pulse proteins in pasta formulations. Most of the studies have also done optimizations for pea and faba proteins. More studies to investigate the feasibility of other pulse proteins such as those from beans, lentils, and chickpea as fortification or gluten-free base in pasta formulations. The studies demonstrated that the process can help improve the pasta texture; optimization studies consisting of protein substitution and varied process parameters could be recommended. Lastly, as soy is more commonly as a protein fortification ingredient, the establishment of formulations using pulse crop proteins can provide a path to cheaper, and less allergenic food products in the market.

Applications in meat analogs and meat products

Consumers have increased their adoption of plant-based diets, and this has led to the increased awareness of pulse-based foods as protein sources. However, a significant number of consumers still regard animal-based products as their main protein source. Hence, the consumption of pulse-based proteins can be increased by creating products with similar sensory and organoleptic properties to those of meat-based foods. These products are called meat analogs, which are products that mimic meat functionality by having meat-like appearance, texture, and sensory attributes (Ismail, Hwang, and Joo 2020). Meat products are mainly composed of water, protein, and fat. The fat binding, water binding, and emulsifying capacity properties of proteins are important in determining their suitability as meat analog ingredients (Hong, Shen, and Li 2022).

De Angelis et al. (2020) explored the physicochemical and sensory qualities of meat analogs made from dry fractionated pea protein (P_{DF}) combined with other protein sources such as pea protein isolate (P_{IS}), oat protein (O_P), and soy protein isolate (S_{IS}). They used a low-moisture extrusion process. Samples made with P_{DF} were characterized to have high specific mechanical energy (SME), lower protein content, high fat content, and high carbohydrate content. In terms of texture, samples made from P_{DF} had a shorter rehydration time, and lower cohesiveness, hardness, springiness, and elastic behavior. Sensory characteristics for P_{DF} were characterized to have more intense taste and odor profiles compared to isolates who had more neutral profiles. Zhu et al. (2021a) studied the use of wet and dry fractionated pea protein fractions for meat analog preparation and reported that dry pea protein-based analogues had more resistance to elastic and viscous deformation, darker color, and lower textural properties (hardness and chewiness) compared to the wet pea protein-based analogs, which had higher protein content, greater hardness, and greater chewiness. These differences were linked to the purity of the two protein fractions as dry fractionated pea proteins had more starch, phenolic compounds, and pigments which contributed to the softer texture and darker color.

Ramos Diaz et al. (2022) combined pea protein isolates and oat fiber to make meat analogs, and reported that it was possible to make high fiber meat analogs with pea protein isolates although samples had lower water holding capacity and structural strength (hardness, chewiness). In another study, Kim et al. (2021) studied the production of high moisture meat analogs (HMMA) by combining pulse protein concentrates (pea – 16%, lentil – 16%, and faba bean – 20%) with pea protein isolate (63% or 59%). They reported darker color, lower textural integrity, higher moisture, and water absorption from texturization compared to a control formulation that used a soy protein concentrate-isolate. It was also recommended that soaking HMMA is a better method for hydration compared to boiling. Soaking encouraged higher moisture, specific density, water absorption, and solubility index. As an alternative to pea proteins, lentil, chickpea, and bean proteins could be used as ingredients for meat analogs. The main challenge in adopting other alternatives to pea proteins is that lentil, chickpeas, and bean proteins had lower gelling and emulsifying capabilities (Kyriakopoulou, Keppler, and van der Goot 2021). Thus, recent studies have focused on improving the functionalities of these proteins using pretreatment methods or protein modification techniques. Chickpeas have better protein functionalities for meat analog applications including good water and oil binding, and good gelling properties (Kurek et al. 2022; Sanjeewa et al. 2010). Sanjeewa et al. (2010) reported that chickpea inclusion in low fat bologna improved its textural properties without significantly affecting its flavor attributes (sensory evaluation).

Aside from being used as a meat analogue, pulse proteins exhibit great potential as meat extenders due to their protein functionalities reducing the meat content required in food products (Pintado and Delgado-Pando 2020). Argel et al. (2020) reported that adding pulse flours (lentil, chickpeas, peas, and beans) at different water-flour ratios (pulse flour: 80 to 150 g; water: 100 to 300 g) improved the cook yield, diameter reduction, and cook loss of low-fat pork burgers compared to the control (all-meat formulation). Holliday et al. (2011) reported that the beans such as navy, red kidney, and small red beans were viable extenders for beef, and pork patties at 50:50 ratio; using beans resulted in optimal weight loss, diameter loss, color, and texture of the beef and pork patties. Shen, Hong, Du, et al. (2022) compared the effect of incorporating 2.5% and 5% pea protein isolate (PPI) or deamidated/conjugated PPI (PGG) on the cookability, physical and texture properties, and sensory attributes of beef patties in comparison with regular patties. Results showed that extending beef patties with PPI or PGG reduced cook loss, and thus increased cooking yield, reduced patty hardness, and increased tenderness. These changes led to decreased juiciness lower beef flavor scores, and increased off-flavor scores. The beef patties containing PGG also showed much softer and more tender texture relative to the control or PPI patties, which would be advantageous features for consumers with such sensory preference.

Although pulse proteins are effective ingredients as meat analogs and meat extenders, their applications are still limited. This is due to the potential changes in sensory quality such as color, flavor, and texture which is important as it affects consumer acceptability of the products. Hence, it is important for future studies to optimize substitution levels that would minimize potential negative effects on sensory attributes and explore processes and techniques that would help improve the protein functionalities.

