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Mild Fractionation for More Sustainable Food Ingredients

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Abstract

With the rising problems of food shortages, energy costs, and raw materials, the food industry must reduce its environmental impact. We present an overview of more resource-efficient processes to produce food ingredients, describing their environmental impact and the functional properties obtained. Extensive wet processing yields high purities but also has the highest environmental impact, mainly due to heating for protein precipitation and dehydration. Milder wet alternatives exclude, for example, low pH–driven separation and are based on salt precipitation or water only. Drying steps are omitted during dry fractionation using air classification or electrostatic separation. Benefits of milder methods are enhanced functional properties. Therefore, fractionation and formulation should be focused on the desired functionality instead of purity. Environmental impact is also strongly reduced by milder refining. Antinutritional factors and off-flavors remain challenges in more mildly produced ingredients. The benefits of less refining motivate the increasing trend toward mildly refined ingredients.

1. INTRODUCTION

Foods are generally created by combining ingredients into a complete food, which is greatly helped by the availability of ingredients that are neutral in taste and constant in quality. This requirement has led to the development of highly refined ingredients, such as oils, sugar, starch, and other carbohydrates like maltodextrins and protein isolates, which are relevant for the conversion into foods. Strong refinement of ingredients requires significant energy for heating and dehydration, and auxiliary chemicals such as salts, acids, and bases. It also inevitably results in the loss of some of the targeted components into waste or sidestreams. Processing therefore has a strong influence on the overall environmental impact, directly through the use of energy and chemicals, and indirectly through the requirement of more raw materials due to losses during processing (Apaiah et al. 2006, Lie-Piang et al. 2021). Subsequently, there is a need to produce more raw materials.

The long-term perspective of food shortage comes from the growth and increasing affluence of the world population (Aiking 2011). This is now aggravated by dramatically rising costs of energy and raw materials as well as unrest in some of the most productive agricultural regions of the world. More efficient production has therefore become paramount. At the same time, consumers tend to feel more negatively toward highly refined food components. Regardless of whether these worries are based on scientific evidence, it is crucial to take note of this. Finally, there is consensus among nutritional scientists that our diets do not contain sufficient dietary fiber (Anderson et al. 2009, Slavin 2008). The use of highly refined ingredients in our foods certainly does not help. The abovementioned trends all point to using milder processing methods, resulting in less pure ingredients that can be used for similar purposes as fully refined ingredients. However, mild processing may also have unwanted side effects, such as the retention of antinutritional factors (Xing et al. 2020a) and microbial or chemical hazards in the ingredients.

Aside from proteins, which are currently in focus because of the trend toward plant-based meat analogs, other important types of refined ingredients are fats and oil. Because plant oils normally contain off-flavors and other unwanted components, extensive refining of oils has been the norm for decades. This process is typically done by (cold and hot) expression of most of the oil and subsequent extraction of the residual oil using hexane. The oil is then degummed to remove phospholipids. The oil may be subjected to an alkaline step to remove free fatty acids and is then dewaxed to remove a high-melting oil fraction because consumers prefer oil that remains clear at all temperatures. The oil-refining steps lead to a loss of micronutrients (Ayerdi & Larbi 2016). Finally, bleaching by adsorption and deodorization by steam stripping yields oils that are neutral in taste but must be stabilized against oxidation.

Both protein isolation and oil refining are currently not efficient in the use of water, auxiliary chemicals, and energy, or in recovery. There are now sincere efforts underway to either create aqueous processes that employ milder conditions in terms of ionic strength and pH or avoid the use of water by using dry fractionation routes. These alternative routes all have different effects on the properties of the ingredients that are produced and compromise between the use of resources and the recovery that they can achieve. It is not yet clear how these alternative routes are related to the properties of the targeted fractions in terms of their technical functionality for incorporation into structured foods, nutritional quality, and retention of antinutritional factors and other hazards. Finally, a good perspective on the overall sustainability footprint of the different milder processing routes is also still missing.

In this review, we highlight the relation between milder processing routes for production of ingredients, resource use efficiency, and (techno-)functional properties of the obtained ingredients. First, different mild fractionation methods are discussed. We then focus on the functional properties that the various fractions have. Then, we assess the footprint of the different routes, and we conclude with a discussion of the trend toward milder fractionation processes and their potential for future applications.

