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Dry fractionation for sustainable production of functional legume protein concentrates

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ABSTRACT

Plant proteins gain increasing interest as part of a sustainable diet. Because plant materials not only contain protein, they are generally isolated via an energy intensive wet fractionation. This review discusses dry fractionation as an alternative and more sustainable route for producing functional legume protein-enriched fractions. Increasing protein purity of dry-enriched fractions is discussed by identification of relationships between legume morphology and ability for separation in the dry state. Finally, functionality and nutritional properties of legume protein fractions and their application in high protein beverage and meat like structures are reviewed.

Introduction

The global protein demand is expected to double in the coming decades due to the increasing prosperity and world population. To keep up with the demand, the transition from an animal to a plant-based protein supply is desirable from long-term economic and environmental perspectives (Jayasena, Chih, & Nasar-Abbas, 2010; Lqari, Vioque, Pedroche, & Millan, 2002; Schutyser & van der Goot, 2011), as the production of animal protein imposes a severe burden on the available arable land, water and fossil fuels. Unfortunately, replacing animal based ingredients and components is not trivial. Animal-derived products have widespread preference because of their excellent taste, which is partly due to the microstructure of meat, but also due to its different composition. Plant materials can contain significant amount of carbohydrates and other components, which strongly influence the taste and nutritional profile. This explains the increased interest in fractionation methods.

The conventional route to obtain plant protein ingredients is wet extraction (Fig. 1). Legumes are an interesting source of plant proteins as they have high initial protein content (>20 g protein/100 g dry matter (Table 1)), dietary fibre content, contain a variety of micronutrients and phytochemicals and have a low level of fat (Messina, 1999) and at the same time they are able to fixate nitrogen. Legumes can be divided into those that use starch for energy storage, and those that store oil for this. Starch-rich legumes, such as peas and many beans are fractionated through dispersing the legumes into water to dissolve the protein and suspend the starch granules. Subsequently, the slurry is treated in a hydrocyclone to separate the proteins from the starch granules. Oil-rich legumes, such as soy and lupine, are subjected to solvent extraction to isolate the oil first. The defatted legume flour is mostly used as feed, but can also be further processed to obtain a protein isolate. Then, the defatted flour is suspended in water and a suspension of protein and fibre is obtained. For both types of legume (starch rich and oil rich) the solubilised proteins are separated from insoluble fibres at pH 9. Soluble fibres are separated by precipitating the proteins at their iso-electric point (pH 4.5–4.8). Subsequently, the pH is readjusted to 7 and a dry protein isolate is obtained after a final drying step (75–90 g protein/100 g dry matter) (Berghout, Pelgrom, Schutyser, Boom, & van der Goot, 2015; Boye, Zare, & Pletch, 2010). This wet process involves the use of large amounts water and chemicals (e.g. for acidification and neutralisation). Typically, the production of lupine protein isolate from the lupine seed requires, more than 80 kg water/kg protein isolate, 22.4 kg hexane/kg protein isolate, 40 g NaOH and 40 g HCl per kg protein isolate (Berghout *et al.*, 2015).

A more sustainable alternative to obtain protein-enriched fractions from legumes is dry fractionation (Fig. 1), which employs milling and air classification. Air classification of legumes has been investigated in the 1970s but since then received less attention (Vose, 1978). Major reasons for the renewed interest into dry fractionation are the wish to establish plant protein extraction routes that are less energy and resource-intensive and that can deliver functional protein fractions for preparing attractive and

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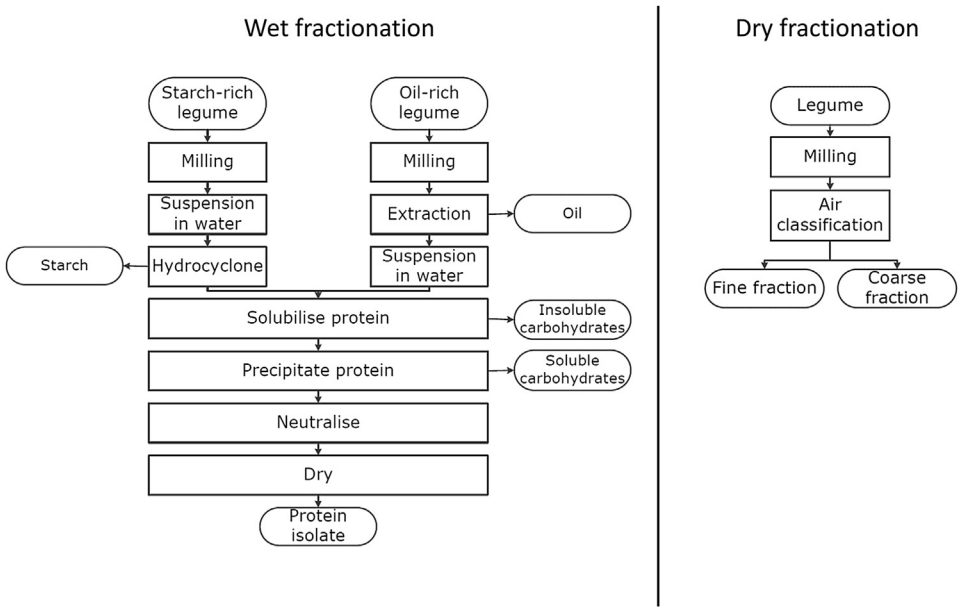

Fig. 1. Schematic illustration of wet (left) and dry (right) fractionation process.

healthy foods. The improved sustainability originates from the fact that dry fractionation does not require the addition of water and thus no energy intensive dehydration (Berghout *et al.*, 2015). Another advantage is that the absence of a drying step together with the absence of the exposure to chemicals retains the native functionality of components. Moreover, air classification is accredited for organic food production and the declaration of its products does not require E-numbers. The major drawback of dry fractionation is the relatively modest enrichment in protein content that can be obtained relative to wet extraction, but it can be expected high purity might not be necessary for most food applications (Schutyser & van der Goot, 2011). Related to the modest enrichment and the absence of heating is the presence of higher amounts of components such as oil, fibres and anti-nutritional components in dry-enriched protein fractions. This has implications in terms of functional and nutritional properties. For example, on the one hand higher amounts of oil are less desired in relation to foaming behaviour, but on the other hand presence of more water binding fibres improves gelling behaviour of the protein fractions.

