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Production of pulse protein ingredients and their application in plant-based milk alternatives

Martin Vogelsang-O'Dwyer [a], Emanuele Zannini [a], Elke K. Arendt [a,b,*]

[a] School of Food and Nutritional Sciences, University College Cork, Ireland
[b] APC Microbiome Ireland, University College Cork, Ireland

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ABSTRACT

Background: Plant-based milk alternatives are surging in popularity, although many examples have poor nutritional value compared to cow's milk. At the same time, protein concentrates and isolates from pea and other pulses are increasingly being recognised for their potential as functional and nutritious ingredients.
Scope and approach: This review contains an overview of pulse proteins and the dry and wet fractionation methods used to produce high-protein ingredients. The influence of pulse type and processing on the techno-functional properties of ingredients is discussed. Additionally, the application of pulse protein ingredients in milk alternatives is explored, with the goal of providing high protein alternatives to cow's milk.
Key findings and conclusions: Pulse proteins ingredients have received much interest for their functionality and potential to replace animal proteins. A considerable amount of research has been generated encompassing novel protein sources, as well as processing methods, with the aim of producing highly functional ingredients. The functional properties of pulse proteins along with the high protein content of isolates/concentrates provide the opportunity to formulate plant-based milk alternatives with higher nutritional value compared to many others currently on the market. Such products containing pea protein are now available, and various other pulse proteins could also be applied in these products as they become more widely available.

1. Introduction

In recent years, the demand for alternative food protein sources that could potentially replace animal proteins has been increasing. There are various driving forces behind this dietary shift, including sustainability, health, and ethical considerations. In order to address the threat of climate change while ensuring food security for the world's growing population, a move towards a more plant-based diet may be unavoidable (Poore & Nemecek, 2018; Willett et al., 2019). However, animal-based foods such as milk, meat and eggs are generally high in protein with well-balanced amino acid profiles, thus care must be taken when providing plant-based substitutes to ensure adequate nutritional quality. Milk substitutes in particular vary widely in nutritional quality; with the exception of soy beverages, most products contain relatively little protein (Jeske, Zannini, & Arendt, 2017; Vanga & Raghavan, 2018). Therefore, there is a need for more plant-based products with protein levels comparable to cow's milk.

Soy-based products can provide a good source of high quality protein. However, soy has negative associations for consumers, including allergenicity and the prevalence of genetically modified varieties (Boye & Maltais, 2011; Fischer, Cachon, & Cayot, 2020), and may not be suitable for cultivation in every climate. Therefore, there is a need for alternative protein sources. Various pulse crops could potentially be used as alternative protein sources in the formulation of milk alternatives and related products, including pea, faba bean, lentil, lupin, chickpea and common bean. Already, high-protein pea protein-based milk substitutes have begun to be commercially available (Mintel Group Ltd., 2018). However, in contrast to soybeans, most pulses are relatively high in starch and other carbohydrates (Boye, Zare, & Pletch, 2010), and most likely require protein isolation/concentration steps to yield high-protein ingredients suitable for milk alternative formulations approaching the nutritional value of cow's milk. At present, the only widely available pulse protein concentrates or isolates are derived from pea. However, alternative protein sources such as chickpea, faba bean and others are being explored (Arntfield & Maskus, 2011). Pulse protein ingredients must possess good functional properties, such as solubility and emulsifying properties, if they are to be applied in milk substitutes or other dairy-type products. These properties may be influenced both

* Corresponding author. School of Food and Nutritional Sciences, University College Cork, Ireland.
E-mail address: e.arendt@ucc.ie (E.K. Arendt).

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by the seed material used and subsequent processing. This review attempts to provide an overview of pulse proteins, their chemistry/structure, manufacture of protein concentrates and isolates, functional properties and potential for application in milk alternative products. While recent reviews have focused on plant-based milk alternatives from various sources (McClements, Newman, & McClements, 2019; Sethi, Tyagi, & Anurag, 2016), this review focuses specifically on pulse protein-based products.

2. Chemistry and structure of pulse proteins

The majority of proteins found in legume seeds are storage proteins, providing free amino acids upon germination, along with ammonia and carbon skeletons (Duranti, 2006). Pulse proteins can be divided into the 'Osborne fractions' based on their solubility in different solvents. These are albumins, globulins, prolamins and glutelins. Albumins are soluble in water, globulins are soluble in saline solutions, prolamins are soluble in concentrated aqueous alcohol solutions and glutelins are soluble in dilute acid or alkali solutions (Day, 2013). The storage proteins of soy and pea have been studied most extensively, however, the proteins found in various legumes share structural similarities. The majority (approx. 70%) of pulse proteins are comprised of globulins, while albumins and glutelins may each account for 10–20% of the proteins (Roy, Boye, & Simpson, 2010).

The albumins include protease inhibitors, amylase inhibitors and lectins, with molecular weights (MW) in the range of 5–80 kDa (Boye, Zare, & Pletch, 2010). The globulins are generally divided into 11S and 7S proteins; this classification is related to their sedimentation coefficients (Oomah, Patras, Rawson, Singh, & Compos-Vega, 2011). The 11S and 7S proteins are known as glycinin and β-conglycinin, respectively in soy (Nielsen, 1984), and legumin and vicilin in pea, respectively. The corresponding proteins in other pulses may also be referred to as legumin and vicilin, or as legumin-like and vicilin-like proteins (Oomah et al., 2011). An additional 7S globulin known as convicilin may also be present (Singhal, Karaca, Tyler, & Nickerson, 2016). Legumin has a MW of ~340–360 kDa. It is comprised of six subunits with a MW of ~60 kDa each, linked by non-covalent interactions. Each subunit can be further divided into an acidic and a basic chain joined by a single disulphide bond, with MW of ~40 and ~20 kDa, respectively (Boye, Zare, & Pletch, 2010; Singhal et al., 2016). Vicilins are typically comprised of a trimer with a MW of ~150–190 kDa, without any disulphide bonds. The subunits of vicilin typically have a MW of ~50 kDa. Considerable variation can be found in size, charge, and glycosylation (Boulter & Croy, 1997). Convicilin, which is present in smaller amounts, is comprised of 3–4 subunits, each with a MW of ~70 kDa (Singhal et al., 2016).

As the properties of legumin and vicilin are different, another important consideration is the legumin/vicilin ratio. While legumin is typically more abundant than vicilin, this ratio can vary considerably. In peas for example, values for legumin/vicilin ratio in the range of 0.2–8.0 have been reported (Singhal et al., 2016). Pulse proteins are relatively high in arginine, glutamic acid, aspartic acid, lysine and leucine, with lower quantities of methionine, cystine and tryptophan (Swanson, 1990). In general, pulse storage proteins are relatively low in sulphur-containing amino acids, but have a high lysine content compared to cereal proteins (Day, 2013; Duranti, 2006), making them suitable for blending with cereal proteins where a complete amino acid profile is required.