2. MILD FRACTIONATION ROUTES

Traditional wet separation often entails either the dissolution of part of the unwanted materials or dissolution and subsequent isoelectric precipitation of the desired component, such as protein, under elevated temperatures (**Figure 1**). Wet extraction enables the concurrent removal of antinutritional factors, such as alkaloids (Rodríguez-Ambriz et al. 2005) and trypsin inhibitors (Swamylingappa & Srinivas 1994). However, the harsh process conditions are thought to disrupt the natural conformation and change the original properties of the proteins as present in the seed

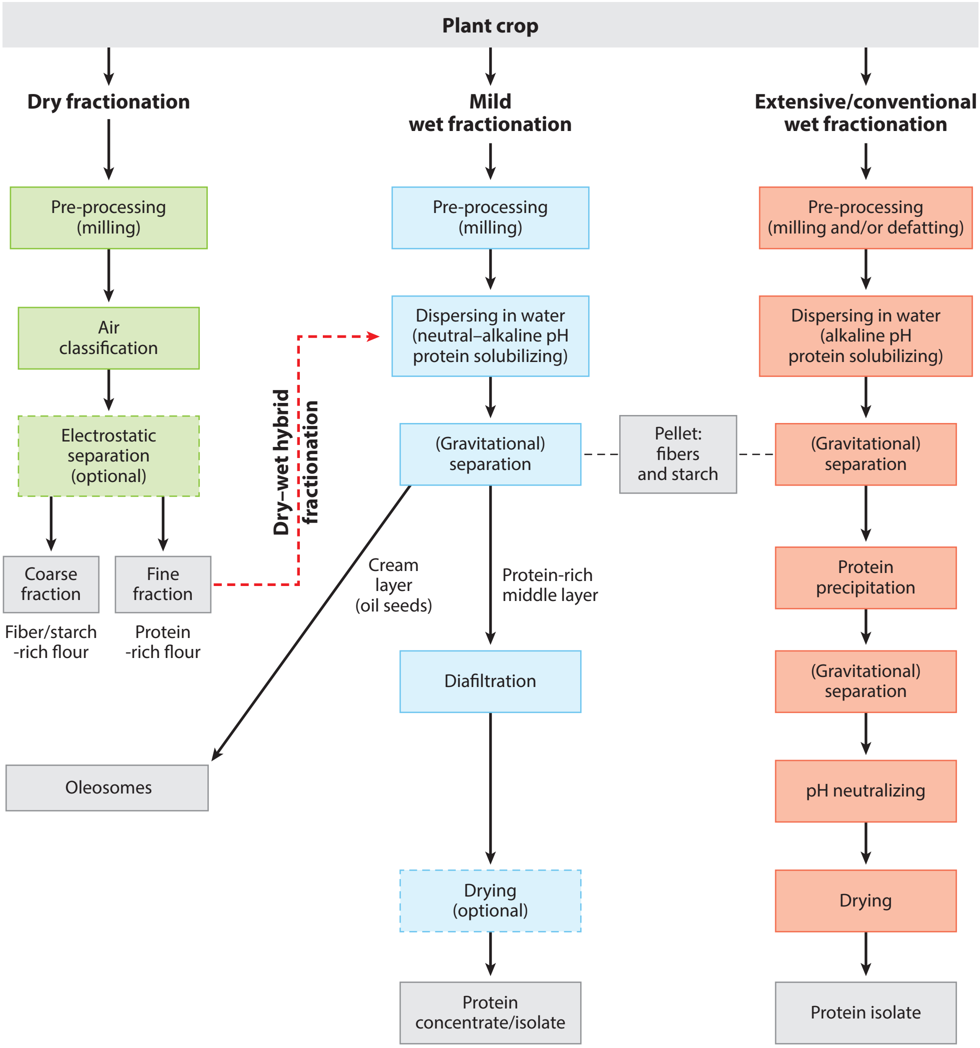


Figure 1

Schematic overview of dry fractionation, mild wet fractionation, and extensive or conventional wet extraction methods to produce plant-based ingredients such as protein- and starch-rich ingredients, oleosomes, or protein isolates.

or bean. Therefore, a logical step is to consider using milder conditions, such as neutral or less alkaline pH, or a method that does not require water at all (e.g., dry fractionation).