Table 1
Protein enrichment by air classification of wheat and several legumes ± absolute deviation.

Legume/ grain	Initial protein content (g/100 g dry matter)	Protein content fine fraction (g/100 g dry matter)
Wheat	12.3 ± 1.8	28.3 ± 4.0
Lima bean	23.7 ± 0.4	48.9 ± 0.8
Cowpea	27.2 ± 0.0	50.9 ± 0.2
Common bean	26.3 ± 1.6	54.7 ± 2.2
Navy bean	27.2 ± 1.6	56.7 ± 6.8
Lentil	23.7 ± 2.1	57.6 ± 4.1
Pea	23.8 ± 1.2	58.5 ± 3.0
Mung bean	27.2 ± 0.4	62.3 ± 1.2
Faba bean	31.0 ± 0.8	69.9 ± 5.2
Lupine	40.4 ± 0.6	59.4 ± 0.6

(Aguilera, Lusas, Uebersax, & Zabik, 1982; Bergthaller, Dijkink, Langelaan, & Vereijken, 2001; Cloutt *et al.*, 1987; Elkowicz & Sosulski, 1982; Jones & Halton, 1959; Kent, 1965; Patel, Bedford, & Youngs, 1980; Pelgrom, Berghout, van der Goot, Boom, & Schutyser, 2014; Pelgrom, Boom, & Schutyser, 2015a; Poel van der, Aarts, & Stolp, 1989; Sosulski & Youngs, 1979; Stringfellow, Wall, Donaldson, & Anderson, 1976; Tyler *et al.*, 1981; Vose, Basterrechea, Gorin, Finlayson, & Youngs, 1976; Wright *et al.*, 1984; Wu & Nichols, 2005; Wu, Stringfellow, 1992).

Overall, it can be concluded that dry fractionation is promising for the novel design of processes were the full legume is used in a more integrated and efficient way, while attention should be paid to the increased variability in composition of the fractions (Abecassis, de Vries, & Rouau, 2014).

This paper reviews the dry fractionation process and the properties of products made through this process. In addition, an evaluation is given of recent developments concerning research on dry fractionation of legumes and paths are identified for future research. Focus is first on legume morphology to give insight in disentanglement of the cellular components, which is required to increase protein content, secondly on sustainability as it is a main driver for this technology, and thirdly on functional and nutritional properties of the fractions for application in food products.

Dry fractionation

Dry fractionation relies on the observation that milling can mechanically detach protein bodies and other cellular compounds into flour with particles of different composition. In starch-rich legumes, such as pea, the cotyledon cells consist of starch granules (±20 μm) embedded in a matrix of protein bodies (1–3 μm) that are surrounded by a fibre-rich cell wall (Tyler & Panchuk, 1982). Ideally, the starch granules are liberated during milling and the protein matrix is fragmented in particles smaller than the starch granules (Fig. 2). Then, the particles and fragments are separated based on size, density or both, as for example by air classification.

Air classification after milling separates the smaller protein-rich fragments from the larger starch granules and fibre-rich fragments. So-called rotor classifiers are mostly used for air classification of finely milled flours. In this classifier the flour is dispersed in a large stream of air. Subsequently, it enters from below and rises upward into a conical vessel containing a rotating classifier wheel with blades at the top. These blades create a centrifugal-counterflow separation zone in which the small and large particles are separated. Also the particle density has influence on the separation behaviour. The drag forces, created by the air flow, and centrifugal forces created by the classifier wheel, together determine the size of the particles that end up in the fine fraction. Particles on which

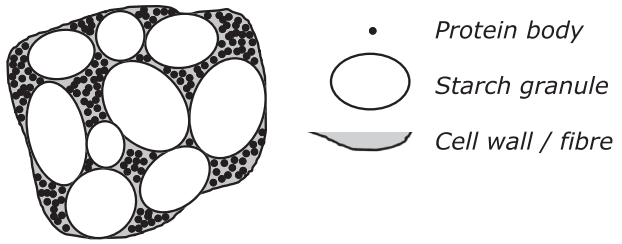

Fig. 2. Schematic drawing of cells of pea and the fragments after milling.

the drag force exceeds the centrifugal force may pass through the openings in the wheel, and enter the fine fraction.

Air classification can be used to make protein concentrates from several legumes and grains (Table 1). The protein content of the legume fine fraction ranges from 49 to 70 g protein/100 g dry matter. Legumes normally give a higher protein purity than grains, like wheat, because, the native protein concentration in legumes is higher and the starch granules of legumes are large and fairly uniform (approx. 25–40 μm) compared to the mixed populations of small and medium-sized granules (so called C, B and A starches) in most cereal grains (Vose, 1978). For products containing small starch particles such as cow peas contain, a lower protein content of the fine fraction will be obtained (Cloutt, Walker, & Pike, 1987). Faba beans contain large starch granules and can therefore be well separated (Cloutt *et al.*, 1987; Tyler, 1984). Therefore, the starch granule size largely determines the potential for dry separation; optimal separation can be obtained when the particle size distribution curve of flour and starch granules overlap maximally, with the protein bodies being smaller particles. Oil may have a negative effect on the separation of the constituents of the legumes as it impairs powder dispersibility, which explains why soy has been reported to be unsuitable for dry fractionation (Elkowicz & Sosulski, 1982; Sosulski & Youngs, 1979).