3. Production of pulse protein ingredients

In soybeans, the most abundant component is protein. They contain more fat than pulses, and relatively little starch, with <1% in mature seeds (Medic, Atkinson, & Hurburgh, 2014). In most pulses, by contrast, the most abundant component is starch. They are lower in protein compared to soybeans, with the exception of lupins, which are relatively high in protein and low in carbohydrate (Bähr, Fechner, Hasenkopf, Mittermaier, & Jahreis, 2014). The macronutrient contents for several common pulses along with soybeans are shown in Table 1. Pulses can contain various components in smaller amounts which should also be considered. These include protease inhibitors, lectins, phytates, phenolic compounds, saponins, oligosaccharides, phytoestrogens and non-protein amino acids (Campos-Vega, Loarca-Piña, & Oomah, 2010; Mohan, Tresina, & Daffodil, 2016). These are important as many are considered to be antinutrients, although some have also been considered to have health benefits (Campos-Vega et al., 2010). It may be necessary to exclude some of these minor components from pulse-based products. Some, such as protease inhibitors, can be deactivated with thermal processing (Campos-Vega et al., 2010). Processing of pulses into protein isolates may help to remove undesirable compounds (Vogelsang-O'Dwyer et al., 2020), depending on the process used.

Generally, if high-protein pulse ingredients are required for food formulations, a protein enrichment or isolation process beyond dehulling/milling will be required. These can be divided into dry or aqueous processes. Typically, high protein fractions from pulses are termed concentrates or isolates, depending on the protein content. Although standards exist for milk protein isolates, which should be 89.5% protein (dry basis) or higher (ADPI, 2019), no universal classification exists in this regard for pulse proteins, and the terminology used can vary (Singhal et al., 2016). In many cases, ingredients with a protein content of >80% are referred to as isolates.

3.1. Dry fractionation

3.1.1. Air classification

Air classification has been used with a variety of different pulses, including pea, faba bean and lupin (Pelgrom, Berghout, van der Goot, Boom, & Schutyser, 2014; Tyler & Panchuk, 1982). Dehulling of seeds may be carried out prior to further processing. Advantages of this include reduction of antinutritional factors (ANFs), removal of bitter/-astringent components, and improved colour. Dehulling may also result in a slight increase in protein content of the seeds (Saldanha do Carmo et al., 2020). The principle behind air classification is based on the separation of particles in an air stream based on their size and density (Sozer, Holopainen-Mantila, & Poutanen, 2017). Pulses must be milled finely enough that cells are disrupted, allowing separation of starch granules from protein bodies. Starch granules should be liberated with minimal damage, while the protein matrix is ground to smaller particles (Boye, Zare, & Pletch, 2010; Schutyser, Pelgrom, van der Goot, & Boom, 2015). Impact milling or jet milling may be used to achieve this (Pelgrom, Vissers, Boom, & Schutyser, 2013).

An overview of the milling and air classification process is shown in Fig. 1. Rotor-type classifiers are generally used for air classification of finely milled flours. The flour is dispersed in an air stream, and is then passed to a rotating classifier wheel, where small and large particles are

Table 1
Macronutrient content of pulses and soybeans (g/100 g dry matter).

	Protein	Fat	Carbohydrate	Ash	Dietary fibre
Kidney bean[a]	17–27	1–5	63–74	3.2–5.2	18–30
Navy bean[a]	19–27	2	67–75	4–4.9	14–25
Chickpea[a]	19–27	1–3	52–71	1.8–3.5	6–15
Lentil[a]	23–31	1–3	42–72	2.1–3.2	7–23
Pea[a]	14–31	1–4	55–72	2.3–3.7	3–20
Lupin[a,b]	32–55.3	5–15	4.5–47	2.6–5.09	14–55
Soybeans[c, d]	32–43.6	8.1–24.7	31.7–35	4.5–6.4	19.7–31.9

[a] Hall, Hillen, and Garden Robinson (2017).
[b] Bähr et al. (2014).
[c] Medic et al. (2014).
[d] Banaszkiewicz (2011).

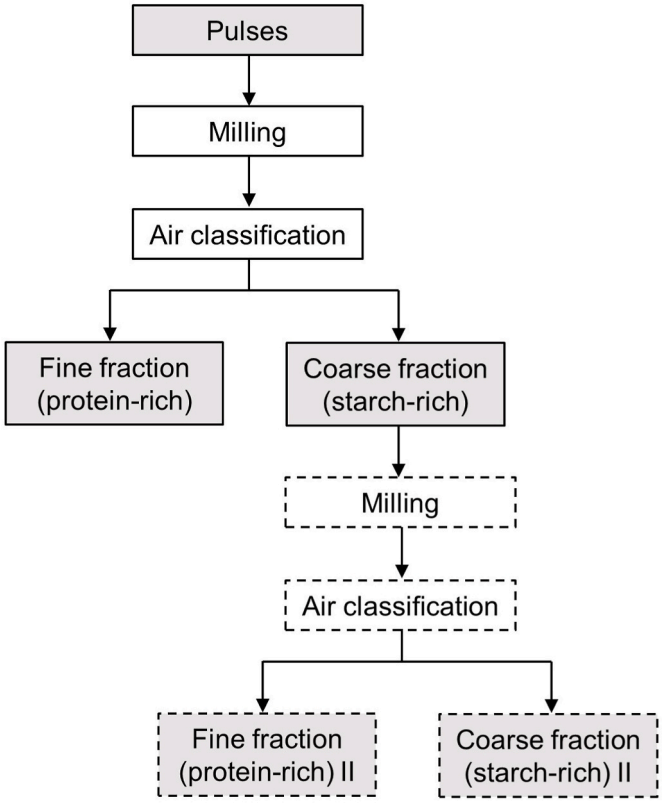

Fig. 1. Flow chart depicting the milling/air classification process. Boxes with broken lines show optional extra steps.

separated centrifugally, the fine fraction is enriched in protein while the coarse fraction is enriched in starch. In some methods the coarse fraction can also be re-milled and subsequently air classified again into coarse and fine fractions in order to increase the yield and purity of each fraction (Schutyser & van der Goot, 2011). Air classification is considered more sustainable than aqueous fractionation, due to far lower energy and water demands, and also does not require a drying process, or the addition of chemicals which are necessary in some aqueous processes (Pelgrom et al., 2013; Vogelsang-O'Dwyer et al., 2020). In addition, air classified protein concentrates may retain more native conformation, and consequently better functionality compared to some protein isolates due to the milder processing conditions involved (Pelgrom et al., 2013; Vogelsang-O'Dwyer et al., 2020). A potential disadvantage of air classification is the lower achievable protein content compared to aqueous processing. Air classified protein concentrates from various pulses have been reported with protein contents in the range of 49–70% of dry matter (Schutyser et al., 2015).