"Mild fractionation" for "food" is a relatively new term, which so far has been mentioned in only 14 published articles on Scopus, with the first published in 2014. Yet mild fractionation techniques have been used under different names. For example, dry fractionation has been applied for decades, for example, by Gueguen (1983). Therefore, it is no surprise that a search for "dry fractionation" for "food" yields 118 articles on Scopus, with the first published in 1984 and focusing on the separation of fats. From 2011 to the present, the focus has shifted to producing sustainable ingredients for consumers. Although these search terms give a good example of the novelty of the definition of mild or dry fractionation, they do not cover all the research going on in this field due to the inconsistent use of definitions. There are several excellent reviews on fractionation techniques themselves (see Barakat & Mayer 2017, Boye et al. 2010, Schutyser et al. 2015, Zhu et al. 2021a). Here, we focus on the principles and the type of fractions that can be obtained using different types of fractionation methods that can all be considered mild.

2.1. Sieving and Air Classification

Fractions rich in protein, starch, or fiber can be obtained by dry fractionation using a combination of milling and separation of the flour particles in two or more fractions. Milling is the most crucial step, as it leads to a physical detachment between cellular microstructures that are rich in protein, starch, and other carbohydrates (Schutyser & van der Goot 2011). Ideally, individual starch granules, proteosomes, and bran fragments are created. Insufficient milling leads to suboptimal separation because the components have not been separated, whereas too strong milling leads to poor separation as well, as small particles aggregate through Van der Waals interactions. Crops with a high level of oil (>35–40%) exhibit liquid bridging by the oil and can be dry fractionated only after defatting. A recent study investigated the effect of oil content on pin milling of soybean (Politiek et al. 2022) and concluded that the exact milling procedure thus requires crop-specific optimization.

Subsequent fractionation of milled flours can be done by sieving, elutriation, air classification, or a combination. Sieving can be done using a single sieve or stacks of sieves (Maaroufi et al. 2000). Elutriation is often operated in conjunction with sieving and employs an upward airflow that takes up smaller particles while larger particles settle downward (Srinivasan & Singh 2008). Air classification with a classifier wheel is a further development of the elutriation principle and involves entrainment of the flour particles in an airflow, which is then blown through a classifier wheel. The advantage of all forms of dry fractionation is that all fractions are dry and therefore remain microbiologically stable. All fractions can be utilized for further application.

Air classification has been developing over many years for the enrichment of flours. Starch-containing legumes, such as field peas, navy beans (Aguilera et al. 1984), lentils, faba beans, cowpeas, and others (Tyler et al. 1981), were found to be especially suitable for air classification. Yet (pseudo)cereals and oil-bearing crops like lupine were also successfully enriched with air classification (King & Dietz 1987, Vasanthan & Bhatty 1995, Wu & Stringfellow 1995, Wu et al. 1994). The reason that starch-containing legumes are suitable is explained by the cotyledon of these legumes, which comprises larger starch granules (±20 μm) that are embedded in a matrix of protein bodies (1–3 μm) and surrounded by a fiber-rich cell wall. These starch granules are liberated by impact or pin milling to enable size-based separation by air classification.

The emergence of crops as promising protein crops for human food and the high-energy-intensive production of ingredients has led to a re-emergence of the technique in the scientific literature from 2011 onward, for example, for barley (Ferrari et al. 2009); oat (Wu & Doehlert 2002); corn (Srinivasan & Singh 2008); cottonseed, wheat, and soybean (Challa et al. 2010); lupine

(Pelgrom et al. 2014); and pea (Pelgrom et al. 2013, Wu & Nichols 2005). The process typically delivers fractions that are enriched in specific components, such as protein and starch, fiber, or other carbohydrates (e.g., arabinoxylans or glucans). Because the process does not require water, the energy consumption is an order of magnitude lower than that for wet processes (Pelgrom et al. 2015b, Schutyser et al. 2015).

2.2. Electrostatic Separation

Air classification separates components that have different combinations of particle size and density but cannot separate components that have similar size and density. For these flours, electrostatic separation may have potential, and the techniques have been described in previous articles (Barakat & Mayer 2017, Wang et al. 2016, Zhu et al. 2021a). The principle of the method entails differential electrostatic charging of the powder particles followed by separation using an electrostatic field.

