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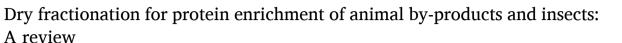
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Review





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ABSTRACT

Due to the global need for sustainably produced protein, optimal usage of animal by-product proteins and novel protein sources like insects are being explored. Dry fractionation, an emerging technology, offers significantly lower energy consumptions and no use of chemicals compared to conventional fractionation technologies. This review evaluates the current state and potential of dry fractionation for animal by-products and insects, with respect to characteristics of raw materials, pre-processing methods, milling, and product-oriented process optimisation.

The reviewed studies focussed on compound enrichment or fractions with distinct functionalities, rather than in depth product and process optimisation linked to composition and functionality. For animal by-products, optimisation should focus on milling and separation, whereas for insects optimisation should concern the entire process chain. A product portfolio and insight in compositional and functional properties after dry fractionation would allow more efficient use of animal by-product and insect fractions, thereby supporting the protein transition.

1. Introduction

Due to the expected growth in world population, 26 % more people must be fed in 2050 (Shepard, 2019). Moreover, the average worldwide meat consumption per capita is expected to increase by 29 % as people are becoming more affluent (Wu et al., 2014). The demand for protein thus grows while the availability of agricultural land decreases due to climate change and over-use of the agricultural fields. We therefore need a transition towards different production and use of novel sources of proteins. While meat and seafood are two important traditional protein sources, 32-57 % (wet basis) of these sources end up as by-products or waste (Jedrejek et al., 2016; Meeker and Hamilton, 2006). These animal by-products are rich in high value nutrients such as proteins. In addition to by-products, novel protein-rich food sources like insects, require less water and land, emit less greenhouse gases, and have higher feed conversion efficiencies compared to conventional meat sources. In addition, insects can be grown on side streams, effectively upgrading these streams (van Huis and Oonincx, 2017). Although the crude protein content of insect species varies greatly, it is similar to that of cattle, poultry, and fish (Akhtar and Isman, 2018). Overall, both animal by-products and insects may well give us additional proteins with high nutritional and technical functionality, if we find ways to concentrate the proteins without large impact on the environment.

Direct use of animal by-products and insects as food is often disliked by consumers and is hindered by the high level of non-protein components such as ash and chitin, which has a negative impact on the digestibility, taste and technical functionality (Moutinho et al., 2017; Tan et al., 2016). To increase the protein content and digestibility of animal by-products and insects, both dry and wet fractionation strategies may be employed. Wet fractionation generally involves selective solubilisation of proteins or other components at elevated pH, low salt concentration (i.e. salting in), or using a mixture of ethanol and water. This is then followed by precipitation at the isoelectric point, salting out, or use of solvents. Such procedures require the use of water and chemicals. If not all proteins dissolve or precipitate a significant part of the proteins may be lost. Generally, the proteins then need to be dried. Dry

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fractionation also involves drying but does not require any additional water or chemicals for the separation. The dried material is fragmented by milling, and subsequent mechanical separation of the fragments using air classification or other methods, such as tribo-electrostatic separation.

As compared to wet fractionation methods, dry fractionation methods have lower energy consumption and use neither water, nor chemicals (Jonkman et al., 2020; Schutyser et al., 2015). For example, the production of fish protein isolates by wet fractionation requires 5-9 parts of water per part of fish, which must then be removed by drying to obtain a protein powder (Shaviklo and Etemadian, 2019). Although animal products contain water that needs to be removed before any dry processing, the amount of water and thus the energy required in drying is much lower than in wet fractionation. As drying is the most energy intensive process of the processes involved in fractionation, a decrease in water that needs to be dried reduces the energy requirements of the whole process considerably (Lie-Piang et al., 2021). Furthermore, dry fractionation techniques induce less structural changes in proteins, as was demonstrated for plant seed proteins (Assatory et al., 2019). However, wet fractionation can yield fractions with a higher purity than dry fractionation, since the dissolution effectively detaches individual molecules from each other, while with dry fractionation the composition of the individual fragments dictates the maximum enrichment that can be obtained. The fractions obtained after dry fractionation typically differ in composition and functionality from wet enriched fractions (Schutyser and van der Goot, 2011). Dry enriched fractions thus fit better in products that do not require pure ingredients and where a cleaner production approach is targeted. All this implies that the decision between dry and wet fractionation techniques should be based on the product requirements and the nature of the raw materials.

Hitherto, dry fractionation of plant seeds, beans, and pulses has gained considerably more attention than dry fractionation of animal byproducts or insects, possibly because animal sources must first be dried before they can be subjected to dry separation processes. The presence of distinct components (i.e. protein, ash, and chitin) could make animal byproducts and insects suitable for dry fractionation. However, there is currently not much known about the transferability of the existing dry fractionation techniques, developed for plants proteins, to animal- and insect products. Furthermore, pre-processing steps, such as drying, largely affect the yield and efficiency of such a process (Assatory et al., 2019). Therefore, it is crucial to choose the right pre-processing, milling, and separation conditions to obtain the desired fractions.

This review evaluates the current state and potential of dry fractionation techniques for processing animal by-products and insects. Animal by-products include meat and bone meal (MBM), fish meal, and shellfish waste. The entire process chain employing dry fractionation, as illustrated in Fig. 1, is described in the present review, starting with the raw materials (§2). Here, the differences between animal by-products and insects in comparison to plants are highlighted. After the raw materials section, pre-processing steps and methods are described (§3). Pre-processing may include deshelling, rendering, biological

decontamination (e.g. blanching), drying, and defatting. After preprocessing, the products must be milled ($\S4$) into a flour. It is described why milling is performed and what effects milling will have on the dry fractionation performance. The flour is consecutively separated via sieving, air classification or electrostatic separation ($\S5$), which is indicated as "dry fractionation" in Fig. 1. Reported work on dry fractionation of animal by-products and insects is elaborated in section 5. Lastly, optimal usage of dry enriched fractions is explored via productoriented process optimisation ($\S6$), to enable the most efficient use of insect- and animal by-product fractions. This section will discuss the possible optimisation approaches of the entire dry fractionation process in industry and indicate routes to maximise fraction usage.

2. Raw materials

To perform dry fractionation on animal by-products and insects, knowledge on the raw materials is crucial. The compositions of various animal by-products and insects are discussed in the following paragraphs and are shown in Table 1. Lupin beans are added as reference, as among the plant products studied in literature for dry fractionation, lupin is the most similar crop based on composition (lipid and protein content; absence of starch) and cell size. As meat and bone meal (MBM) and fish meal may come from different animal species, typical values are shown (Garcia et al., 2006). In the present review, a distinction was made between the animal by-products and the insects. In contrast to the by-products, insects are often grown specifically for production of protein, and their processing can therefore be better tailored towards the dry separation procedures. Furthermore, insects are processed as a whole, while animal by-products are specific parts of the animal, such as bones. This implies potentially different dry fractionation strategies.