3.1.2. Tribo-electric separation

Tribo-electric separation is a relatively novel process for fractionation of flours, and has been explored for the fractionation of navy bean (*Phaseolus vulgaris*) flour (Tabtabaei, Jafari, Rajabzadeh, & Legge, 2016; Tabtabaei, Vitelli, Rajabzadeh, & Legge, 2017) and gluten-starch mixtures (Wang, de Wit, Boom, & Schutyser, 2015). A similar technique was explored for protein enrichment of pea and lupin flours, as well as their air classified fine and coarse fractions (Pelgrom, Wang, Boom, & Schutyser, 2015). The principle of this technique involves the entrainment of particles in a gas flow through a channel, where collision with the walls of the channel causes them to become charged. The application of an external electric field then allows the particles to be separated based on the difference in charge (Wang et al., 2015). Further development of this method may be necessary due to the lower protein content of the protein-rich fraction compared to air classification. Tabtabaei et al. (2016) reported a maximum protein content of 42% for the protein-rich fraction of navy bean flour.

3.2. Aqueous fractionation

Aqueous fractionation involves the extraction of protein from either flaked or milled pulses in an aqueous solvent, followed by recovery/isolation of proteins. A de-fatting step may be carried out before extraction, depending on the type of pulse used (Boye, Aksay, et al., 2010). Sometimes an air classified high protein fraction is used as the starting material (Sumner, Nielsen, & Youngs, 1981). The protein extract is usually dried to facilitate storage and transport. While for dry fractionated concentrates, the protein content is generally less than 70% of dry matter, aqueous processes generally result in higher protein content compared to dry fractionation, often 80–90% (Arntfield & Maskus, 2011). Although the protein content may be lower in some cases, for simplicity all ingredients described in this section will be referred to as isolates. An outline of the processes mainly used to produce pulse protein isolates is shown in Fig. 2.

3.2.1. Isoelectric precipitation

Isoelectric precipitation (IEP) is the most commonly used method for production of pulse protein isolates and has been used with a wide variety of different pulses (Karaca, Low, & Nickerson, 2011; Singhal et al., 2016). This method takes advantage of the different solubility of pulse proteins depending on the pH environment. The lowest solubility is observed near the isoelectric point, pH ~4–5. At higher or lower pH values, away from the isoelectric point, the protein solubility is higher, where the proteins gain a net negative or positive surface charge (Karaca et al., 2011). Most commonly, pulse proteins are extracted in mild alkaline solution, and subsequently recovered by IEP. The extraction pH, usually achieved with addition of NaOH, is typically in the range of 8–11, but may also be higher (Berghout, Boom, & van der Goot, 2014; Boye, Aksay, et al., 2010; Liu, Damodaran, & Heinonen, 2019; Yang, Liu, Zeng, & Chen, 2018). Extraction pH, temperature and time as well as flour/solvent ratio may be optimised to deliver maximum yield and/or protein content (Jarpa-Parra et al., 2014). As well as the extracted protein, this mixture contains insoluble seed material including starch and insoluble fibres, which must be removed using filtration/sieving or centrifugation. The protein is then precipitated with the addition of acid such as HCl, typically around pH 4–5 where the solubility of the majority of the proteins is minimal (Boye, Zare, & Pletch, 2010; Singhal et al., 2016). The precipitated protein must then be separated from the supernatant, typically using centrifugation. The purity of the protein sediment may be increased by washing steps with water or acid solution. The recovered protein is then resuspended, most often neutralized with alkali addition, and a heat treatment step may be carried out to improve microbial quality (D'Agostina et al., 2006). The liquid protein isolate is typically dried, to give a product which can be stored for later use. At laboratory scale, freeze-drying followed by milling may be used, whereas at pilot or industrial scale, spray-drying is typically used (Burger & Zhang, 2019; Chen et al., 2019). The smaller fraction of acid soluble proteins remaining in the supernatant after IEP (rich in albumins), may be processed separately, isolated using ultrafiltration/diafiltration (UF/DF), to give an acid soluble protein isolate. This has been carried out in a lupin protein isolation process, and was referred to as 'type F' lupin protein isolate due to its excellent foaming properties (D'Agostina et al., 2006).

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

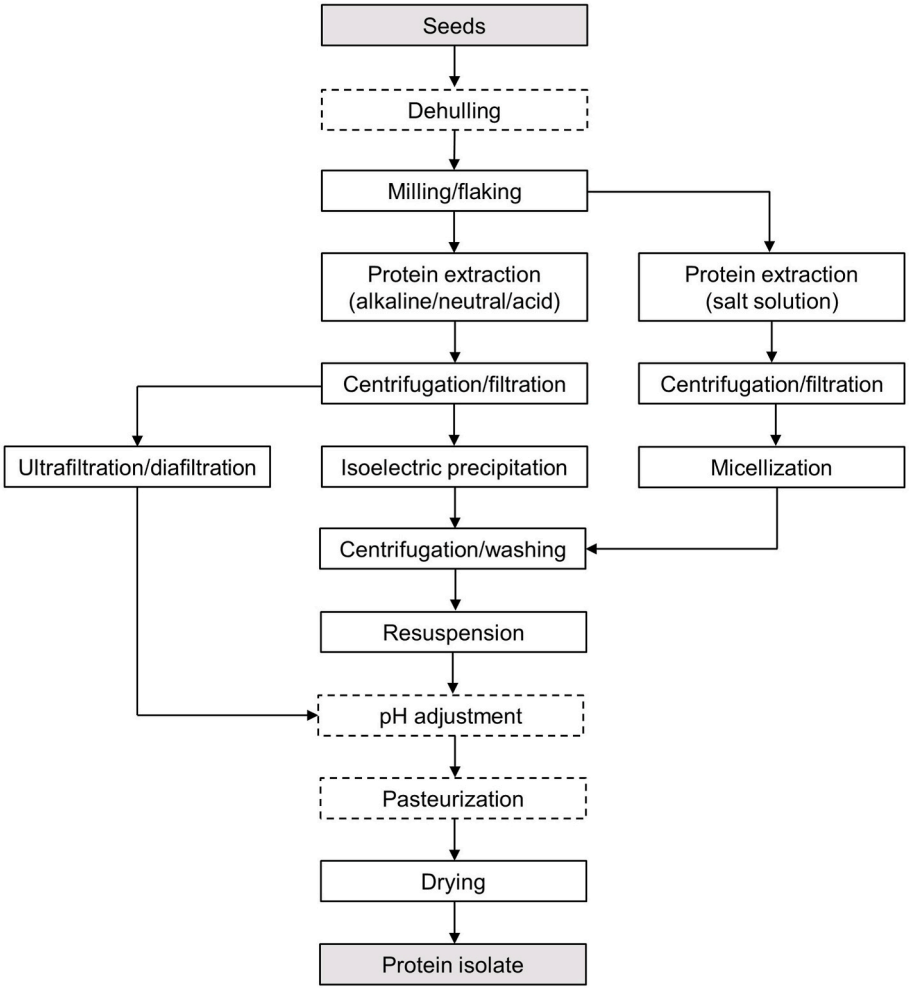

Fig. 2. Process flow chart showing some common methods and steps used for production of pulse protein isolates. Boxes with broken lines represent steps which are not necessarily always included.