Applications as plant – based dairy alternatives and emulsifying agents

Pulse proteins are also viewed as a potential ingredient for dairy alternatives. Currently, soy-based dairy products have been the most common dairy alternatives to cow's milk due to their higher protein content compared to other alternatives such as nut-based products (e.g. almonds) (Vogelsang-O'Dwyer et al. 2021). Dairy alternatives that use pulses are limited by the composition of the raw material used. The use of pulse protein concentrates will allow formulated dairy alternatives to achieve similar nutritional profiles to dairy milk. In this application, pulse proteins act as an emulsifier and nutritional ingredient in pulse-based milk alternatives (McClements, Bai, and Chung 2017). A study by Jacobs et al. (2016) produced a lupine-based milk alternative with ultra-high temperature (UHT) processing which resulted in acceptable sensory profile due to reduced lipoxygenase activity. Jeske et al. (2019) used lentil protein isolate and sunflower oil in a similar process which resulted in a product with comparable sensory profiles to other milk alternatives. Commercial products that used pulse protein ingredients in dairy alternatives have been available in the market, as described in a review by Vogelsang-O'Dwyer et al. (2021).

The variety of pulse protein ingredients each bring different changes to the sensory quality of the product. Hence, it is important to adjust the formulations with the aim of improving consumer acceptability. The emulsification properties of pulse proteins are also useful in the development of salad dressings (Shen, Babu, et al. 2022). Ma, Boye, and Simpson (2016) optimized the formulation of salad dressing using either lentil, pea, or chickpea protein isolates as a substitute emulsifier to egg yolk. They reported that optimizing protein levels led to improvement in rheological and textural properties of the dressing resulting in comparable characteristics to commercial products. Abdel-Haleem, Omran, and Hassan (2022) reported that in protein isolates from faba bean, white bean, and cowpeas, solubility and net surface charges were significant factors in protein emulsifying capabilities. Faba bean proteins were observed to have the best properties compared to white bean, and cowpeas. In addition, the 3% faba bean vegan mayonnaise formulation exhibited better stability, and economic value (~$7.58) compared to 3% cowpea which was not as satisfactory. Lafarga et al. (2020) utilized various pulse protein isolates such as lentils, cowpeas, faba beans, chickpeas, runner beans, beans, and peas. They reported that the protein isolates exhibited acceptable water and oil holding capacities, emulsifying, and foaming capacities, which show their potential in emulsification and foaming applications. A study by Shen, Babu, et al. (2022) reported that the use of pea proteins conjugated with guar gum had excellent emulsifying and viscoelastic properties in mayonnaise applications; the modified protein ingredient used at 6 and 8% showed better functionalities compared to egg yolk, and pea protein isolates. Hence, pulse proteins possess great potential as emulsifiers which can replace animal-based proteins in product formulations. The potential of dry fractionated pulse proteins as emulsifiers could be further explored, as well as the use of modification techniques to further improve protein functionality (Shen, Hong, Singh, et al. 2022; Shen, Du, et al. 2022).

Future research opportunities and conclusion

Pretreatment methods have been widely studied for different pulse crops. Research could be done to find optimal pretreatment methods for major pulse seeds (lentil, pea, beans, or chickpea) considering the differences in seed characteristics and composition. The fractionation methods discussed in this paper have several advantages and disadvantages when used to produce plant protein ingredients. For wet fractionation, the disadvantages are the loss of certain protein fraction and functionality and sustainability issues with regards to its process. As for dry fractionation, the disadvantages are its modest protein enrichment and lower purity, which lead to decreased palatability and nutritional benefits. However, dry fractionated protein ingredients were also reported to have superior functional properties compared to wet fractionated proteins. These disadvantages have been lessened with the use hybrid fractionation methods which combine some steps from the individual fractionation processes providing a good compromise for quality, purity, and sustainability. Future research could focus on optimizing and modifying the pretreatment process as well as the dry and hybrid fractionation methods to improve sustainability and protein quality. Pulse protein ingredients were also incorporated in the formulations for various food commodities. However, the use of pulse ingredients led to changes in product quality (texture, color, and flavor changes) which influenced consumer acceptability. Research could be implemented on better optimization of formulations involving pulse protein concentrates and removal of anti-nutritional factors in protein concentrates. These could help improve the palatability and digestibility of pulse protein concentrates, which can increase their suitability in food formulations. For example, Vatansever et al. (2021) reported that using supercritical CO_2 with ethanol extraction lowered the levels of off-aroma compounds in pea flour after processing. Fractionation processes are essential to produce protein-rich ingredients from plant sources. These fractionated ingredients could then be used in food formulations to help meet the projected increase in food supply demands of the increasing population. Thus, it is important for future research to improve the yield of fractionation process, increase palatability and nutritional value, and optimize food applications of pulse protein concentrates.

Acknowledgements

This is contribution No. 23-004-J from the Kansas Agricultural Experimental Station. This research was supported in part by the USDA Pulse Crop Health Initiative grants (Grant Accession No. 0439205 and No. 0439200) and the USDA National Institute of Food and Agriculture Hatch project (Grant Accession No. 7003330).

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This is contribution No. 23-004-J from the Kansas Agricultural Experimental Station. This research was supported in part by the USDA Pulse Crop Health Initiative grants (Grant Accession No. 0439205 and No. 0439200) and the USDA National Institute of Food and Agriculture Hatch project (Grant Accession No. 7003330).

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