Since dry fractionation is currently mostly applied for plant materials, it is important to consider the similarities and differences between plant protein sources and animal products. The structure of animal tissue is not the same as the structure of plant tissue. On average, plant cells (10–100 μm) are larger than animal cells (10–30 μm) but have higher structural rigidity due to their cell walls. Another important difference is that plant cells contain specific storage bodies, such as protein or starch bodies, which facilitates separation by mechanical means (Pelgrom et al., 2014). Dry fractionation has been extensively studied for pulses like yellow field pea and navy bean. However, the compositions of vellow field pea and navy bean are very different from that of animal by-products and insects, mainly due to their high starch contents. In contrast, lupin has similar sized cells (30–35 µm) without large starch granules and has a fat content approximately similar to that of the animal products reviewed in this study, as can also be seen in Table 1 (Aguilera and Garcia, 1989). The outcomes of studies on lupin will be used as a reference throughout the manuscript. The findings of the studies on starch rich pulses will merely be used to explain the principle of the dry fractionation process and highlight optimisation possibilities and functionalities achieved.

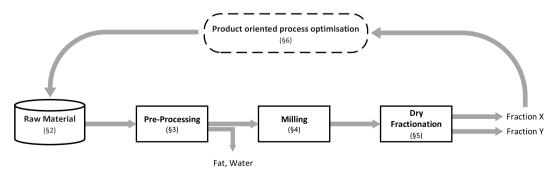


Fig. 1. General visualisation of processing the raw materials (i.e. animal by-products and insects) into the final fractions. The different sections of the current review are indicated by the section signs (§).

Table 1
Proximate composition based on dry weight of animal by-products, insects, and lupin.

Raw material		Protein content (%)	Lipid content (%)	Carbohydrates/fibre/chitin content (%)	Ash content (%)	References
Animal by- products	Pure meat meal	75–87	ND	ND	8–14	Garcia and Phillips (2009)
	Pure bone meal	29–32	ND	ND	62–66	Garcia and Phillips (2009)
	Meat and bone meal	43–63	8–16	ND	16–40	Adedokun et al. (2014) Garcia et al. (2006)
						Garcia and Piazza (2015)
						Parsons et al. (1996)
						Shirley and Parsons (2001)
	Fish meal	56–82	5–16	ND	10–33	FAO (1986)
						Flynn et al. (2020)
						Hansen et al. (2010) de Lima et al.
						(2014)
	Shrimp waste Crab waste	10–40 10–35	0–14 1–2	15-46 (chitin) 13-29 (chitin)	30–60 38–58	Nirmal et al. (2020)
						Tan et al. (2020)
						Antunes-Valcareggi et al. (2017)
						Hamdi et al. (2017)
						Jung et al. (2007) Muralidhara and Maggin (1985)
Insects	Yellow mealworm larvae	45-65 (crude)	13–35	4-15 (fibre)	3–7	Boulos et al. (2020)
						Purschke et al. (2018)
						Roos (2018)
						Rumbos et al. (2019)
	Adult house crickets	55-75 (crude)	7–23	3-23 (fibre)	2–14	Boulos et al. (2020)
						Kulma et al. (2019)
						Roos (2018)
						Rumbos et al. (2019)
						Rumpold and Schlüter (2013)
Plant seeds	Lupin	33–37	6–8	52–57	2–3	Erbersdobler et al. (2018)
						Pelgrom et al. (2015a, 2015b)

ND = no data available.

2.1. Animal by-products

Slaughtering creates two main types of product streams: whole meat and by-products that are further processed into for example tallow (i.e. extracted fat), degreased bones (for gelatine production) and protein meals (e.g. fish-, meat- and bone meal) (Hicks and Verbeek, 2016). From the whole animals that enter the slaughterhouse, 32-57 % (wet basis) end up as by-product or waste (Jedrejek et al., 2016; Meeker and Hamilton, 2006). When considering that in 2019 over 68 million tonnes of cattle meat alone was produced, one can imagine the potential to extract components of value, such as proteins, out of this side stream (FAO, 2020). The edibility of an animal by-product stream depends on the category of the starting material and the hygienic conditions during processing. Furthermore, legislation determines whether animal by-products can be fit for human consumption. According to European law, animal products and animal by-products are classified into three different categories before slaughtering. Both category 1 and 2 comprise material that is not fit for human consumption (e.g. infected material, spinal cord and brain), while category 3 material is fit for human consumption (EFPRA, 2020b; Jedrejek et al., 2016). Meat and bone meals from category 3 material are also known as processed animal protein (PAP) in Europe, if processed according to strict regulations (EFPRA, 2020a). This is due to safety considerations that are a consequence of the BSE outbreak in the 1990s. However, the regulations might soften in the near future, enabling wider use of these by-products (Ricci et al., 2018). This review will focus on category 3 material.

Pure meat meal, made exclusively from soft tissue particles like muscle cells, has consistent crude protein (75–87 %) and ash (8–14 %) contents regardless of the species, which also holds true for the protein (29–33 %) and ash (62–66 %) contents of pure bones (Table 1). Bone tissue is mainly composed of collagen, crystalline minerals and other minor proteins, aggregated into fibrillar structures (~500 nm in diameter). The fibrils are stacked and mineralised to form larger fibres (micron to millimetre scale), to form either the inner light porous bone or the outer dense and protective bone (Kane and Ma, 2013). As explained previously, meat and bone meals (MBMs) are often mixtures

of dried soft tissue and bones. Therefore, various compositions were reported for meat and bone meal, as seen in Table 1. For similar reasons, fish meal compositions are also reported with varying protein (56–82 %), fat (5–16 %), ash (10–33 %) and dry matter content (90–98 %), as is summarised in Table 1. MBM and fish meal usually have a high protein quality. The protein quality can be assessed via several methods, for example the protein digestibility-corrected amino acid score (PDCAAS). The PDCAAS can range between 0 and 1, in which 1 is the best score, indicating a high-quality protein. Seafood and animal proteins have in general a high PDCAAS of 1 or close to 1 (Huang et al., 2018; Tan et al., 2018). Specific proteins might have a less favourable amino acid profile. Collagen for example is deficient in some essential amino acids resulting in a lower PDCAAS of 0.52 for young children (2-5) and 0.94 for adults (Dong et al., 2014). This does not implicate that these proteins are not of use, for example collagen has very high technical functionality as a gelling agent (after hydrolysis into gelatine). They can also be combined with other protein sources to obtain an overall better nutritional balance.