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

3.2.2. *Ultrafiltration*

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

3.2.3. *Salt extraction/micellization*

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

changes, and therefore potentially less protein denaturation during the process (Muranyi et al., 2016).

4. Functional properties of pulse protein ingredients

The term functional properties can have various meanings with regard to proteins; however, here the meaning will be taken as 'techno-functional' properties. The functional properties of a protein ingredient are of great importance, as they determine the types of food applications for which the ingredient can successfully be used. Reliably measuring these properties allows comparison of different ingredients, and can potentially help predict their behaviour in food systems. Some of the most important functional properties for food and beverages include solubility, emulsifying, foaming, gelation and water/fat binding (Boye, Zare, & Pletch, 2010; Foegeding & Davis, 2011). In order to select or design an ingredient for a particular application, it is necessary to identify which functionality is required, and how this may be achieved. Regarding milk alternatives, solubility and emulsion properties are critical. Storage stability can present difficulties with regard to plant-based products. The presence of insoluble components, especially large particles, leads to sedimentation during storage (Sethi et al., 2016). Thus, in high-protein beverages, solubility of the protein isolate or concentrate is particularly important. In formulations containing oil, creaming can be an issue, and proteins used as emulsifiers must be able to form and stabilise an emulsion; the proteins must be capable of migrating to the oil/water interface and forming an interfacial layer. The protein stabilised fat globules must also be capable of repelling each other, in order to avoid flocculation or coalescence (McClements, Bai, & Chung, 2017). Various factors can impact the functionality of pulse protein ingredients. These include the species and/or variety used, ingredient processing steps, as well as the food product processing and environment (Karaca et al., 2011; Singhal et al., 2016).

4.1. Influence of pulse type

Various studies have examined the influence of the input material, i.e. the type of pulse used, on the properties of the final protein ingredients. It is reasonable to assume that differences in seed protein composition may affect protein ingredient functionality. Numerous studies have compared different pulses in this regard. At present pea protein ingredients are the only widely available commercial pulse proteins; however, a wide range of pulses could potentially be used to develop functional isolates and concentrates, hence the importance of these studies. Shevkani, Singh, Kaur, and Rana (2015) compared protein isolates from various kidney bean (*Phaseolus vulgaris*) and pea varieties with regard to structural and functional properties. Electrophoresis suggested vicilin was the dominant protein fraction in the kidney bean protein isolates, whereas both legumin and vicilin were more equally apparent in the pea protein isolates. Both kidney bean and pea protein isolates showed similar ranges for solubility and foaming properties. Emulsifying activity index was greater for kidney bean protein isolates, whereas emulsion stability index was higher for the pea protein isolates. Some studies have focused on commonly used pulse proteins sources such as pea, and how the variety chosen affects the physicochemical and functional properties of protein isolates or concentrates. Stone, Avarmenko, Warkentin, and Nickerson (2015) produced protein isolates from 7 different pea cultivars, and found protein solubility to be significantly different between some of the cultivars, whereas no significant differences were observed for most of the other properties measured, including water and fat holding capacity. Lam, Warkentin, Tyler, and Nickerson (2017) compared protein isolates from six different pea cultivars, from two different locations in Canada and two different years. They hypothesized that variation in the legumin/vicilin ratio due to growth environment would influence the functionality of the protein isolates. Although some differences were found between cultivars in solubility and foaming capacity, no major trends between the different factors were observed for the functional properties tested, and the authors concluded overall that these differences may be of limited importance to ingredient processors. A somewhat similar study carried out by Martinez et al. (2016) on air classified faba bean protein concentrates found significant effects of environment, genotype, and environment × genotype on some functional properties including protein solubility. However, they also concluded that these differences were insufficient to justify screening of varieties before processing into concentrates. On the other hand, more extensive differences in functionality due to cultivar have also been reported, including the study of Li, Shu, Yan, and Shen (2010). They compared protein isolates produced using IEP from sixteen different varieties of mung bean, and found significant differences in functional properties such as foaming, emulsifying properties and solubility. The highest and lowest nitrogen solubility values reported were 65.52% and 28.70%, respectively. Differences in functional properties have also been found between the legumin and vicilin fractions from peas (Koyoro & Powers, 1987). Legumin was found to have higher emulsion capacity, while vicilin was found to have higher solubility. Therefore, this might be an important factor to consider for the selection of raw materials.

4.2. Influence of ingredient processing

The processing steps used to manufacture high protein ingredients from pulses can have a major effect on the functional properties of the final ingredients. As previously mentioned, the ratio of the different seed proteins present can influence various properties. Depending on the conditions during ingredient manufacture, different fractions may be concentrated or removed to some extent. In the case of dry fractionated protein concentrates, it may be assumed that the protein composition will remain unchanged relative to the raw material used, the level is simply concentrated due to removal of starch granules (Schutyser et al., 2015). The use of aqueous processing may enrich different fractions due to their solubility in a given environment. In the case of IEP processes, the initial extract is comprised mainly of globulins and albumins. Globulins are then concentrated in the precipitated material, while the proteins remaining in the supernatant can be assumed to be higher in albumins. On the other hand, UF processes allow for the recovery of albumins along with the globulins (Arntfield & Maskus, 2011). Boye, Aksay, et al. (2010) demonstrated different functionality for UF isolates compared to those produced using IEP, depending also on the pulse type. In the case of red lentil for example, the UF isolate had higher protein solubility at neutral pH compared to the IEP isolate, whereas in the case of desi chickpea higher solubility was observed for the IEP isolate. Relatively little difference was observed in the emulsifying properties.