Besides the protein and starch also the other components will distribute towards the coarse and the fine fractions. For example for peas it was found that while the oil is originally 1.9 g/100 g dry matter in the flour, it increased to 3.8 g/100 g dry matter in the fine fraction and decreased in the coarse fraction to 1.3 g/100 g dry matter (Pelgrom, Boom, & Schutyser, 2015b). With respect to the fibre content, 26 g fibre/100 g dry matter was found in flour and 42 g fibre/g dry matter and 21 g fibre/g dry matter in the fine and coarse fractions, respectively. With respect to the ash content, 5 g ash/100 g dry matter was found in flour and 9.6 g fibre/g dry matter and 2.3 g fibre/g dry matter in the fine and coarse fractions, respectively. Similar trends were observed by others (Tyler, Youngs, & Sosulski, 1981; Wright *et al.*, 1984). It is emphasized that the functionality of the fractions and their utilization behaviour are strongly related to the exact compositions and therefore these should be considered next to the protein content.

Recent developments and future directions for dry fractionation

Improving dry fractionation by better understanding of the legume morphology

Protein-enrichment by air classification is achieved by using differences in size and density between protein-rich and protein-poor particles in legumes. Proper milling should therefore disentangle the smaller protein bodies from the larger starch granules and cell wall fibres. Even though starch-rich legumes have lower protein content than oil-rich legumes, the maximum protein content that can be attained with dry fractionation is similar for the starch-rich and the oil-rich legumes. The protein bodies in these legumes typically have an intrinsic protein content of 70–88 g protein/100 g dry matter (Plant & Moore, 1983; Weber & Neumann, 1980), which defines a theoretical maximum for dry fractionation. So far, a protein content of 60 g protein/100 g dry matter is obtained practically, indicating room for improvement by more precise milling and separation. For oil-rich legumes the maximum protein content is reached at a larger particle size than for starch-rich legumes because the protein bodies in oil-rich legumes are larger (Pelgrom *et al.*, 2014; Pelgrom *et al.*, 2015a).

The highest protein content for both starch-rich and oil-rich legumes is obtained when only the smaller particles are collected in the fine fraction; however this is accompanied by a decreased yield (Fig. 3). Two causes for yield losses were established. First, material may remain in the equipment (an effect that will become less important when processing larger materials streams), but second protein present in the coarse fraction also negatively influenced the yield. A relatively modest protein yield in the enriched fraction is therefore a characteristic of dry fractionation processes.

The protein content of the fine fraction can be increased by pre-treatments like increasing moisture content or defatting of the oil-rich legumes before fractionation (Pelgrom, Boom, & Schutyser, 2015c). The influence of moisture content is based on the notion that the local fracture behaviour is dependent on the physical state, i.e. glassy or rubbery, of the starch and protein domains. Electron scanning microscope images showed that more disentanglement takes place when the protein is in the rubbery state, which was

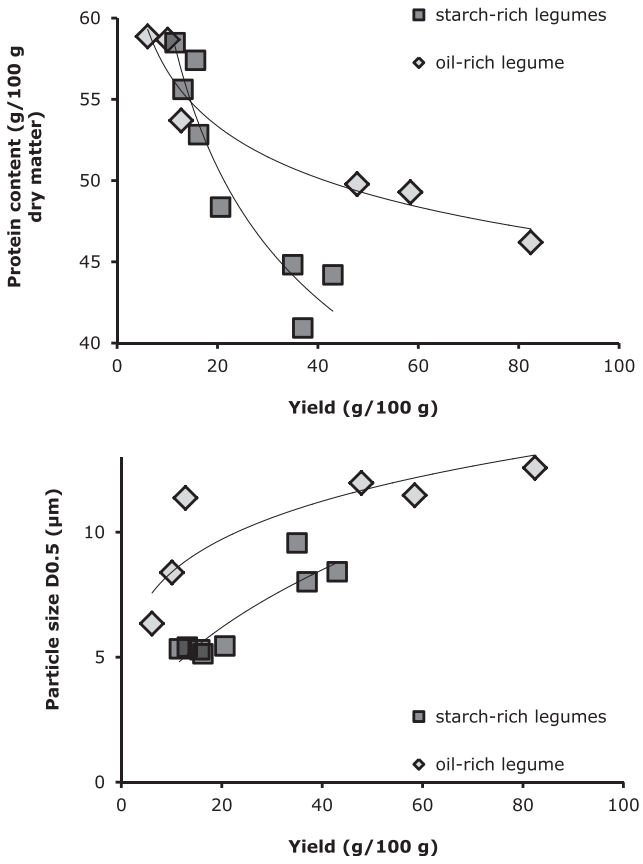

Fig. 3. Relation between yield and protein content or particle size of air classified pea and lupine fine fraction (Pelgrom *et al.*, 2014; Pelgrom *et al.*, 2015a).

later confirmed by higher protein separation efficiencies after milling rubbery pea and lupine (Pelgrom, Schutyser, & Boom, 2013).