Next to mammals and fish, animal by-products also originate from shellfish, that are aquatic invertebrates with an exoskeleton. These include amongst others crustaceans like shrimps, crabs, and lobsters, which are genetically related to insects (Mishyna and Glumac, 2021). The shellfish processing industries produce a significant amount of by-products. Depending on the species, 50-75 % of the total weight of shellfish ends up as waste (Saima et al., 2013). It is estimated that in 2012 the major lobster processing countries (Canada, United States of America and Australia) produced over 50,000 tons of lobster by-products (Nguyen et al., 2017). The crustacean exoskeleton or shell is an important part of these by-products. This exoskeleton is made of a cuticle consisting of four different layers (Nagasawa, 2012). In general, shellfish by-products contain, as seen in Table 1, 10-40 % proteins, 30–60 % ash (mainly calcium carbonate), and 13–46 % chitin along with other compounds like pigments and lipids. Shellfish also include mussels and other molluscs. Mussel by-products are not included in this literature review, as to our knowledge no research has been done towards dry fractionation of mussel by-products, although this could potentially be of interest. Dry fractionation could be used to separate protein, calcium carbonate, and/or chitin, which are the main constituents of mussel shells (Naik and Hayes, 2019; Varma and Vasudevan, 2020).

2.2. Insects

In contrast to animal by-products, for insect processing typically the whole insect is used. Currently, over 2000 edible insect species are known (Jongema, 2017). Although insects are already consumed in many tropical countries for millennia, Western countries currently have strict regulations on insects for food and feed consumption. However, the legislation with respect to insects for food and feed, amongst others in the European Union, is easing, which increases the possibilities for the food and feed industry to include insects in their products (Belluco et al., 2017; Meijer and van der Fels-Klerx, 2017). Currently, the insect industry is growing rapidly, and this growth is predicted to continue for the coming years (Wade and Hoelle, 2020).

Insects are in general a good protein source with a protein content and quality similar to that of cattle, poultry, and fish (Churchward-Venne et al., 2017). Insects contain different types of proteins, including cuticular proteins in the exoskeleton, muscle proteins, and haemolymph (Yi et al., 2013). Furthermore, insects contain more polyunsaturated fatty acids, iron and zinc than conventional meat sources (Rumpold and Schlüter, 2013). Insects are also a source of fibre, which is mainly found in the exoskeleton in the form of chitin, together with the cuticular proteins (Finke and Oonincx, 2017; Yi et al., 2013). These compositions vary largely between insect species and within individual insect species caused by factors like the feed and the developmental stage of the insect. In the developmental stage of insects, one of the important aspects is the difference between larvae and adults. Larvae and adults have different amino acid profiles, yellow mealworm (Tenebrio molitor) adults contain for example more protein and chitin, while the larvae contain more lipids (Finke and Oonincx, 2017; Nowak et al., 2016). Table 1 shows the composition of yellow mealworm larvae (T. molitor) and adult house crickets (Acheta domesticus). Here, the crude protein contents are given, which also include nitrogen originating from chitin, based on dry weight (Churchward-Venne et al., 2017; Roos, 2018).

The composition and structure of animal by-products and insects are different from plant sources commonly used for dry fractionation. Furthermore, there are large distinctions in the composition of various animal by-products and insects. However, based on the composition and protein quality of the animal by-products and insects there is a high potential to upgrade these products into safer and more versatile ingredients, with increased sustainable and nutritional aspects. The presence of different compounds, i.e. protein and non-protein components like ash and chitin, suggest that the materials are suitable for dry fractionation.

3. Pre-processing

Raw materials (i.e. animal by-products and insects) must be preprocessed and milled before dry fractionation. For animal by-products and insects, pre-processing steps may include deshelling, rendering, biological decontamination defatting, and drying. The purpose of these pre-processing steps is to minimise safety concerns and to prepare the material for the dry fractionation process. The pre-processing steps chosen depend on the raw materials and will affect the properties of the products. Deshelling and rendering are usually already carried out with current by-products processing but need to be addressed here as these processes affect the product properties. After pre-processing, milling is a necessary step to fragment the material into a flour to enable subsequent dry fractionation (Schutyser and van der Goot, 2011).

3.1. Deshelling

Of all the materials of interest for the present review, both insects

and shellfish have a shell, i.e. exoskeleton. Shellfish are deshelled, while insects are generally too small and too soft for deshelling. However, the chitin from the insect shells can later be removed via dry fractionation. Deshelling is done to separate the meat from the shells, which is the byproduct. There is great potential to extract the residual proteins in the by-products, but also to extract the chitin. Chitin could be used in, amongst others, food applications and agriculture (Barikani et al., 2014). Deshelling is either done manually or mechanically. Before the deshelling process, shell loosening is often necessary. For shrimps, this is traditionally done by letting the shrimps mature in ice or brine for several days. To shorten this process, alternative techniques for deshelling are now emerging, like high pressure, ultrasound and enzyme treatments. In addition to efficient loosening of shells, these techniques allow the preservation of food quality and ensure food safety. For example, instead of boiling lobsters and crabs to loosen the shell and reduce the number of microorganisms, high pressure can be used (Dang et al., 2018).

3.2. Rendering

After cleaning and slaughtering of vertebrates, by-products are rendered, such as cattle skeletal bones. Rendering plants must strictly follow approved methods with specified processing conditions (i.e. time, temperature and pressure applied) and meet standards for the final product quality, such as microbial counts (Ricci et al., 2018). In general, all rendering processes consist of heating for decontamination, moisture removal and consecutive separation of fat and other solids, yielding crude animal fat and protein meal (Hicks and Verbeek, 2016; Meeker and Hamilton, 2006). Moisture is removed to improve the product stability, the animal fat is then further cleaned in a separate step and, depending on the risk category of the starting material, used as edible fat in food or as inedible fat for other applications (Alm, 2020). Water and/or steam is used in wet rendering, but dry rendering is also performed. In this method, the raw material is cooked to melt the fat and to condition the animal fibrous tissue. The cooked material is then drained and pressed to separate the fat from the protein material (Prokop, 1985). During the heating of the materials, the proteins are most likely denatured. Therefore, further biorefining to separate those streams into materials of value should be driven by sustainability instead of by preserving native functionality.

3.3. Microbiological and enzymatic stabilisation

Microbiological stabilisation extends the shelf-life and minimises safety concerns related to the further use of the raw materials. Various methods can be applied to decontaminate the raw materials, but the most common methods to inactivate micro-organisms and enzymes involve a heat treatment, for example blanching for insects. Similarly, vertebrate by-products are rendered to amongst others provide biological stabilisation, which was discussed section 3.2. While a heat treatment is effective, it also denatures proteins and thereby degrades the quality in terms of native functionality, as can result in for example a reduced solubility. On the other hand, a heat treatment can, depending on the type of material, improve both the digestibility and taste of the product. Thermal inactivation of enzymes as proteases and lipases, prevents or minimises amongst others enzymatic browning in insects, which is undesired for food products with lighter colours (Janssen et al., 2019; Purschke et al., 2018). When heat treatments are undesired, milder processes that apply milder temperatures (<40 °C) may be used for microbiological and enzymatic stabilisation. Examples of relatively mild processes include high pressure processing and atmospheric cold plasma (Barba et al., 2017). High pressure processing and cold plasma treatments have been performed with yellow mealworm (T. molitor) larvae (Rumpold et al., 2014). Drawbacks of milder processing include their higher cost and their limited applicability, e.g. only surface treatment by cold plasma is possible.