Another important consideration is the preservation of native protein functionality in isolates and concentrates. In general, producing a high protein ingredient with good functionality requires the proteins to retain a globular structure. In this conformation, exposure of hydrophobic groups to the surface is minimized, favouring solubility. Properties such as foaming, emulsification and gelation may also be dependent to a certain extent on protein solubility (Jiang et al., 2016). Dry fractionation has the advantage of preserving native protein conformation as the proteins are not subjected to heating or extremes of pH, which in turn can result in better functionality, including solubility and gelation (Assatory, Vitelli, Rajabzadeh, & Legge, 2019; Pelgrom, Boom, & Schutyser, 2015; Vogelsang-O'Dwyer et al., 2020). However, due to the lower protein purity of dry fractionated concentrates, the higher protein content of isolates may be required for some applications. However, the extreme conditions involved in commonly used isolation processes, such as alkaline and acidic pH during extraction and precipitation, along with high temperatures during spray-drying, can cause protein denaturation. This in turn may limit the functionality of the protein isolates. The extent of denaturation can be measured using differential scanning calorimetry (DSC) (Hickisch, Bindl, Vogel, & Toelstede, 2016). It has been observed for lentil protein isolate that alkaline extraction at pH 9.5 resulted in

increased denaturation compared to extraction at pH 8 (Lee, Htoon, Uthayakumaran, & Paterson, 2007). By contrast, Jarpa-Parra et al. (2014) found that increasing extraction pH led to slightly higher solubility for lentil protein isolate. The method of protein recovery is also important in this regard. When salt extraction/micellization was employed for chickpea protein isolates, higher protein solubility was observed compared to the IEP isolate (Paredes-López et al., 1991). This was also observed for lupin protein isolates, and higher denaturation for the IEP isolate compared to the micellized isolate was apparent in the DSC thermograms (Muranyi et al., 2016).

In the case of pea protein, it has been recognised that a gap in functionality exists generally between commercial protein isolates and lab-prepared isolates (Arntfield & Maskus, 2011; Burger & Zhang, 2019). This has been attributed to the harsher conditions experienced during processing for commercial isolates, in particular as a result of the high temperatures experienced during spray drying (Burger et al., 2019; Chen et al., 2019). In general, commercial isolates tend to have lower solubility when compared to isolates prepared at laboratory scale. Pilot scale-produced pea protein isolate prepared using UF was shown to have considerably better solubility, emulsifying and gelling properties compared to a commercial pea protein isolate (Taherian et al., 2011). Processing parameters of commercial pulse protein isolates may need to be optimised in order to match the functional requirements for applications such as milk alternatives.

5. Application in plant-based milk alternatives

At present the only widely available milk alternative beverages with protein content comparable to cow's milk, are soy-based products, while other popular beverages such as almond-based milk alternative often contain less than 0.5% protein (Jeske et al., 2017; Singhal, Baker, & Baker, 2017). Ideally, plant-based milk alternatives should possess a similar nutritional profile to cow's milk, while also resembling cow's milk in terms of taste, texture and appearance. Cow's milk is a complex colloidal dispersion, consisting of emulsified fat globules and casein micelles surrounded by an aqueous medium containing dissolved components, including sugars, proteins, and minerals (McClements et al., 2019). The overall composition of whole cow's milk may vary slightly; however, it is composed of approximately 87.5% water, 3.4% protein, 3.9% fat, 4.8% lactose and 0.8% minerals (Tetrapak, 2020). The fat can be standardised to various levels to give whole, low fat or skim milk. By contrast, plant-based milk alternatives vary considerably in their nutritional composition, as well as appearance and taste (Jeske et al., 2017; McClements et al., 2019). This is not surprising, due to the variety of raw materials used in their production. Therefore, the composition and properties of plant-based milk alternatives are not strictly defined.

5.1. Traditional production method for plant-based milk alternatives

The well-established traditional method for production of soy-based and other beverages involves first milling the seeds (wet or dry milling) which may have been pre-soaked, extracting seed material in water, separating insoluble material, homogenisation, and heat treatment, with the optional addition of extra components such as flavourings, sugar, and stabilisers (Jeske, Zannini, & Arendt, 2018). The composition of the final product is limited to a large extent by the composition of the input seed material. In this regard soybeans, due to their composition, are suitable for production of beverages with similar protein and fat content to cow's milk (Jeske et al., 2017; Vanga & Raghavan, 2018). This approach has been used for various pulses; however, with most pulses this will result in a product lower in protein and higher in carbohydrate compared to soy beverages. Also, due to the low oil content of most pulses, oil would need to be added to the formulation in order to provide a similar fat content to cow's milk or soy beverage. Chickpea has been used to prepare a milk alternative in the manner outlined above, and while its sensory acceptance was comparable to a soy based beverage, it was characterized by considerably lower protein and higher carbohydrate contents, due to the relatively high starch content of chickpeas (Wang, Chelikani, & Serventi, 2018). Caygill, Jones, and Ferber (1981) used a similar process to produce different beverages using mung bean, cowpea, chickpea, pigeon pea and black gram. The protein contents of the products were in the range of 1.35–1.85%. Akinyele (1991) produced cowpea-based milk alternatives with protein content of 1–2% depending on the process conditions. Lupin is perhaps the only type of pulse which could provide a similar protein level to soy beverage using this process, due to its high protein and low starch content. Xia et al. (2019) produced a milk alternative using lupin flour as the starting material, using high pressure homogenisation with multiple passes to improve stability. The nutritional composition of the product was not reported.

5.2. Designing milk alternatives with pulse protein ingredients

While the traditional method for plant-based milk alternative production is limited by the composition of the whole or dehulled seed material used, the use of pulse protein concentrates/isolates opens up the possibility of formulating products to a target nutritional composition. An oil in water emulsion can be formed, which potentially could be tailored to match the protein and fat content of cow's milk, whether it be whole or low-fat milk, etc. (Jeske, Bez, Arendt, & Zannini, 2019). Depending on the composition of the protein ingredient, it is also possible to deliver a lower carbohydrate content than cow's milk if desired. With this approach, the protein functions both as a source of nutrition and as a natural emulsifier (McClements et al., 2017). A basic overview of the typical process is depicted in Fig. 3. This process involves preparing a protein dispersion and formation of a pre-emulsion with the aid of high shear mixing. This pre-emulsion is then subjected to high pressure homogenisation to form an emulsion with decreased particle size. Homogenisation is a critical step to improve the colloidal