The sharpness of the transition from rubbery to glassy state is however debatable. The flour particle size that is obtained by milling increases gradually as a function of the moisture content, as well as the protein content of particles smaller than 20 μm. Based on the glass transition curves of pea it is expected that at room temperature (20 °C) the glass transition of protein takes place at 14 g water/100 g dry matter and that the glass transition of starch occurs at 19 g water/100 g dry matter.

However, these values do not take into account any uneven water distribution and inhomogeneities in the pea seed. Moisture sorption isotherms indicated that at fixed water activity the moisture content of the starch phase is higher than that of the protein phase (Pelgrom, Vissers, Boom, & Schutyser, 2013). When the glass transition curves of the isolates are adapted for the moisture distribution in a seed, the difference in glass transition between protein and starch becomes smaller (Fig. 4). Moreover, the glass transition curve did not always coincide with the brittle–ductile transition curve for several foods, i.e. of fish meat (Watanabe, Tang, Suzuki, & Mihori, 1996) and gelatinized starch (Nicholls, Appleqvist, Davies, Ingman, & Lillford, 1995). The reasons could be due to a number of extrinsic factors including strain rate, stress state, specimen geometry, and the presence of notches and flaws (Rahman, 2006). However, in other cases the glass transition curve of pea was shown to coincide with ductile–brittle transitions (Pelgrom, Schutyser, *et al.*, 2013).

Further research on legume morphology and dry fractionation

As indicated by Schutyser and van der Goot (2011) and Abecassis *et al.* (2014) better understanding of the limiting factors in dry fractionation is needed for further increase of the protein purity and use of dry separated fractions. Better protein enrichment by dry fractionation could enable a wider applicability of the dry-enriched protein ingredient fractions in foods. The milling behaviour needed to disentangle protein bodies and other cellular components may be improved by increasing our understanding of the morphology of the seed and its (micro)breakage behaviour upon impact or deformation (Topin, Radjai, Delenne, Sadoudi, & Mabille, 2008). One approach would be to further increase our knowledge on the exact properties and compositions of the different regions and the morphology inside legumes and relate that to more precise milling without starch damage. Other approaches involve the development of pre-treatments or use or develop legume varieties that better facilitate dry fractionation, or use improved dry separation strategies. These approaches are discussed as well and possibilities for further research are given.

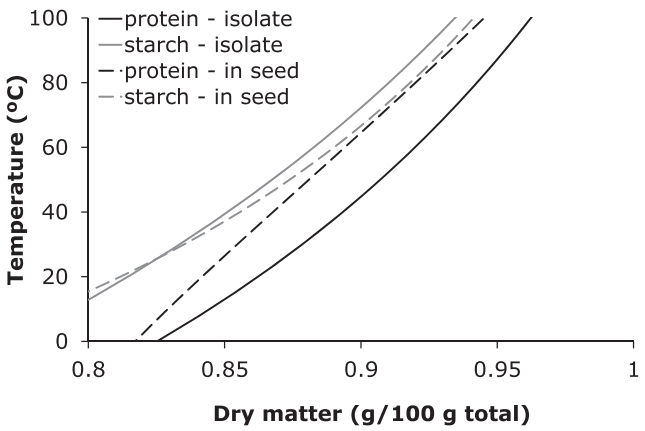

Fig. 4. State diagram of pea. Solid curves are DSC results modelled with the Gordon–Taylor equation, dashed lines are adapted for the moisture distribution in the seed based on the GAB sorption isotherms (Pelgrom, Schutyser, *et al.*, 2013; Pelgrom, Vissers, *et al.*, 2013).

Our knowledge on legume morphology can be extended with more knowledge of the adhesion and hardness of fibre, protein bodies and starch granules. The aim would be to use this information to influence break behaviour, improve disentanglement and design criteria for the equipment. For example, increasing the size of the fibre particles compared to protein-rich particles could reduce the fibre content in the fine fraction. Moreover, the hardness of the protein bodies and starch granules can be determined at various temperature and moisture combinations. This information will complete the state diagram and will aid in determining milling temperature and moisture combinations that yield optimal disentanglement between the cellular components.

Atomic force microscopy (AFM), laser-induced breakdown spectroscopy (LIBS) methods and advanced modelling techniques are discussed here as methods to study legume morphology. AFM can be used to characterize the local distribution of the mechanical properties of flat surfaces and has been applied on wheat grains recently. These mechanical properties combined with structural features were found relevant to improve milling behaviour (Chichti, George, Delenne, Radjai, & Lullien-Pellerin, 2013). The LIBS technique uses a laser to ablate material from a sample's surface from which the physical and mechanical properties can be estimated (Singh & Thakur, 2007). Using this technique, grain milling behaviour was related to the mechanical properties of wheat tissue (Martelli, Brygo, Delaporte, Rouau, & Barron, 2011). A similar approach might be followed to assess adhesion forces between tissue constituents of legumes. Particle-based modelling approaches may be used to study the parameters that are of influence to the breakage behaviour of legumes. For example, the fracture behaviour of wheat was modelled with the protein matrix as a continuous phase and the starch phase being the granular phase, using a lattice-element method (Topin *et al.*, 2008). A toughness parameter was used to describe the starch-protein adherence and the protein content.