3.4. Defatting

Depending on the fat content of the raw material, fat may be released upon milling, which may impede the milling process. A defatting step before milling may prevent agglomeration during milling and dry fractionation. The extracted animal fat can be used as an ingredient in food and feed. Meat and bone meals are already defatted by rendering. However, additional defatting is sometimes performed to further enhance the flowability. Nevertheless, thorough defatting can result in increased dust release during dry fractionation and thus again lead to larger product losses. Therefore, chilling below the melting trajectory of the fat, or the addition of anticaking agents may be alternatives to traditional defatting (Garcia et al., 2007; Garcia and Piazza, 2015). Defatting has been used more frequently with plant seed fractionation. For instance, Xing et al. (2018) achieved successful soybean protein enrichment of 15 % by dry fractionation after defatting. Protein enrichment was also reported for rapeseeds, sunflower seeds, and lupin (Basset et al., 2016; Laguna et al., 2018; Pelgrom et al., 2014).

Mechanical pressing and organic solvent extraction are widely used methods to remove oil. Both processes may lead to protein degradation. Solvents such as ethanol and hexane tend to denature proteins, but also the force and temperature applied during mechanical pressing may affect the protein nativity in the obtained cake. Furthermore, application of organic solvents (e.g. hexane) has downsides concerning sustainability, cost, and safety restrictions. Alternatively, milder and more sustainable extraction techniques can be used. For example, cold pressing better preserves the product quality, albeit at the cost of some oil yield (Nde and Foncha, 2020). Cold pressing has been used for fat extraction of black soldier fly (Hermetia illucens) larvae (Matthäus et al., 2019). Supercritical carbon dioxide (SC-CO₂) extraction causes minimal protein denaturation and may retard lipid oxidation, while the oil yield is similar to the oil yield after pure hexane extraction (Russin et al., 2011). The effectiveness of SC-CO2 was for example demonstrated for defatting of yellow mealworms (Purschke et al., 2017). However, the use of SC-CO2 also has some disadvantages, including high capital and operational costs (Russin et al., 2011). To conclude, defatting is applied prior to dry fractionation to extract oil as a fraction from the raw materials, to reduce agglomeration and to increase particle flowability. The chosen method impacts the final product quality in terms of sustainability, composition, and protein functionality after dry fractionation.

3.5. Drying

For dry fractionation, the feed material must be dry. In some cases, the material is already dry enough (i.e. rendered animal protein meals), while in other cases an additional drying step needs to be performed. The moisture content must be low enough to allow the creation of a freeflowing flour that is sufficiently fine for resolution between the different constituents, but not yet giving rise to clumping and aggregation due to interparticle interactions. There is no golden standard for the moisture content needed for dry fraction, as this depends on the raw material and the exact process to be applied. For example, the optimum moisture content for air classification of legumes was between 7 and 9%, while for other applications, like debranning of wheat grains, higher moisture contents are allowed (Owusu-Ansah et al., 1991; Schutyser and van der Goot, 2011). Drying methods for animal by-products and insects include contact-, oven- or microwave drying. These thermal methods will likely induce similar protein denaturation as in the stabilisation step. If this is undesired, alternative drying methods can be used that involve temperatures below the denaturation temperature. An example of such a method is dehumidified air drying, in which relatively low temperatures of below 40 °C can be used (Djaeni et al., 2018). Another example of a lower-temperature drying method is freeze-drying. An additional advantage of freeze-drying in case of insects is that the matrix becomes porous, which will ease the subsequent milling (Purschke et al., 2018). However, disadvantages are that freeze drying is quite energy intensive,

expensive and time consuming, and it has been reported to reduce the solubility of proteins as well (Berghout et al., 2015; Ratti, 2001).

In summary, before dry fractionation of animal by-products and insects, pre-processing steps are applied dependent on the raw material. Deshelling of shellfish and rendering of animal by-products are usually already performed. These processes are important to take into account as they strongly affect the final product properties. After deshelling and rendering, additional pre-processes can be applied to alter the powder properties before milling and separation. As insects are mainly processed whole, there is more freedom to apply the desired pre-processing steps for the purpose of dry fractionation. Within the pre-processing steps described, a wide range of methods is available. These methods can have different effects on the final quality of the materials, depending on the method and the raw material. When selecting pre-processing methods, the quality needs for the final product application must be considered.

4. Milling

Milling is considered perhaps the most critical step to enable subsequent dry fractionation. During milling, small fragments with different composition are created. This is critical as without physical detachment, dry mechanical separation would be impossible (Schutyser and van der Goot, 2011). Milling is often done in two consecutive steps. First rough milling is performed to increase the surface area, to allow for defatting and for the second fine milling. This fine milling is performed to give the material the desired particle size distribution for the dry fractionation process, and to release cellular components. Different mill types can be used. One distinction can be made between classifier mills and other mills. In a classifier mill, particles larger than the desired particle size are recirculated until they have reduced to the desired size. When materials are temperature sensitive, contain volatile components, or are too elastic or soft, cryogenic milling can be used, in which materials are milled while immersed in a cryogen, usually liquid nitrogen. The type of mill used might also affect the final separation efficiency, as was observed by Vitelli et al. (2020). The milling method used has to be tuned to the characteristics of the raw materials and the desired particle size reduction. A classification of the main milling methods is given by Gao et al. (2020).

4.1. Consequences of ineffective milling

There are only limited studies on the effects of milling on the dry fractionation efficiency of animal by-products and insects; most studies have been performed on the optimal milling settings for dry fractionation of plant products. During fine milling of starch-containing pulses such as yellow field peas, large starch granules (~22 µm) and small protein bodies (1-3 µm) in the cotyledon are released (Pelgrom et al., 2013). It is critical for the subsequent separation that these components are physically detached. Both too coarse and too fine milling can lead to unsuccessful dry separation. Too coarse milling leads to poor cell breakage and insufficient release of individual components, which results in lower yields of the components and lower purities of the fractions. Too fine milling on its turn causes damage of larger cellular materials, like starch granules, resulting in similarly sized starch granule fragments and protein-rich particles. Too fine milling also causes poor flowability and the formation of aggregates due to increasing van der Waals forces, ultimately leading to poor dry fractionation results (Pelgrom et al., 2013). Even though animal products do not contain starch granules, milling into an optimal particle size is still essential, as agglomeration will take place upon too fine milling and microstructures will not be detached upon too coarse milling.