Electrostatic separation for food materials is relatively new. Plant materials that have been enriched by electrostatic separation are lupine (Wang et al. 2016), soybean (Xing et al. 2018), navy bean (Tabtabaei et al. 2016), rapeseed (Basset et al. 2016), oat bran (Sibakov et al. 2014), and wheat bran (Hemery et al. 2011). Ingredients produced from legumes were enriched in protein, whereas ingredients derived from oat and wheat bran were enriched in β-glucans and/or arabinoxylans. In other fields, such as the mining industry and waste recycling, triboelectric separation is an established technology. For example, large-scale electrostatic separation is used to treat coal fly ash and mineral beneficiation, including beneficiation of iron, phosphate, talc, and calcium carbonates on a larger scale (Bittner et al. 2014).

Because the principle is different from air classification alone, good results can be obtained by carrying out air classification and electrostatic separation in sequence. Such 2D separations can result in protein concentrations of up to 65%, albeit after repeated electrostatic separation steps, as was shown in fractionation of yellow pea and lentil (Xing et al. 2020b). Xing et al (2020b) enriched air-classified pea flour by electrostatic separation from 57.1% to 63.4–67.6% protein on a dry basis with an ingredient yield between 15.8% and 4.0%. More recently, researchers proposed the application of a magnetic field on top of the electric field to further enhance the separation of air-classified pea flour by electrostatic separation (Zhu et al. 2021b). This could enrich air-classified pea from 59.7% to 72.1% protein content on a dry basis with an ingredient yield of 9.2%.

There seems to be a theoretical limit to the concentration at around 75% for yellow pea because this is the concentration of proteins in the proteosomes (Plant & Moore 1983); this limit is expected to be similar for other seeds. Concerning the relatively low yield of protein concentrate, it should be realized that generally electrostatic separation devices applied in reported studies are lab-scale devices not optimized for high yield. An advantage of dry fractionation is that all fractions (i.e., also starch- or fiber-enriched fractions) are not wasted as such but can be used depending on their functionality, such as thickening or water binding.

2.3. Mild Wet Fractionation

Boye et al. (2010) provided a comprehensive overview of the main wet separations of plant proteins. Salt-induced or micellar extraction and precipitation avoids extreme pH values but employs a solution with high ionic strength to solubilize proteins. Subsequent dilution then precipitates the proteins. The remaining ionic strength supposedly protects the proteins from full denaturation (Tanger et al. 2020). Another strategy is to use just water for extraction. The yield of a water-only method is lower than with adjusted (more alkaline) pH or ionic strength, and thus the extraction is often repeated. The protein concentration varies from 54% for chickpea to 67% for pea, and therefore qualifies as a concentrate (Cai et al. 2001). This process step was also applied to yellow pea to produce starch- and protein-rich fractions (Geerts et al. 2017c, Pelgrom et al. 2015a). Ultrafiltration or diafiltration can also be used to concentrate proteins in the supernatant after mild wet fractionation. Möller et al. (2022) applied multiple washing steps and ultrafiltration to the protein- and starch-rich fractions to increase the protein yield from approximately 78% to 87%. Another method without protein precipitation was applied by Kornet et al. (2020, 2022), in which the proteins were first extracted using elevated pH, then purified and concentrated using ultrafiltration. They showed that avoiding a precipitation step by using ultrafiltration can recover the albumins from the raw materials, which normally do not precipitate because of their better water solubility and therefore are wasted. The fractions obtained by such a mild process yielded fundamentally different properties with respect to emulsion and foam stabilization (Kornet et al. 2022) and gelation (Kornet et al. 2020).

Besides protein and starch, milder processes to extract oil from crops are also in development. The isolation of oils from oil-bearing seeds is typically done by the expression and use of organic solvents, such as hexane. Hot pressing and solvent-based extraction make proteins less soluble (Mosenthin et al. 2016) and oxidize other components in the seeds and therefore do not allow optimal use of the complete raw material. Because the use of organic solvents in the food process is under public scrutiny, the use of mild wet oil extraction is explored, as it allows the simultaneous extraction of oils, proteins, and other components.