Plant breeding and pre-treatments may lead to a seed composition that is better suited for mechanical disentanglement, improved dispersibility or increased size differences between the cellular components. Legume varieties have been developed with increased protein content of legumes (Day, 2013). In addition, natural differences in starch granule size have been observed between varieties of legumes (Hoover & Ratnayake, 2002). Varieties with larger starch granules can reduce the level of milling required. The milling behaviour can also be enhanced by selection of softer seeds. This was found from recent dry fractionation results for lentil, which has lower seed hardness compared to pea, bean and chickpea, and yielded a higher protein content in the fine fraction (Pelgrom *et al.*, 2015a). Some chickpea cultivars contain more fibre, which increase the adhesion between cellular components, requiring smaller particle size for the detachment of the starch granules during milling (Wood, Knights, Campbell, & Choct, 2014). In this case the fibres have to be milled too fine and will enter the fine fraction. Therefore one may select cultivars that contain tougher fibre or specifically degrade the fibres by pre-soaking with additives such as sulphur dioxide, sodium hydroxide, sulphuric acid or enzymes. The use of the chemicals or enzymes may however degrade the material and will compromise the sustainability of the fractionation process.

Finally, further development of the air classifiers is desirable to provide a sharper separation between the coarse and the fine fractions. The classifier wheel house may be redesigned to reduce the amount of material that is blocked there and hinders separation. One could for example develop a round house containing a

rotating wall scraper. Next to that, the angle and the design of the vanes could be optimized for legume flour (Huang, Liu, & Yu, 2012). Alternatively, dry separation may be improved by using new driving forces for separation such as electrostatic separation. This method relies on electrostatic charging behaviour instead of size and density. Electrostatic separation has been successfully used to fractionate wheat and oat bran. Fibres from the pericarp and the aleurone layer were separated, which led to the production of nutritionally interesting food ingredients (Hemery *et al.*, 2009; Sibakov, Abecassis, Barron, & Poutanen, 2014). Recently, electrostatic separation has been used to obtain protein enriched fractions from lupine flour by separating protein bodies from fibres. Enriched pea protein concentrate obtained from air classification could be further enriched by the removal of the fibres (Pelgrom *et al.*, 2015c).

Sustainability assessment of dry and wet fractionation

Renewed interest into dry fractionation of plant protein is amongst others motivated by its much lower energy and water use compared to wet fractionation. This is confirmed by estimations of the energy and water use per kg end product (Fig. 5). Even though industrial dry fractionation facilities exist with typically a capacity of 90 000 tons per year, wet fractionation is the mainstream technology for plant protein extraction. Wet fractionation of starch-rich legumes typically starts by diluting flour to a suspension of 13 g flour/100 g solution. A second dilution step is carried out before spray drying (Fig. 5) (Passe, Fouache, Verrin, & Bureau, 2008). These two dilution steps result in a consumption of 50 kg water/kg recovered protein. For oil-rich legumes, water consumption of 90 kg water/kg protein has been reported (Berghout *et al.*, 2015). In contrast, dry fractionation by definition consumes no water. Part of the added water during wet fractionation is removed by spray drying, which is the main cause for the difference in energy use between dry (3.6 MJ/kg recovered protein) and wet (54 MJ/kg recovered protein) fractionation.

Wet fractionated plant protein still embodied much less water compared to the production of animal protein. It is estimated that the primary production of 1 kg of animal protein requires about 200 m^3 water compared to the water use of 0.5–2 m^3 for the cultivation of 1 kg of cereal protein (Pimentel & Pimentel, 2003). The water required to extract the proteins is much less, approximately 2.5–20% of the water use of the cultivation. The protein delivery efficiency was calculated by the ratio of protein to invested life cycle energy in foods and indicates that animal products can provide 4–11 g protein per MJ while legumes can provide 41–77 g protein per MJ (González, Frostell, & Carlsson-Kanyama, 2011). The protein delivery efficiency is thus much larger for legume-based foods compared to animal-based foods. However, when further protein extraction is taken into account, the ratio for wet extracted pea protein reduces to 14.6 g protein per MJ. For dry fractionated pea this it is still 55.8 g protein per MJ. The latter calculation shows that the ratio for wet extracted protein is nearly equal to that for animal foods, which emphasizes the importance to explore the more efficient dry fractionation and the development of novel food products based on those fractions.

Development of a hybrid dry and aqueous fractionation process

Dry fractionation is a more efficient and sustainable process in terms of water and energy use compared to wet fractionation, but the protein purity of the dry-enriched fractions is lower than that of the wet-enriched fractions. For some applications a high purity can be essential. In those cases, a hybrid process involving subsequent dry and aqueous fractionation was very recently introduced that yielded a higher protein content and still requires less water and energy than wet fractionation only (Pelgrom *et al.*, 2015b). The differences between conventional wet fractionation and the new hybrid process are that suspension of flour takes place at a lower dilution, no chemicals (HCl, NaOH) are used and no dilution takes place before spray drying. The energy and water use of this combined process are graphically represented per kg product in Fig. 6. The fine fraction obtained by dry fractionation is suspended in