4.2. Effective milling for dry fractionation

To discuss effective milling conditions to be used for dry

fractionation, we use three examples from various plant materials that were milled to different degrees. It must be noted that the relation between the wheel speed and the obtained particle size distribution is machine specific and depends on factors such as the air speed and the wheel type and size, so the specific settings give only an indication for comparison purposes. In the first example, for air classification of lupin an optimal wheel speed of 1000 rpm was found during impact milling (ZPS50 impact mill Hosokawa Alpine) and for electrostatic separation an optimal wheel speed of 2500 rpm was found. In the second example, impact milling (ZPS50 impact mill Hosokawa Alpine) of different plant seeds showed optimal classifier wheel speeds of 2200 (lentil), 2900 (chickpea), and 4000 (yellow pea) rpm (Pelgrom et al., 2015a). In the third example, impact and shear milling (UPZ100 impact and shear mill Hosokawa Alpine) of rapeseed meal and sunflower meal also resulted in different ideal grid sizes for electrostatic separation of respectively 0.1 mm (average particle size of 23.7 μ m) and 0.5 mm (average particle size 105 μm) (Laguna et al., 2018). Therefore, these three examples indicate that the optimal particle size and best milling settings are dependent on both the fractionation method applied and the raw material used. Typically, one has to find the balance between good separation and minimising losses in the system due to clumping, as discussed above.

For plant materials, the required milling intensity increases with a higher hardness, a lower brittleness, and/or a higher crude fibre content (Assatory et al., 2019). When two substructures (e.g. protein bodies vs. starch granules in pulses) differ in brittleness (e.g. one in the rubbery state and one in the glassy state), this will benefit disclosure during milling and thus favour separation (van Donkelaar et al., 2015). Furthermore, pre-treatments influence the final particle size after milling, as was observed for lupin (Pelgrom et al., 2015b). In general, animal cells (10-30 μ m) are smaller than plant cells (10-100 μ m), which may require higher milling speeds than for plant material. However, it is not clear whether results from dry fractionation of plant materials can be translated to animal tissue, as plants have for example rigid cell walls, which are absent in animal cells. Furthermore, components in animal by-products and insects are organised in larger structures such as the exoskeleton, which may require milling strategies targeted at larger particle sizes.

In summary, the degree of milling critically affects the efficiency of the dry fractionation process. An optimal particle size distribution should be found for each raw material. The best settings for animal byproducts and insects, are yet to be identified.

5. Dry fractionation of finely milled material

After milling, dry fractionation can be achieved using different

techniques, such as sieving, air classification and (tribo)electrostatic separation. Sieving is directly based on the size of the particles, where larger particles remain on top of the sieve and smaller particles pass through. Air classification is based on the combination of the particle size and density (Boye et al., 2010). Particles with smaller size and lower density are separated from larger and more dense particles by an upward airstream. In electrostatic separation, particles are separated based on their tribo-electric charging properties. Tribo-electric charging of particles is induced by particle-particle interactions and particle-wall interactions (Hemery et al., 2011). From these interactions, proteins are expected to gain a positive charge where most carbohydrates are expected to gain a slightly negative charge (Tabtabaei et al., 2016b). When subjected to a transversal electrostatic field, the fractions can be collected at respectively the ground and the positive electrode.

The three dry separation methods require different pre-processing and milling. Table 2 summarises the driving forces for separation, the effects of milling and defatting and general examples of usage of the three separation techniques. The impacts of milling and defatting are divided into "large", "medium" and "small". The effect of milling and defatting is smaller for sieving purposes as sieving is suitable for separation of larger particles. A medium to large effect of defatting was indicated for air classification and electrostatic separation as the effect varies between materials. For example, defatting had no impact on dry fractionation of yellow pea, whereas the protein separation efficiency increased for defatted lupin (Pelgrom et al., 2015b). The degree of milling is expected to have a larger influence on the separation efficiency of air classification and electrostatic separation than defatting. This because the separation is not effective when milling is not performed correctly, independent of the material.

As already mentioned in the introduction, the advantages of dry fractionation in comparison with wet fractionation include that no chemicals and water are required, the process is more energy efficient, and the process preserves the native structure and thus functionality of proteins (Assatory et al., 2019; Jonkman et al., 2020; Schutyser et al., 2015; Zhu et al., 2021). Disadvantages include a lower obtained protein purity, and the need of a difference in triboelectric or other physical properties for separation purposes (Schutyser and van der Goot, 2011; Tabtabaei et al., 2016b). Next to general advantages and disadvantages of dry fractionation, each of the three different separation methods also have their specific advantages and disadvantages, which are listed in Table 2. While specific for the separation method, these advantages and disadvantages are valid for most materials, including animal products. To mitigate the risk of powder explosion as indicated in Table 2, many studies use an inert gas (e.g. nitrogen) to convey the powder during electrostatic separation (Zhu et al., 2021). When more than two

Table 2
Three dry fractionation methods, their corresponding driving forces, the effect of milling and defatting on their separation efficiency, general examples of usage, and their specific advantages and disadvantages (Assatory et al., 2019; Schutyser and van der Goot, 2011; Xing et al., 2020b; Zhu et al., 2021).

	Sieving	Air Classification	Electrostatic Separation	
Driving force(s)	Particle size	Particle size and density	Tribo-electric charging properties	
Effect of milling	Medium	Large	Large	
Effect of defatting	Small to medium	Medium to large	Medium to large	
General example(s) of usage	Particles with larger sizes	Separation of protein and ash Separation of protein and starch	Separation of proteins and fibres	
Advantage(s)	Mature technology with well understood separation mechanism Well suitable for multicomponent separation Low capital investment	Relatively mature technology with well understood separation mechanism	Translation to food materials is relatively recent Combination with air classification is straightforward and can lead to superior separation	
Disadvantage(s)	Sieve blinding and bridging Only suitable for powders with larger particles Scalability is limited	Large gas volumes needed Separation mechanism not yet completely understood Finely milled particles may aggregate due to electrostatic- and Van der Waals forces Prior defatting necessary to avoid capillary bridging Strongly dependent on the humidity of materials and gas Risk of powder explosion if run with air		

fractions with different particle sizes are required for optimal separation, sieving may be a suitable method, as an additional sieve can easily be added. For air classification an additional air classifier unit behind the first one should be added or a multi-product air classification system (e.g. elbow jet air classifier) should be used (Furchner and Zampini, 2009; Yuan et al., 2013). For electrostatic separation more than two fractions can be obtained by adding collection bins and/or areas, the use

of multiple electrodes, or the addition of a second electrostatic separator (Tabtabaei et al., 2016a; Wang et al., 2016; Xing et al., 2018).