Nikiforidis & Kiosseoglou (2009) and later Romero-Guzmán et al. (2020a) explored the use of elevated pH and centrifugation to extract not just the proteins, but also oils in their native oleosome form. These are the intact vesicles that contain the oils in the plant cells and are still surrounded by a phospholipid monolayer, membrane-bound proteins, and generally some storage proteins (Abdullah et al. 2020). Owing to their hydrophilic surface, they are dispersible in an aqueous environment. For instance, intact oleosomes were extracted by blending rapeseed and sunflower seeds in dispersion (De Chirico et al. 2018, Karefyllakis et al. 2019). Ntone et al. (2020) showed that both oleosomes and proteins could be extracted simultaneously, as well with elevated pH and centrifugation steps, and recovered separately. Moreover, Romero-Guzmán et al. (2020c) found that water with some ionic strength could extract these oleosomes, although the resulting emulsion flocculated depending on the cation used.

Regarding all extensive and mild wet processes, the thermal load by drying tends to denature or otherwise negatively influence the properties of the fractions. Proteins lose at least part of their solubility when dried (van der Goot et al. 2016), even in freeze drying (Berghout et al. 2015b). van der Goot et al. (2016) therefore implied that the omission of a drying step and storage and application as a liquid concentrate could benefit the properties of the materials as well as significantly reduce the overall environmental footprint of the extraction process.

2.4. Hybrid Dry–Wet Fractionation

Even after air classification and/or electrostatic separation, the obtained fractions would qualify at most as concentrates rather than isolates. The use of a mild wet step specifically on the target fraction may help remove most of the residual fiber, starch, and other materials. This was shown for the separation of pea protein to be as simple as wet suspension and selective centrifugation of this fraction in water without pH adjustment (Pelgrom et al. 2015a, Schutyser et al. 2015). The protein was concentrated in the top layers, which can be decanted and further processed by microfiltration and diafiltration to obtain a wet native protein concentrate of high purity. The hybrid procedure may yield purities in the range of those from isolates but at the cost of a somewhat lower yield. This hybrid separation has been evaluated for yellow pea, mung bean (Yang et al. 2022c), and quinoa (Avila Ruiz et al. 2016). For quinoa, the wet phase separation was further improved by the addition of salt to enhance protein solubility and thus increase extraction yield. Interestingly, for faba bean, a hybrid fractionation process was proposed in which the coarse fraction after classification was subjected to a subsequent wet separation to increase protein recovery (Dumoulin et al. 2021).

3. FUNCTIONALITY OF MILDLY FRACTIONATED INGREDIENTS

The (techno)-functional properties of mildly processed fractions are different from those of ingredients made with more intensive processing. In the following sections, the effect of different processing steps on protein, oil(-bodies), and starches and other carbohydrates is discussed. An overview of fractions obtained by extensive and mild fractionation methods is provided in **Figure 2**.