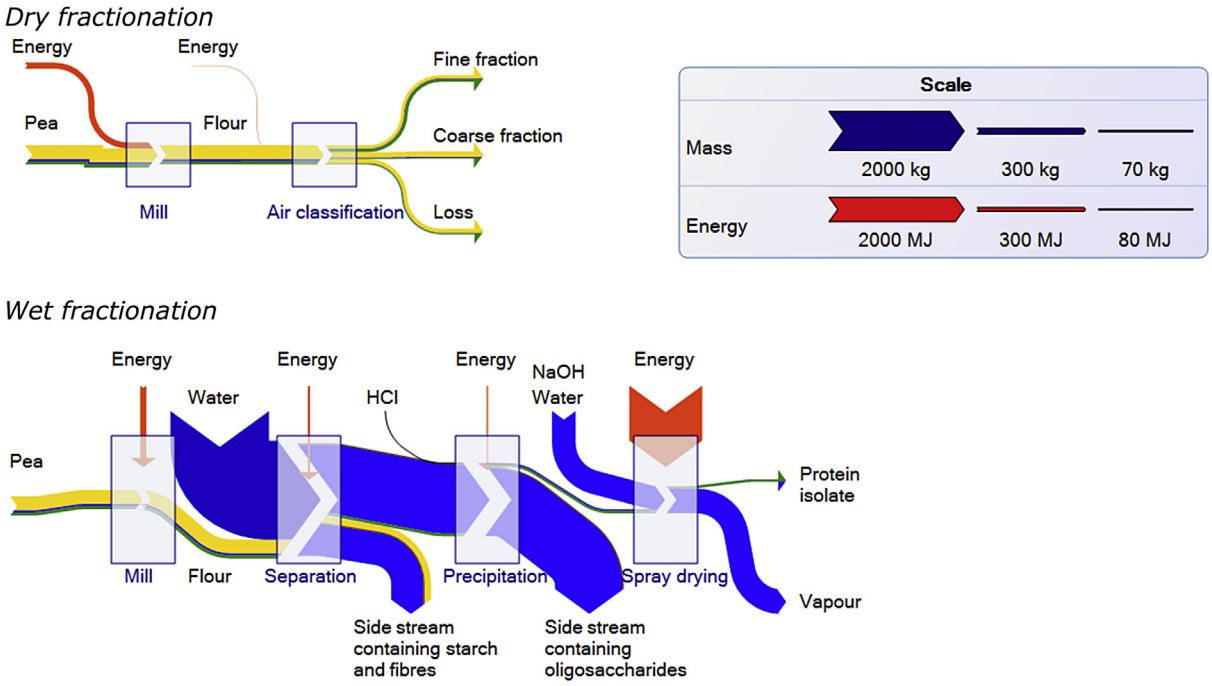

Fig. 5. Sankey diagrams of dry fractionation (left) based on (Pelgrom *et al.*, 2015a) and wet fractionation (right) based on (Passe *et al.*, 2008). Process streams containing water (blue), protein (green), starch, fibre, oil and ash (yellow) and energy (orange) are depicted. For simplicity reasons mass flows and energy are given in the same diagram.

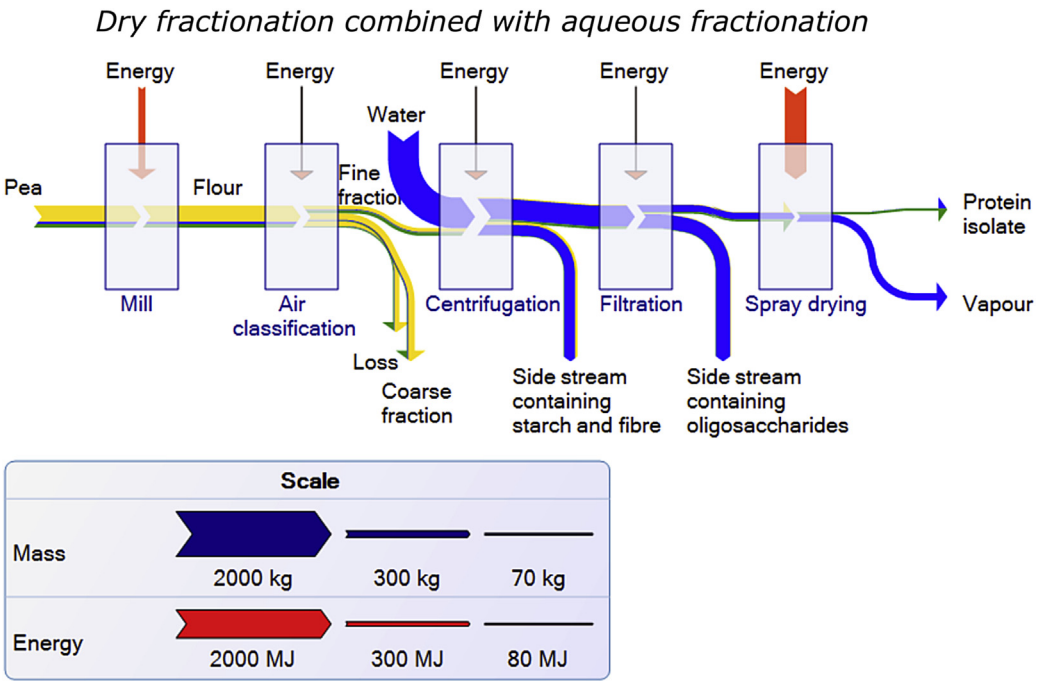

Fig. 6. Sankey diagrams of the combined dry and aqueous fractionation process based on (Pelgrom *et al.*, 2015b). Process streams containing water (blue), protein (green), starch, fibre, oil and ash (yellow) and energy (orange) are depicted. For simplicity reasons mass flows and energy are given in the same diagram.

water and the top aqueous layers were further purified by a centrifugation and a filtration step.

The water consumption may be reduced to 13 kg/kg protein because less powder has to be suspended; the fine fraction is 35% of the total amount of flour, but contains 81% of the proteins present in the flour. Reducing the water consumption by combining dry and aqueous fractionation is not specific for pea, but has also been proposed for lupine (Berghout *et al.*, 2015). Following this approach, the energy consumption can be reduced to 20 MJ per kg recovered pea protein (29.1 g protein per MJ), mainly because no water is added before spray drying. This water addition is needed in the conventional wet fractionation process to reduce the viscosity (17.8 ± 2.2 Pa s at 25 °C, shear rate of 100 s^{-1} and 30 g solids/100 g sample) (Bouvier & Campanella, 2014; Pawlowski, 2008). The viscosity of the protein solution obtained with the hybrid process is lower at similar conditions (0.3 ± 0.0 Pa s at 25 °C, shear rate of 100 s^{-1} and 30 g solids/100 g sample) because the proteins remain in their native state (Pelgrom *et al.*, 2015b). An overview of estimated energy use per tonne processed material of various process steps in the fractionation routes is provided in Table 2.