The dry separation methods applied can be cascaded or combined to alter the product properties in terms of composition and the component yields (Assatory et al., 2019). For example, sieving can be a pre-screening technique to eliminate larger fragments like the exoskeleton of insects. This can then be followed by fine milling and air

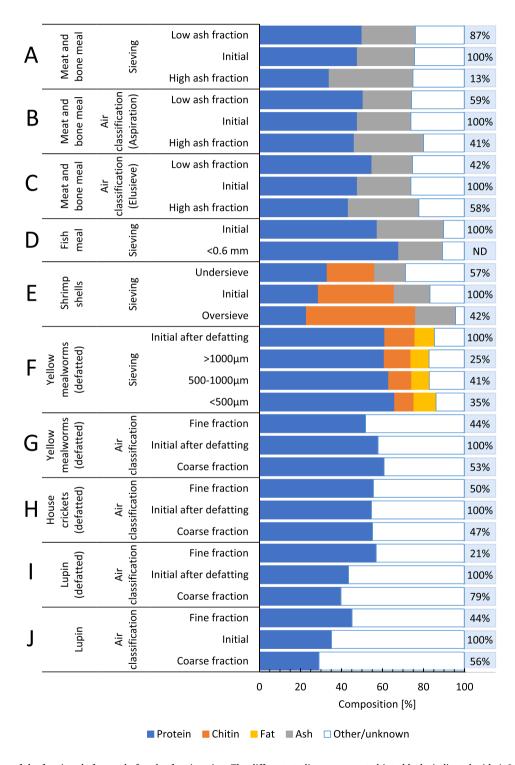


Fig. 2. Composition of the fractions before and after dry fractionation. The different studies are separated into blocks indicated with A-J. The percentages on the right indicate the mass percentage compared to the initial mass. ND indicates that no mass data was given (A-C: Garcia and Piazza, 2015; D: de Lima et al., 2014; E: Aye and Stevens, 2004; F: Purschke et al., 2018; G-H: Sipponen et al., 2018; I-J: Pelgrom et al., 2015b). The reader is referred to the online version for a colour representation.

classification or electrostatic separation. The advantage of this is that the purity can be improved and that more than two components (e.g. protein, chitin, and ash) can be separated by combining sieving, air classification and/or electrostatic separation. Next to this, repeated separations and intermediate re-milling can be used to improve the purity or yield (Wang et al., 2016; Xing et al., 2020b).

5.1. Meat and bone meal (MBM)

Meat and bone meal can be fractionated into protein rich fractions and ash/mineral rich fractions. Fig. 2 is a visualisation of the dry fractionation results of different raw materials. The first 3 blocks, indicated with A, B and C, show the compositions and yields before (indicated as initial) and after three different dry fractionation processes of MBM (indicated as low and high ash fractions). For example, sieving MBM (47.7 % protein, 27.9 % ash) resulted in a high ash fraction (yield 13.3 %, 34.0 % crude protein and 40.8 % ash) and a low ash fraction (yield 86.7 %, crude protein 50.0 % and ash 26.0 %), as can be seen in Fig. 2A (Garcia and Piazza, 2015).

Research on air classified meat and bone meal mainly focused on the functionalities of the air classified fractions rather than the process itself. The few studies that did focus on the fractionation will be discussed first. In a study on air classification of meat and bone meal by Garcia et al. (2005), protein and ash were separated using an aspirator. An aspirator is a specific air classification system where the particles are separated based on the terminal velocity of particles in an air stream. For this study meat and bone meal (55.8 % crude protein, 34.5 % ash, 9.7 % fat) was obtained commercially; the particle size was not reported. A slow feed rate and a low negative pressure yielded the highest protein content (60.9 %) while the ash content dropped (24.8 %), as illustrated in Fig. 2B. Garcia and Piazza (2015) combined sieving and subsequent air classification. The separation of commercially obtained meat and bone meal with this process resulted in ash and protein enriched fractions. The tested meat and bone meal had a broad particle size distribution with a mean particle size of 343 µm. Even though the particles were quite large, the protein content in the protein-rich fraction increased from 47.7 % to 54.8 % and the ash content of the high ash fraction increased from 26.1 % to 34.4 % (Fig. 2C). Moreover, the combined process provided a better separation (i.e. higher ash/protein shift) of particle types with different compositions than sieving or air classification alone.

Other studies on air classified meat and bone meal focussed on improving the digestibility by reducing ash levels. Bureau et al. (1999) found that low ash air classified MBM had an apparent crude protein digestibility that increased from 83 % to 87 %. Shirley and Parsons (2001) found a higher PER (protein efficiency ratio), a measure for protein quality, for 16.5 % ash MBM than for 35.2 % ash MBM. The authors suggested that the higher protein digestibility in low ash MBM was caused by a lower levels of collagen. These studies show that air classification may lead to a better functionality, in this case a higher digestibility of animal by-products, even if there is no overall increase in the protein content as such.

Air classification of meat and bone meal has been reported in literature and is already applied in industry, but electrostatic separation of meat and bone meal has, to our knowledge, not yet been reported in scientific literature. However, industrial trials with electrostatic separation were carried out for meat and bone meal: in one patented industrial trial, oven dried and sieved bovine bone meal (41 % protein, 50.5 % ash) was fractionated into a protein enriched fraction (65.5 % protein and 25.1 % ash) and an ash enriched fraction (38.7 % protein and 54.4 % ash) (Flynn et al., 2020). This shows the potential of electrostatic separation of meat and bone meal. In conclusion, sieving, air classification and electrostatic separation can be used separately or in combination, to separate meat and bone meal into fractions that are enriched in specific components, or have better functionality, but the available studies are still quite sparse and more work is needed to

explore the potential more fully.

5.2. Fish meal

Fish meal can be further fractionated into a protein rich fraction and an ash rich fraction. Sieving through a 0.6 mm sieve increased the protein content to 68 % (initially 57 %) and decreased the ash content to 21 % (initially 32 %), although fraction yields were not reported (Fig. 2D; de Lima et al., 2014). Air classification and electrostatic separation of fish meal have not been reported.

Some industries claim successful ash reduction with air classification, but no sufficient data on the extent of the reduction is shown. In one example the initial ash content (21 %) was reduced to 14–18 %, where the initial protein content (62 %) was increased up to 64–68 % (Hannibal Solutions International, n.d.). Flynn et al. (2020) achieved protein enrichment from fish meal (average particle size of 81 μ m) using an industrial patented electrostatic separation process from 73.4 % to 80.4 % protein with a yield of 81.3 %. Combination of different methods can result in a better functionality or higher purities, as was observed for pea by Xing et al. (2020b). In conclusion, the current data on sieving, air classification and electrostatic separation of fish meal is very limited, but some successes have been claimed, and there seems to be substantial potential.