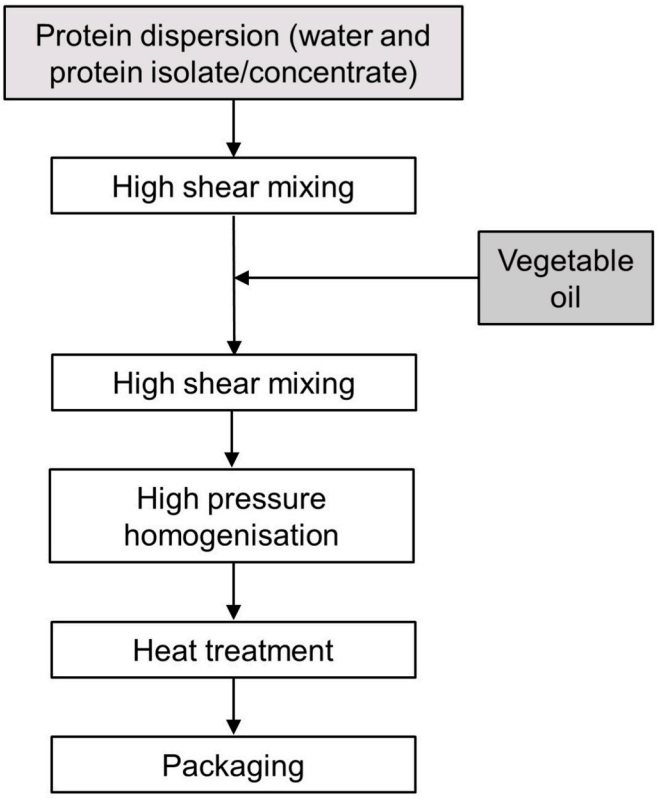

Fig. 3. Process flow chart showing the production of a milk alternative using pulse protein and vegetable oil.

stability of the product, allowing reduction of fat droplet size to the sub-micron range. The product can then be heat treated to improve microbial stability.

Several studies have focused on the production of milk alternatives using pulse protein isolates/concentrates. Sosulski, Chakraborty, and Humbert (1978) prepared various pulse protein isolates using IEP and used them in the manufacture of imitation milk products, in order to compare the suitability of the different protein sources. The formulation was intended to simulate milk and included protein isolate, hydrogenated coconut oil, lactose, polysorbate 80 and a salt mixture. Homogenisation of the mixture was achieved with high shear mixing. The protein isolates used were derived from soybean, lupin, pea bean, mung bean, field pea, Great Northern bean, baby lima bean, lentil, faba bean, and chickpea. Of the pulse protein-based products, lima bean was found to be the most stable to creaming, while pea was the least stable, after 24 h of storage. In a sensory test, all of the products were considered relatively poor compared to cow's milk, with lima bean and mung bean scoring the highest, and faba bean scoring the lowest. Various colours were also observed for the different products, including white, grey and yellow. Jacobs, Stephany, Eisner, and Toelstede (2016) produced a lupin-based milk alternative using the method outlined in Fig. 3, composed of 2% lupin protein isolate, 4% coconut oil and 4% dextrose. Ultra-high temperature (UHT) treatment resulted in a product with excellent microbial quality. Sensory quality was reported to be pleasant overall, which was attributed to the lower lipoxygenase activity of the UHT product compared the unheated product. A similar method was also used by Jeske et al. (2019) for the production of a pasteurised milk alternative with lentil protein isolate as the source of protein, and sunflower oil as the fat source. The product was formulated with a protein and fat content comparable to full-fat or low-fat cow's milk, i.e. 3.3% protein, and 3.3% or 1.5% fat. It was compared with other plant-based milk alternatives in a sensory acceptance test, including almond, rice and soy and hemp-based beverages, and acceptance was found to be comparable overall with the other products tested.

Recently, milk alternatives formulated with pulse protein ingredients have become available commercially, some of which provide a protein content similar to cow's milk and soy-based beverages. Examples of such products are shown in Table 2. Some of the products have a protein content of 3.2% or higher, which is comparable to the level found in cow's milk (Chalupa-Krebzdak, Long, & Bohrer, 2018), while none of them are below 2% protein. The pulse protein isolates/concentrates used are from pea or lupin, while a variety of vegetable oils are used. Pulse protein can also be used in conjunction with other protein sources, e.g., pea, barley and rice protein. Many of the products also include other ingredients such as sweeteners, stabilisers, vitamins and minerals.

5.3. Technological aspects of pulse protein emulsions

There is relatively little literature regarding plant-based milk alternatives manufactured with protein isolates or concentrates compared to the 'traditional' method; however, interest in the topic is growing, as demand increases for healthy, plant-based and high-protein products. At the same time, there is much research on the emulsifying properties of various pulse proteins from a more fundamental perspective, rather than application focused. Various studies have focused on pulse protein-stabilised oil in water emulsions, their physical and chemical properties and stability, and may provide essential knowledge for those who intend to manufacture milk alternatives from pulse protein ingredients. In order to be a successful emulsifier, the protein must have adequate solubility, ability to adsorb quickly at the oil-water interface during homogenisation, provide a reduction in interfacial tension, and form a viscoelastic interfacial layer with repulsive forces, including electrostatic and steric repulsion (McClements et al., 2017). For proteins, the balance of hydrophobic and hydrophilic amino acids is important, as well as their ability to alter their conformations to increase the contact of hydrophobic regions with the oil phase (Gumus, Decker, & McClements,

Table 2
Some commercially available milk alternatives based on pulse proteins, with ingredients and protein content.

Product	Ingredients	Protein content (g/100 mL)
Ripple Original Unsweetened[a]	Water, ripptein® (water, pea protein), sunflower oil, vitamin A palmitate, vitamin D2, vitamin B12, tricalcium phosphate, dipotassium phosphate, sunflower lecithin, sea salt, natural flavor, guar gum, gellan gum	3.3
Mighty Pea M.lk Unsweetened[b]	Water, Pea Protein (4%), Sunflower Oil, Calcium Carbonate, Tapioca Starch, Natural Flavourings, Emulsifier (Sunflower Lecithin), Sea Salt, Acidity Regulator (Potassium Carbonate), Stabilizer (Gellan Gum), Iodine, Vitamins (B12, D)	3.2
Sproud Original[b]	Water, pea protein (2,5%), agave syrup, rapeseed oil, dipotassium phosphate, calcium carbonate, calcium phosphate, gluten-free oat oil, salt, vitamin B12, riboflavin (B2) and vitamin D2	2
Princess and the Pea Unsweetened[b]	Water, Pea Protein, Rapeseed Oil, Maltodextrin, Calcium Phosphates, Natural X Flavourings, Lecithin, Sea Salt, Gellan Gum	3.2
Qwrkee Unsweetened Plant-Based Pea M'lk[b]	Water, Pea Protein, Rapeseed Oil, Maltodextrin, Inulin, Natural Flavourings, emulsifier (Sunflower Lecithin), Sea Salt, Gellan Gum, Minerals (Calcium, Tricalcium Phosphate), Potassium Iodide, iron (II) -L2 hydroxy propionate, Vitamins (Riboflavin, Ergocalciferol, Vitamin B12, Vitamin A)	3.2
YoFiit Miylk10[c]	Chickpea base (water, organic ground chickpea), non GMO pea protein, organic flax seed oil, calcium, natural flavor	4
Made with Luve Natur[d]	Water, lupine preparation (8.0%; water, lupine protein isolate), maltodextrin, coconut oil, sugar, acidity regulator: potassium phosphates, stabilizer: gellan	2
Unsweet Silk Protein[e]	Almondmilk (Filtered Water, Almonds), Cashewmilk (Filtered Water, Cashews), Pea Protein, Sunflower Oil, Calcium Carbonate, Salt, Sunflower Lecithin, Gellan Gum, Ascorbic Acid, Natural Flavor, Vitamin E Acetate, Vitamin D2	4.2
Australia's Own Like Milk Unsweetened[f]	Water, Pea Protein Isolate (4%), Sunflower Oil, Minerals and Vitamins (Calcium Phosphate, Vitamin B2, Vitamin A, Vitamin D, Vitamin B12), Natural Flavours, Stabilisers (418, 415), Salt	3.3
Bolthouse Farms Plant Protein Milk Unsweetened[g]	Water, Pea Protein, Sunflower Oil, Sea Salt, Sunflower Lecithin, Natural Flavor, Gellan Gum, Carob Bean Gum, Tricalcium Phosphate, Vitamin A Palmitate, Vitamin D2, Vitamin E (D-	4.2