Table 2
Energy use per tonne processed material of various process steps used in dry, aqueous or wet fractionation. An energy efficiency factor of 0.5 was used for heating and drying processes. Cooling and transport energies are not taken into account.

Process step	Energy (MJ/tonne)	Reference
Mill	500	(Schutyser & van der Goot, 2011)
Air classification	23	(Schutyser & van der Goot, 2011)
Centrifugal decanter/ hydrocyclone	15	(Grimwood, 2011; Haverinen, 2014)
Nozzle	6	(Hui, 2008)
Spray dryer	4800[a]	(Schutyser & van der Goot, 2011)
Ultrafiltration	14	(Cheryan & Kuo, 1984; Ramirez, Patel, & Blok, 2006)

[a] For spray drying per tonne of evaporated water.

These estimations of the energy consumption do not take further utilisation of side streams into account. The side streams of wet fractionation contain more water and therefore require more energy to be processed into products. Overall, better utilisation of side streams will make processes more sustainable. The coarse fraction is rich in starch and is thus valuable for many applications (Gómez, Doyagüe, & de la Hera, 2012). Fibre-rich side streams can be used as additives to health promoting foods and beverages (Dalgetty & Baik, 2003). Moreover, pea fibres are increasingly used to enrich gluten-free products that are made from rice, corn or potato flour (Tosh & Yada, 2010). Next to that, side streams can be used for non-food applications, such as food packaging material (Mikkonen & Tenkanen, 2012; Al-Abbas, Bogracheva, Topliff, Crosley, & Hedley, 2006), bioethanol, biogas or animal feed (Draganovic, van der Goot, Boom, & Jonkers, 2013). In conclusion, the combination of dry and wet fractionation could be explored further for possible scale-up as the protein purity and yield are comparable to the traditional wet fractionation process but at reduced water consumption.

Functionality of dry-enriched legume protein fractions

Dry fractionation yields protein concentrates that exhibit native properties, but at a lower protein purity compared to wet fractionated isolates. The native properties are reflected in better solubility, foam stability, digestibility and lower viscosity compared to conventional protein isolates (Pelgrom *et al.*, 2014; Pelgrom *et al.*, 2015b; Pelgrom, Vissers, *et al.*, 2013). However, differences may be observed between functional properties of various legumes. The viscosity of the fine fraction of lupine is lower than that of the fine fraction of pea, but the viscosity of lupine flour is higher than that of pea flour (Pelgrom *et al.*, 2014; Pelgrom, Vissers, *et al.*, 2013). These differences can be explained by the higher intrinsic solubility of lupine protein and more water absorbing fibres in lupine flour. Moreover, lupine protein forms very weak heat-induced gels, while

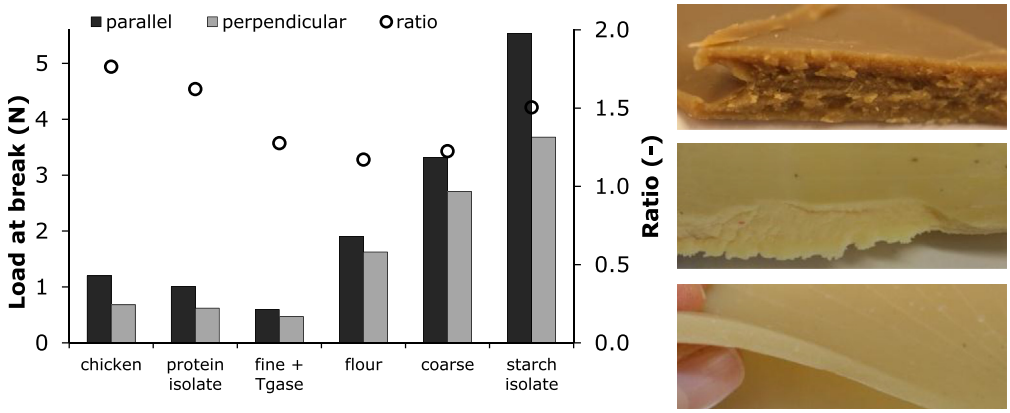

Fig. 7. Strength of structures formed after shear deformation (140 °C, 15 min, 30 rpm or with transglutaminase 50 °C, 35 min, 30 rpm) from various pea fractions (44 g/g sample, 1 g/g salt and 55 g/g tap water, and to the fine fraction transglutaminase was added to obtain an enzyme to protein ratio of 1:20) (Pelgrom, 2015). Load at break was determined using a Texture Analyser. Samples were cut to a dog-bone-shape in the direction parallel and perpendicular to the shear or for chicken breast filet parallel and perpendicular to the direction of the meat fibre. Images on the right represent protein isolate, fine fraction with transglutaminase and coarse fraction (bottom).

pea protein can form strong heat-induced gels (Berghout, Boom, & van der Goot, 2014; Pelgrom *et al.*, 2015b).