5.3. Shellfish by-products

Two studies reported on dry fractionation of shellfish by-products, aimed at protein and chitin. Aye and Stevens (2004) studied dry sieving of black tiger shrimp (*Penaeus monodon*) shells. The dried shells (55 °C for 24 h, moisture content 3–5%) were ground into pieces with a diameter of 2–5 mm. Sieving was performed with a 0.85 mm screen to separate the proteins and the chitin. Of the ground shrimp shells, 57.1 % were able to pass the sieve (i.e. undersieve), and the protein content increased from 28.7 % to 33.0 % (Fig. 2E). The undersieve fraction still contained some chitin (\sim 36 % of the total chitin). The oversieve fraction was decalcified and deproteinated to produce chitin, as chitin is cross-linked with protein, possibly reinforced with calcium carbonate (Calvert, 1987). Even though about one third of the chitin was lost in the fine fraction, the authors conclude that this loss is easily compensated by the financial value of the protein powder.

Muralidhara and Maggin (1985) separated crab picking waste into a chitin-rich and a protein-rich fraction. Blue crab waste was dried and crushed to pass a 6.4 mm hardware cloth. Subsequent air classification yields a fraction (65 %) that had approximately the same composition as the feedstock (13–15 % chitin, 30–35 % protein, and 50 % CaCO₃), and a second one (35 %) that was sieved. The authors could achieve up to 58 % protein in a fraction representing 18 % of the dried starting material. It is likely that the process may be further improved to obtain a higher yield and/or purity, as the separation efficiency depends on the settings.

5.4. Insects

As of today, there are two articles to our knowledge about dry fractionation of insects to obtain protein-rich fractions. Purschke et al. (2018) used various pre-processing methods (blanching, drying, and defatting) on the dry fractionation behaviour and the physico-chemical properties of yellow mealworm (T. molitor) larvae. Sieve classification with various mesh sizes was used as a separation step. Both freeze drying and partial defatting by supercritical CO_2 extraction resulted in a significantly higher proportion of smaller particles (<500 μ m) as compared to coarse particles (>1000 μ m), due to the lower mechanical hardness of the material before milling. The highest proportion of smaller particles was found in the partially defatted powder, which was most likely caused by a reduced stickiness, preventing agglomeration of the fine particles. The chitin content was lower in the fine fractions, except for non-defatted blanched oven-dried powders. The lower chitin

content in the fine fractions was probably caused by the larger chitin-protein complexes in the insect exoskeleton ending up in the coarse fraction. Logically, the highest crude proteins contents (up to 66 %) were found in the partially defatted powders due to the lower fat content, while the fat containing powders consisted of up to 58 % crude protein. The resulting protein enrichment of 5.4 % is thus limited (Fig. 2F). The size of the particles in the smallest fraction (<355 μm) is still large compared to the typical size of animal cells, and we thus expect that the fragments are not broken down enough. However, the authors do suggest that chitin can be separated at these larger particle sizes, as it will end up in the coarser fractions. Enrichment of the proteins then may require re-milling of the finest fractions.

Sipponen et al. (2018) used supercritical carbon dioxide (SC-CO₂) extraction and pin milling, followed by air classification on house crickets (A. domesticus) and yellow mealworm larvae (T. molitor). At a rotor speed of 6000 rpm (Minisplit Classifier British Rema), a minor protein enrichment was observed for defatted yellow mealworms and no large differences in overall protein content were observed for defatted house crickets (Fig. 2G and H). However, the fractions differed in amino acid compositions, solubility, and sensory characteristics. Contrary to animal by-products, protein was here enriched in the coarse fraction, which contained more chitin, as assessed with fluorescence microscopy. The coarse fraction will therefore be enriched with protein originating from the exoskeleton. This coarse fraction had a lower protein solubility than the fine fraction. In terms of sensory profiling, the fine fraction was rated significantly powderier and had significantly higher meat-like flavour ratings, but there were no significant differences in flavour intensity and saltiness. The particle size and size distribution (2–350 μm) of the milled particles was comparable to the particle size and size distribution of milled lupin (Pelgrom et al., 2014), for which an optimal rotor speed of 8000 rpm was found in a tested range between 6000 and 10000 rpm (50 ATP classifier Hosokawa Alpine) (Xing et al., 2020b). Silventoinen et al. (2018) found that for barley the protein content increased when increasing the classifier wheel speed from 4000 rpm to 10000 rpm (50 ATP classifier Hosokawa Alpine), remained constant between 10000 rpm-18000 rpm, and then increased again from 18000 rpm-21500 rpm. Although Sipponen et al. (2018) tested a rotor speed of 21000 rpm, though at a lower air flow (50 m³/h instead of 120 m³/h) and a different classifier (50 ATP classifier Hosokawa Alpine), no rotor speeds between 6000 and 21000 rpm were investigated, which may indicate that the ideal settings have yet to be found, and the yield and/or purity may still be improved.

5.5. Dry fractionation optimisation for animal by-products and insects

Dry fractionation on animal by-products and insects until now is still in the phase of proving the principle. Further process optimisation is rarely performed, so the full potential to obtain higher yields and/or purities of the desired fraction(s) may not yet have been reached. For one, the relation between the milling intensity and good detachment of fragments for air classification and sieving is still lacking. In several of the discussed articles on air classification, the particle size was significantly larger than the average size of animal cells, which cannot result in optimal detachment of components (Aye and Stevens, 2004; Garcia and Piazza, 2015; Muralidhara and Maggin, 1985; Purschke et al., 2018). This is in agreement with the relatively small increases in protein content that were found.

Secondly, the protein content is negatively correlated to the protein yield, with a higher protein purity resulting in a lower protein yield. It is known from plant materials such as lupin, that insight in the relation between protein purity and yield in dry fractionation is obtained by tuning the degree of milling and the separation settings (Pelgrom et al., 2014, 2015b). An example of protein enriched full-fat lupin and defatted lupin is given in Fig. 2I and J. The separation settings, such as the rotor speed and air flow, were also varied in some studies on air classification of animal by-products and insects (Garcia and Piazza, 2015; Sipponen

et al., 2018). However, compared to the fine tuning and the insight gained that took place on literature for plant seeds, the knowledge gained on insects and animal by products is only minor in this respect. Since the milling intensity was not systematically varied in the articles on dry fractionation of animal by-products and insects, there might be an opportunity in optimisation of air classification of animal by-products.