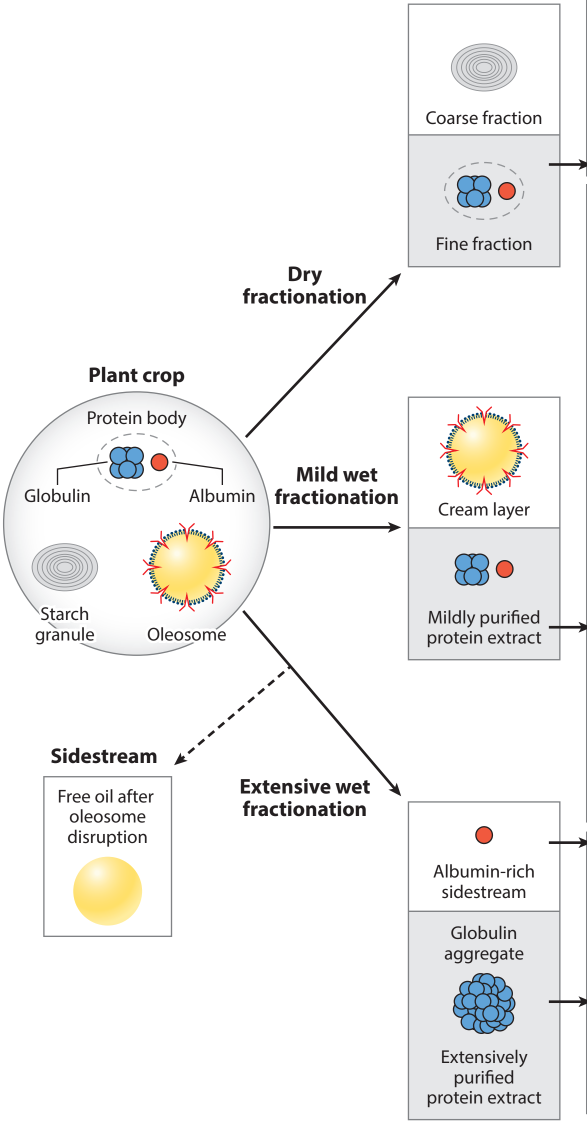
3.1. Proteins

The general focus in plant protein extraction is on the storage proteins (Sari et al. 2015), which are stored in spherical organelles called protein bodies or proteosomes (Pernollet 1978). Although wet methods disrupt these proteosomes, they can be extracted whole using dry fractionation methods (Möller et al. 2021, Pelgrom et al. 2013). Pelgrom et al. (2014) showed that native air-classified lupine concentrates have better foam stabilization and similar digestibility compared to the heated protein concentrate. Dry-fractionated lentil protein extracts could be used to form stable oil droplets in emulsion systems (Funke et al. 2022). Dry fractionation thus bears promise for obtaining protein extracts with good functional properties.

Protein purities around 90% can be achieved by wet extraction (Sari et al. 2015). However, more extensive protein extraction generally leads to lower protein recovery (Loveday 2020). In addition, the molecular properties (e.g., composition, structure, and solubility) can be affected by the extraction method. Generally, the focus in wet extraction is on water-soluble protein groups such as globulins and albumins. Globulins often exist as large quaternary structures, such as trimers and hexamers, whereas albumins are substantially smaller. In addition, globulins of many sources were reported to have a pH between 4 and 5, whereas albumins are soluble over a larger pH range (2–9) (Gonzalez-Perez et al. 2005). The conventional extensive protein extraction method is therefore mainly designed to extract plant globulins, as the alkaline pH solubilizes the globulins and albumins, but the precipitation step precipitates only the globulins. This leads to lower protein recovery, as albumins may compose up to 25% of the total protein content of a plant seed (Yang et al. 2022b).

The separation of albumins and globulins influences the functionality of the ingredients, as they possess markedly different properties in, for instance, gelling, foaming, and emulsification. In the gelation of pea proteins, globulins can form stiffer gels compared to albumins, but albumins showed better gelation when mixed with dairy proteins (Kornet et al. 2021a). Albumins were also superior for foaming, as they form very strong interfacial films (similar to dairy proteins), thus giving high foamability and foam stability (Wong et al. 2013, Yang et al. 2022b). Globulins only form weak interfacial films, leading to poor foaming. Albumins form more stable emulsions in a mixture with globulins than with single proteins (Ntone et al. 2021). Albumins are largely overlooked and should receive more attention in future studies. For example, the albumin-rich sidestream has high value in (i.e., tofu or soy whey) tofu and soy protein production (Chua & Liu 2019).

Water-only protein extraction can recover both albumins and globulins at the same time. Dry fractionation of protein bodies also yields the coextraction of albumins and globulins, which explains the better foaming properties of dry-fractionated compared to wet-extracted lupine protein extracts (Schutyser et al. 2015). Another advantage of mild extraction methods is higher protein solubility, as the precipitation step in wet fractionations generally leads to protein aggregation and thus lower protein solubility (Geerts et al. 2017b, Kornet et al. 2021b). High solubility is essential



Dry fractionation		
+ High solubility due to protein native state	Lupine	Schutyser et al. 2015
+ High foamability and stability due to presence of albumins		
+ High emulsion stability	Lentil	Funke et al. 2022
+ Lower least-gelling protein concentration compared to extensively purified extract	Bambara groundnut	Diedericks et al. 2020