(continued on next page)

Table 2 (*continued*)

Product	Ingredients	Protein content (g/100 mL)
	Alpha-Tocopheryl), Vitamin B12, Dipotassium Phosphate	
Take Two Barleymilk Original[h]	Barleymilk (water, barley and rice protein), coconut cream, organic cane sugar, chicory root extract, organic sunflower oil, pea protein, calcium carbonate, natural flavor, sea salt, organic locust bean gum, gellan gum, vitamin D2, organic sunflower lecithin	2.1

[a] ripplefoods.com (2020).
[b] Mintel Group Ltd. (2018).
[c] yofiit.com (2020).
[d] madewithluve.de (2020).
[e] silk.com (2020).
[f] australiasownfoods.com.au (2020).
[g] bolthouse.com (2020).
[h] taketwofoods.com (2020).

2017). The emulsion should be stable and resist creaming for the duration of its intended shelf life, ranging from several weeks for pasteurised products to months in the case of ultra-high temperature (UHT) treated products. Selection of a protein ingredient with the correct properties is critical, but also the environmental conditions must be taken into account, e.g., pH must be far enough from the isoelectric point to provide electrostatic repulsion and prevent aggregation (Boye, Zare, & Pletch, 2010). Another consideration is the ratio of protein/oil. There must be sufficient protein available to coat the total surface area of the oil droplets and provide effective stabilisation, and increasing protein concentration up to a certain level can improve emulsion stability (Chen et al., 2019). However, the protein/oil ratio used in high-protein milk alternatives is typically higher than found in many studies, so this should not be a concern, e.g. 1:1 and 2:1 to give the approximate composition of whole and low-fat milk, respectively (Jeske et al., 2019). At the same time, increasing protein content may also have a negative effect on emulsion stability depending on the protein source and environmental conditions (Burger et al., 2019). Tabilo-Munizaga et al. (2019) found that lentil protein-based nano emulsions homogenised at 300 MPa with 1:1 protein/oil showed the longest stability based on visual assessment with a result of 21 days, compared to 5 days and 8 days for 1:2 and 2:1 protein/oil ratios, respectively.

5.3.1. Homogenisation

As with cow's milk, homogenisation is a critical step for producing milk alternative emulsions with good stability and resistance to creaming. High shear mixing can be effective in constructing a coarse emulsion; however, the disruption generated may not be sufficient to produce the small size of oil droplets necessary for longer term stability. It is, however, a useful tool for the preparation of pre-emulsions, which can subsequently be subjected to further homogenisation treatment (Qamar, Bhandari, & Prakash, 2019). Adequate reduction of droplet size is essential for stability, as the rate of phase separation is strongly influenced by droplet diameter (Håkansson, 2019). High pressure homogenisation is a commonly used technology which can produce emulsions with good stability. The pre-emulsion is forced through a narrow gap; turbulent interactions as the jet of fluid exits the gap results in the breakup of oil droplets (Håkansson, 2019; Tabilo-Munizaga et al., 2019). Typically, either 1-stage or 2-stage homogenisers are used, the purpose of the second stage being separation of aggregates formed after the first stage (McCarthy et al., 2016). With high pressure homogenisation, generally the effects of homogenisation pressure and the number of passes on droplet size are of interest. Tabilo-Munizaga et al. (2019) found that increasing pressure up to 300 MPa generally reduced creaming and improved stability of lentil protein emulsion, although this was also dependent on other factors. Two passes resulted in smaller mean droplet size compared to 1 pass; however, no further reduction was observed with 3 passes. Jeske et al. (2019) found a significant reduction in droplet size accompanied by increased stability for lentil protein emulsion homogenised at 90 MPa compared to 18 MPa. Mean droplet size (z-average) was below 0.5 μm for all samples. Other technologies which can be used to form emulsions include microfluidization and ultrasonication (McClements et al., 2019). In a microfluidizer, the fluid is accelerated into an interaction chamber, where high-velocity microstreams are generated and oil droplets are broken apart with high shear and impact forces to form an emulsion (McCarthy et al., 2016). This method has been reported to produce smaller droplets than high pressure homogenisation (McClements et al., 2019). Ultrasonication works by using sound waves to produce pressure differential cycles in the fluid. Voids are created which then collapse during the high-pressure cycle, causing turbulence and shear forces which reduces the droplet size (McCarthy et al., 2016). Qamar et al. (2019) found that microfluidization produced more stable pea protein emulsions compared to ultrasonication. It should also be mentioned that homogenisation aids in the solubilisation of protein ingredients. Homogenisation of a lentil protein isolate dispersion at 90 MPa resulted in decreased particle size and an increase in protein solubility from 54% to 98% (Jeske et al., 2019). Saricaoglu (2020) improved the solubility of lentil protein isolate with homogenisation up to 100 MPa, while higher pressures resulted in aggregation and decreased solubility.