As indicated earlier, the relative modest enrichment during dry fractionation leads to higher amounts of other components in the protein concentrates, such as oil and fibres, which has impact on the functional properties. Native pulse proteins are relatively rich in albumins and therefore show very good foaming properties equivalent to those of egg white (Day, 2013). When exploring the foaming properties of lupine protein concentrates obtained by dry fractionation, it was found that after defatting the foaming properties of the concentrate improved drastically (Pelgrom *et al.*, 2014). In contrast, in the same study a commercial wet processed concentrate appeared to yield no foam at all; this was explained by denaturation and aggregation of the proteins. In another study again good foaming properties were observed, but in that case lupine proteins were obtained via a more mild wet isolation procedure (Day, 2013). Concluding, high solubility of the protein is key to achieve high foamability, but presence of other components play a role as well.

As discussed above, added value of dry fractionated protein concentrates can be found in products that require their high solubility, like beverages (high protein drinks) or products in which emulsification, water and fat absorption and adhesive properties are required, like baked goods, pasta, granola bars, meat products, vegetarian burgers and texturized products (Tulbek, 2010). For most of the applications mentioned, 100% purity is not required, so these protein concentrates may indeed be applied (Boye *et al.*, 2010). Protein concentrates are also associated with health benefits compared to completely refined proteins, although presence of specific components may also have an adverse health effect if not processed adequately (Jacobs, Gross, & Tapsell, 2009). Anyhow, one may move from the use of pure protein isolates to less refined concentrates, such as dry enriched fractions. This would result in ingredients that require far less resources to produce, while at the same time providing better composition for our health (fibre, micronutrients). Challenges that accompany this transition would be in new product formulations, more variability in the composition of ingredients and in taste and nutritional value; however it would also generate new freedom in these fields, for new types and qualities of products.

Differences in taste and nutritional value can originate from nutritionally active components that are partly deactivated during wet fractionation, but not during dry fractionation. Examples are protease inhibitors (trypsin inhibitors), amylase inhibitors, lectins, polyphenols, saponin and phytic acid (Asgar, Fazilah, Huda, Bhat, & Karim, 2010). Some of these components may influence the uptake of nutrients during digestion (Elkowicz & Sosulski, 1982; Guillamon *et al.*, 2008; Schlemmer, Frølich, Prieto, & Grases, 2009).

Recently, some of these components were associated with health promoting properties, like anti-oxidant and anti-carcinogenic activity (Guillamon *et al.*, 2008; Schlemmer *et al.*, 2009; Shi *et al.*, 2004). Toasting is frequently applied to treat dry enriched ingredient fractions to remove their bitter or astringent taste, for which saponins are responsible (Curl, Price, & Fenwick, 1985). Upon post-processing, like cooking, protease inhibitors, amylase inhibitors, lectins and polyphenols are deactivated or removed (Asgar *et al.*, 2010; Trugo, Donangelo, Trugo, & Bach Knudsen, 2000), and the amount of saponin is reduced by 7–53% (Shi *et al.*, 2004), while phytic acid is heat stable (Schlemmer *et al.*, 2009; Trugo *et al.*, 2000).

Air-classified ingredient fractions usually also contain lipoxygenase providing a beany flavour to the legumes. This enzyme may be deactivated during mild heating at 60 °C, thus without having a detrimental effect to the degree of protein denaturation (Asgar *et al.*, 2010). Additional research could indicate how to further balance the functionality, nutritional value and taste of the dry ingredient fractions.

Outlook on the development of solid meat-like structures

Dry enriched pea fractions gelate under non-flow conditions and phase separate upon suspension in water (Pelgrom *et al.*, 2015b). This means that the pea fraction possesses the two main properties needed to form anisotropy by gelation under shear-flow conditions (Manski, van der Goot, & Boom, 2007).

Soy concentrate and wheat gluten have been reported to form fibrous structures under shear flow (Grabowska, Tekidou, Boom, & van der Goot, 2014; Krintiras, Gobel, Bouwman, van der Goot, & Stefanidis, 2014). The presence of two separate biopolymeric phases is generally thought to be needed for structure formation. The two phases are deformed and aligned by the shear flow, leading to the formation of aligned or layered zones. The phase separation after suspension in water indicates that dry fractionated pea protein concentrates contain incompatible biopolymers and experiments show that it forms anisotropy under deformation (Fig. 7) (Pelgrom, 2015). Even though the ratios of the material strength parallel and perpendicular to the shearing direction are similar for the coarse fraction and the fine fraction using transglutaminase as a cross-linking agent, the latter formed a layered structure while the coarse fraction gave no layers.

Structure formation using pea protein was also observed after high moisture extrusion cooking (Osen, Toelstede, Wild, Eisner, &

Schweiggert-Weisz, 2014). In conclusion, there is potential for the formation of aligned structures, like meat replacers, from dry-enriched pea fractions, which justifies further research in this field.

Conclusions

We reviewed dry fractionation as a route to sustainably produce functional legume protein fractions. While dry fractionation has a protein delivery efficiency of 55.8 g protein per MJ it is only 14.6 g protein per MJ for conventional wet fractionation. A disadvantage is the lower purity of the dry enriched protein fractions. For this a hybrid fractionation route is evaluated, which still delivers 29.1 g protein per MJ. Different strategies have been reviewed to further increase purity of dry enriched legume protein. Specifically, the connection between legume morphology and ideal milling conditions requires further exploration. Moreover, selection of legume varieties and using improved dry separation techniques could contribute to further increase of protein purity. Finally, dry enriched fractions retain their native properties and exhibit much better solubility than conventional protein isolates. This makes them suitable for high protein drinks. Additionally, pea fractions can be gelatinized under specific conditions and were shown to have potential for preparing structured solid protein foods, such as meat replacers.

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