Mild wet fractionation		
+ High solubility due (partly) to protein native state	Pea	Kornet et al. 2021b
+ Formation of homogeneous and stiff gels		
+ Similar emulsion formation and stabilization as extensively purified proteins	Pea	Kornet et al. 2022
+ Higher foamability and stability compared to extensively purified proteins	Bambara groundnut	Yang et al. 2022a
+ Albumin and globulin have a synergistic effect in emulsion formation and stabilization	Rapeseed	Ntone et al. 2021
+ Albumin and globulin have a synergistic effect in emulsion-filled gel systems	Rapeseed	Ntone et al. 2022
+ Starch can be present: increased emulsion thickening and increased gel stiffness	Pea, faba bean	Geerts et al. 2017a; Johansson et al. 2022
= Oleosomes can be present: could decrease foam stability; can be controlled by higher protein contents	Rapeseed	Yang et al. 2021

Extensive wet fractionation		
– Aggregation of globulin protein, leading to lower protein solubility	Pea	Johansson et al. 2022, Geerts et al. 2017b
– Weak and inhomogeneous gel formation by globulin	Pea	Kornet et al. 2021b
+ Good emulsion properties by globulin	Pea, rapeseed	Cheung et al. 2014, Hinderink et al. 2020
– Poor foaming properties by globulin + High foaming properties by albumin	Mung bean, pea, lupine	Wong et al. 2013, Yang et al. 2022b

Figure 2

Overview of fractions that can be obtained with dry fractionation, mild wet fractionation, and extensive wet fractionation methods and their functional properties, such as solubility, foaming, or gelation.

for functionalities such as foaming and heat-induced gelation. However, heat-induced aggregation might be positive for extrusion. Therefore, the functionality of native proteins can be improved by heating, enzymatic hydrolysis, or chemical modification. For an extensive overview, we refer to previously published work by Nikbakht Nasrabadi et al. (2021).

Mild purification may lead to lower protein purities. (Dia)Filtration can remove other components to yield higher protein purity. Removal of phenols ensures high protein functionality, as they interact with proteins, leading to aggregation of the proteins (Keppler et al. 2020, Shahidi & Senadheera 2019). Phenols impact taste, color, and digestibility as well. Rivera del Rio et al. (2022) investigated the impact of mild refining on the digestibility of pea proteins, measured with in vitro assays. Although alkaline process conditions reduce the digestibility, it was concluded that a heat treatment inactivates the trypsin inhibitors. A mild thermal treatment increased the digestibility of initially insoluble proteins but decreased the digestibility of initially soluble proteins. The protein isolate was chosen as the benchmark and was not always the least digestible. However, additional heating reduced the digestibility of the protein isolate even more, probably because of extensive aggregation of the denatured proteins.

In summary, the protein extraction method has a major impact on protein functionality and protein recovery. This relationship provides us with a unique tool, as the fractionation method can be used to tune the desired properties of the final protein extract.

3.2. Oils and Oleosomes

Many plant seeds are mainly grown for their high oil content. The lipids are stored in storage organelles called oleosomes, also known as oil bodies or lipid droplets. Oleosomes are natural oil droplets of triacylglycerols that are surrounded by a monolayer of phospholipids with anchored membrane proteins (Tzen & Huang 1992). In the current oil-extraction processes, the oleosomes are disrupted by pressing to extract the oil. The result is a pressed cake, from which oil can be further extracted using solvents (often hexane) to increase the oil yield. The remains are referred to as defatted meal (or cake), which is high in protein and often used for protein extraction.

Milder extraction of the oleosome extract could yield new possibilities in the formulation of oil-containing plant-based products while retaining protein nativity. The coextraction of oleosomes and proteins was shown for rapeseed (Ntone et al. 2021) and Bambara groundnut (Yang et al. 2022a). Both materials were extracted at an alkaline pH (9–9.5), leading to highly negatively charged oleosomes and proteins, thus preventing interaction between the two components. A gravitational separation step resulted in three layers: (*a*) a top oleosome-rich cream layer, (*b*) a protein-rich middle layer, and (*c*) a pellet with insoluble material.