5.3.2. Heat treatment

Heat treatment is usually required for products such as milk and milk alternatives, in order to ensure safety and prevent spoilage during the product's shelf life. Pasteurisation should significantly reduce spoilage bacteria and eliminate pathogens, while UHT treatment should effectively sterilise the product (Tetrapak, 2020). Heat treatment can also have other effects, such as changes in colour (Jeske et al., 2019) or aroma (Trikusuma, Paravisini, & Peterson, 2020). For pulse protein emulsions, heat stability is an important consideration, as globular proteins may denature and aggregate during thermal treatment, which could result in a less stable product. Excessive heat treatment could cause sedimentation due to formation of insoluble protein aggregates, or flocculation of oil droplets. Therefore, it is important to consider the protein ingredient used as well as heating time/temperature, and environmental conditions (Bogahawaththa, Bao Chau, Trivedi, Dissanayake, & Vasiljevic, 2019; McClements et al., 2019). In low oil emulsions, the oil droplets may be more susceptible to aggregation and flocculation during heating, due to the presence of non-adsorbed protein in the continuous phase. This protein may interact with adsorbed protein at the oil/water interface, promoting aggregation (Diftis & Kiosseoglou, 2006). On the other hand, heat treatment has also been shown to improve stability in some cases. With microfluidized pea protein emulsions with lecithin, UHT treatment (140 °C for 2 s) resulted in lower creaming compared to unheated samples (Qamar et al., 2019). For lupin-based milk alternative, pasteurised and UHT treated samples both had a smaller average particle size after 24 h storage compared to the unheated product (Hickisch, Bindl, et al., 2016). This was thought to have been due to coalescence during storage of the unheated milk alternative. It is also important to consider other factors which can affect heat stability, such as pH and the presence of minerals. Alonso-Miravalles, Zannini, Bez, Arendt, and O'Mahony (2020) found that the heat stability of lentil protein emulsions decreased with increasing calcium addition, and also decreased when pH was lowered within the range of 7.2–6.3. Alternative technologies including pulsed electric fields, ultra-high-pressure homogenisation and high-hydrostatic-pressure should also be explored as means of extending the shelf life of pulse protein-based milk alternatives in cases where thermal treatment has a detrimental effect (Munekata et al., 2020).

5.3.3. Enzymatic treatment

Enzymatic treatment may be a useful tool to improve the functionality of pulse proteins and the properties of the emulsions. Limited hydrolysis of proteins can be an effective method for improving protein solubility and emulsion stability. Liu, Bhattarai, Mikkonen, and Heinonen (2019) used limited hydrolysis with Alcalase to improve the stability of faba bean protein-stabilised oil in water emulsions. Eckert et al. (2019) evaluated the use of various proteases for improvement of faba bean protein functionality. They found pepsin to be particularly effective in improving solubility, with an increase from 24.4% to 88.8% at neutral pH, and from close to 0% up to 81% at pH 5. Foaming capacity and oil holding capacity were also significantly improved with this treatment. Tamm, Herbst, Brodkorb, and Drusch (2016) found that limited hydrolysis of commercial pea protein isolate with trypsin was effective in improving emulsion properties by reducing droplet size, while Alcalase treatment had a negative effect on stability. Limited hydrolysis can improve the emulsifying properties of proteins, decreasing molecular weight, exposing previously buried hydrophobic groups, and increasing molecular flexibility (Tamm et al., 2016). Due to the relatively high protein/oil ratio in high-protein milk alternatives, it is likely that excess protein could be present which could sediment over time, if poorly soluble. Enzymatic hydrolysis could possibly be used to mitigate this by increasing protein solubility. In the case of air classified protein concentrates, significant amounts of insoluble starch may also be present. Liquefaction with amylase treatment could be used to prevent sedimentation in this case, as this is commonly used in the production of milk alternatives from starchy seeds (Bonke, Sieuwerts, & Petersen, 2020; Makinen, Wanhalinna, Zannini, & Arendt, 2016). Enzymatic treatments, when incorporated in product processing steps, could be a promising method for improvement of commercial isolates displaying poor functionality.

5.4. Related products

Cow's milk can be further processed into products such as yogurt, cream, ice cream and cheese. It may be possible for pulse protein-based milk alternatives to be used as a base for some of these product types; however, major structural and functional differences exist between pulse proteins and milk proteins. These differences could lead to very different properties in pulse protein-based products compared to dairy products (McClements et al., 2019). Hickisch, Beer, Vogel, and Toelstede (2016) produced yogurt form a lupin-based milk alternative which was produced using lupin protein isolate. Strains of *Lactobacillus plantarum*, *Pediococcus pentosaceus* and *Lactobacillus brevis* were used in the fermentation. The type of heat treatment (UHT vs pasteurisation) as well as the bacterial strain used were shown to affect the textural and rheological properties of the yogurt. Further analysis on the network formation of these yogurts revealed that the type of heat treatment as well as bacterial strain used influenced the type of gel network formed. UHT treatment of the milk alternative resulted in formation of a denser network in the yogurt due to more extensive denaturation, which promoted increased formation of disulphide bonds. While soy-based yogurts have been commercially available for many years, pea-protein based yogurts have more recently become available (ripplefoods.com, 2020). Various dairy alternatives produced with lupin protein isolate are also commercially available, including ice cream, yogurts and spreads (madewithluve.de, 2020). The wide range of formulations along with frequent use of non-dairy components in conventional ice cream, perhaps widens the scope for variability in plant-based ice cream type products using pulse proteins. More research and development on different milk alternative product types will likely be carried out as novel pulse protein isolates or concentrates become available.

6. Outlook and challenges

Pulse protein ingredients present a valuable opportunity to improve the nutritional value of plant-based milk alternatives, while avoiding some of the negative aspects of dairy products.

However, several challenges may need to be addressed. Protein isolate manufacturing processes need to be further developed to improve functionality, which may entail limiting the extent of denaturation during processing. Some pulse protein ingredients also show poor sensory characteristics such as off flavours. In addition, protein sources may need to be developed to allow increased availability and affordability for manufacturers, as the acceptability and widespread consumption of high protein pulse-based milk alternatives will no doubt be limited if their cost is prohibitive compared to conventional products. Furthermore, other issues such as vitamin and mineral fortification, protein quality, and clean label/natural image may need to be addressed.

7. Conclusions

With the large variety of pulses potentially suitable for processing into protein concentrates and isolates, and various dry and wet processing options available, there is considerable scope for the development of highly functional protein ingredients. As the demand for high protein plant-based milk alternatives grows, it could be met by the development of suitable pulse protein concentrates and isolates. This may involve improvement in the functionality of widely used commercial protein ingredients such as pea protein isolate, and also the development and commercialisation of more novel pulse protein sources such as lentil, chickpea and faba bean. Dry fractionated pulse protein concentrates should also be explored as alternatives to protein isolates. Pulse proteins may have an important role to play in producing sustainable plant-based milk alternatives along with other products, due to their potentially excellent functionality compared to other plant proteins.

Declaration of competing interest

The authors declare no conflict of interest.

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