Very limited research has been done on electrostatic separation in animal products and no scientific data have been published. Still, electrostatic separation brings interesting possibilities as it allows separation of particles of similar size, but different in composition. When two materials are brought into contact via triboelectric charging, a positive charge will be induced to one material and a negative charge to the other (Xing et al., 2020a). This charge can be induced by the material of the charging tube but also by particle-particle interactions. Based on the data for meat and bone meal, fish meal and plant seeds, it may be expected that the ash present in shellfish and insects will most likely gather a negative charge. Chitin and protein may both obtain a positive charge, due to the presence of amino groups in both chitin and protein. The intensity of the positive charge of chitin and the proteins can differ, and this may still allow separation, for example by using a plate-type tribo-electrostatic separator with separate collection sections (Tabtabaei et al., 2016b). A theoretical example of an industrial fractionation scheme including electrostatic separation of black soldier fly (H. illucens) larvae is given by Ravi et al. (2020). After selecting appropriate electrostatic separation equipment, separation can be further optimised by, for example, varying the particle size, air flow rate, voltage, angle of the charging plate(s), charging tube size, shape, length and material, as was also done for lupin and navy bean (Xing et al., 2020a, 2020b; Tabtabaei et al., 2016a; Wang et al., 2014). Cascading the milling and/or the separation of a certain fraction (i.e. recycling), can result in both higher protein yield and higher purity (Wang et al., 2016; Tabtabaei et al., 2017). Combinations of different dry fractionation methods can also be used to improve the product yield and purity (Assatory et al., 2019). In a few articles on dry fractionation of animal by-products and insects, combined techniques were already used (Garcia and Piazza, 2015; Muralidhara and Maggin, 1985).

6. Product-oriented process optimisation

Dry fractionation results in multiple fractions with different compositions (e.g. rich in protein or chitin) and physicochemical and functional properties. These fractions can either be used as they are, or they can be further purified using consecutive wet fractionation. The combined process of dry and wet fractionation is also referred to as hybrid fractionation. Hybrid fractionation might be desirable when a protein with a high purity is needed. Hybrid fractionation will be more sustainable than only wet fractionation, as there is less material at the start of the wet fractionation step, so less water and energy are involved (Lie-Piang et al., 2021).

There are several aspects on which the optimisation can be based, such as the composition and/or the required functionality of the final product. The essential processing steps and key optimisation strategies for dry fractionation are shown in Fig. 3. For optimisation of the fractionation parameters based on composition, the raw material source and processing method should be considered. It is expected that optimisation of dry fractionation of insects will differ from optimisation of animal byproducts. Insects are bred specifically for food consumption, so the entire process chain can be altered specifically for the purpose of dry fractionation. In case of animal by-products, the pre-processing of the raw materials are given, and the available of animal by-products and their composition determine the feedstock for dry fractionation. Therefore, optimisation possibilities for insects can be found in the entire process chain, while for animal by-products optimisation can mainly be performed by altering the milling intensity and the settings during the separation step.

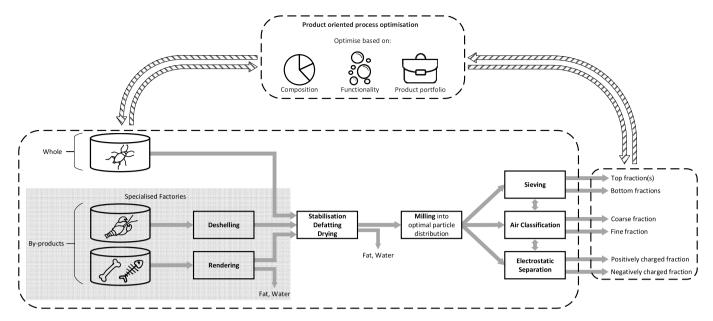


Fig. 3. Visualisation of processing the animal by-products and insects into the final fractions, including key optimisation strategies. The grey arrows indicate process streams, double headed arrows indicate possibilities for combined dry-fractionation routes. The striped arrows and boxes indicate feedback routes for product oriented process optimisation. The transparent grey area indicates that the products are already pre-processed in specialised factories. Note that rendering already includes decontamination, defatting and drying and the resulting by-products can be directly milled.

Compositional differences between tissues of the same species are important to consider. For example, tuna frames and tuna trimmings are both by-products from the tuna industry, where frames include the bones with residual meat, while the trimmings include the waste from cutting the fish. Tuna frames with 28.7 % protein and 44.1 % ash may yield larger compositional improvements than dried milled tuna trimmings with 80.7 % protein and 3.4 % ash (Abbey et al., 2017). We expect that air classification could be suitable to obtain a protein-rich fraction with reduced ash contents. This will likely cause larger improvements in the tuna frames than in the tuna trimmings. Dry fractionation of insects can result in protein rich and chitin rich fractions. Also, here the initial composition varies widely and might for example result in larger improvements in purity for higher chitin rich insect species. Recycling of fractions can be used as a method to optimise the composition and increase yields.

It is interesting that a number of studies showed that the processes could achieve different functionality, even when there was no big change in the overall protein content. This was for example shown in section 5.4: the fine and coarse fractions of air-classified insects did not differ greatly in protein content but did have different solubility and sensory properties (Sipponen et al., 2018). The exploration of these differences in functionality instead of just the protein content could well be an important route to take. This will require better insight into the interaction between different types of functionality, the composition, and the chosen processing conditions. For this, the industry that processes animal by-products and insects may follow the lead from recent innovations in the plant protein processing field. Jonkman et al. (2020) showed that it is possible to substantially reduce the use of water and energy by usage of a portfolio of food products with not only purified fractions, but by using mildly modified fractions in the product portfolio: the same product compositions can be achieved while saving significantly on the separation intensity. In case of animal by-products and insects, examples of products that might be included in a product portfolio are collagen drinks, food packaging, and insect burgers. Different dry fractionation methods may be combined or compared to obtain the fractions that combine in the best possible way into a product portfolio.

7. Conclusion

Research on dry fractionation has significantly advanced over the last decade but has so far mainly focused on plant materials. In general, little research has been performed on dry fractionation of animal byproducts and insects, and on many insect species and animal byproducts (e.g. mussel by-products), dry fractionation has not even been performed at all. The research that has been performed primarily concerned its feasibility instead of process optimisation. However, first results for fractionation of animal by-products and insects were promising. In some cases, dry fractionation could yield significant increases in protein (or other) content. In other cases, the separations yielded different functional properties such as solubility and sensory properties, without a substantial change in overall protein content. This may give rise to different dry fractionation strategies that are not solely based on yield and purity but also on functionality. Research that takes the effect of particle size for dry fractionation of animal products into account is clearly needed, as it is crucial to achieve required purities, yields and functionality. Different optimisation strategies will be required for different raw materials. For insects all pre-processing steps can be specifically aimed towards dry fractionation, but for animal by-products the origins of the materials and the established rendering and deshelling steps narrow the possibilities to tune the materials for the separation. Therefore, especially for insects development of the pre-processing steps and methods for dry fractionation is important.

While pre-processing and milling are crucially important, the settings during dry fractionation, such as the air classifier wheel speed during air classification, are also of great importance. Cascading the separation steps, for example by re-milling and combined or repeated dry fractionation steps, can further improve the material yield and purity. Furthermore, a structured and systematic approach about the effects on physicochemical and functional properties is currently also lacking. Insight in these properties and the use of a product portfolio would enable more efficient use of animal by-product and insect fractions. This can reduce the environmental impact and be essential in the protein transition to reach the sustainability goals.

Declaration of competing interest

None.

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