Several options are available here, as the supernatant (cream + middle layer) can be recovered as such, leading to an extract (milk) that is high in lipids and proteins. A second option is the separation of the cream layer and middle layer. The cream layer can be directly used as a dense emulsion system or diluted to desired concentrations. Currently, oleosomes are disrupted to extract the plant oils, which are later homogenized with lecithin or plant proteins to create oil droplets in, for example, a cream. The disruption of oleosomes can be omitted as these are already naturally present oil droplets. Another promising feature is the oleosome membrane, which provides high stability against droplet coalescence, lipid oxidation, and heating (Ding et al. 2020a,b; Kapchie et al. 2013). The membrane proteins interact strongly with phospholipids by electrostatic and hydrophobic forces. Another functional property of oleosomes is the ability to take up free oil or encapsulate hydrophobic components (Fisk et al. 2013, Ishii et al. 2017). Several studies addressed potential applications, such as oleosome-based emulsions (Romero-Guzmán et al. 2020b) or pork-fat replacement with rapeseed oleosomes (Bibat et al. 2022). The protein-rich middle layer can be recovered and further purified by diafiltration to yield a protein extract with 65% protein and 15% oil (Ntone et al. 2020). A positive outcome here is a mild extraction of the proteins and the coextraction of both albumin and globulin proteins. This mild protein extract from oil seeds possesses good functional properties such as gelling, emulsifying, and foaming (Ntone et al. 2021, 2022; Yang et al. 2020).

3.3. Other Components

Conventional extensive processing is generally oriented toward the recovery of a particular component, such as protein or oil. However, the conditions that give maximum yield of oil are not optimal for extraction of proteins: hot expression and solvent extraction degrade the proteins and may induce covalent bonds with polyphenols (Karefyllakis et al. 2017). Avoidance of these conditions reduces the yield of the components of primary interest but increases the quality and yield of other components. This implies that mild processing is better suited for the total use of raw materials than conventional modes of processing.

Next to oil and protein, carbohydrates or fiber are the most important constituents. Karefyllakis et al. (2019) stabilized oil droplets by using the fiber-rich fraction, which was obtained as an additional stream from (mild) sunflower protein extraction. Also, fiber-rich sidestreams could be utilized in bakery product applications (Martins et al. 2017, Subaşı et al. 2021). Geerts et al. (2017a) used a starch-rich fraction to stabilize thickened oil-in-water emulsions due to the cooperation between starch and protein. A protein- and fiber-rich ingredient was produced from brewers' spent grain, which showed potential in a fiber-rich pasta, with improved firmness and tensile strength. The presence of fiber also decreased the glycemic index (Sahin et al. 2021).

With respect to all components, fractionation in general should not be aimed at isolating one particular component but should have the perspective of total use: fractionation of the raw material in as many high-value, functional fractions as possible. Although most attention has gone to the isolation of proteins and, to a lesser extent, oils, the refinement processes also yield other fractions, which can be valuable.

3.4. Antinutritional Factors

The absence of antinutritional factors and off-flavors is important for food applications. Common antinutritional factors are protease inhibitors, amylase inhibitors, and lectins, whereas off-flavors, such as grassy or beany flavor, may be generated by enzymatic oxidation (Asgar et al. 2010). A potential disadvantage of milder fractionation methods is that the obtained ingredients are still raw and require further processing, e.g., heat treatment or fermentation, to remove antinutritional factors (ANFs) and tailor other properties such as taste and functionality. For instance, the beany taste of air-classified ingredient fractions is caused by lipoxygenase-catalyzed oxidation, which can be prevented by a mild heat treatment (Schutyser et al. 2015).

Heating or toasting is a widely used method to remove both off-flavors and antinutritional factors, but milder methods have also been developed. Dehulling of legumes can already remove some antinutritional factors, as reviewed by Asgar et al. (2010). The type of milling and the settings of the classifier wheel speed can influence the concentration of antinutritional factors (Amin et al. 2022). An example of a post-treatment on these ingredients is solid-state fermentation. Xing et al. (2020a) applied solid-state fermentation on air-classified chickpea flour using lactic acid bacteria. They could substantially reduce the antinutritional α-galactosides (raffinose, stachyose, and verbascose) by 88–99%, depending on the galactoside. The phytate level was reduced, and the resulting sourdough was stable and showed increased protein solubility. Fermentation is a sustainable and natural approach to removing undesired components and can even generate compounds that positively influence the taste and/or nutritional value of the protein concentrate.

4. SUSTAINABILITY ASSESSMENT

4.1. Chain Assessment

Both exergy and life cycle assessments (LCAs) are used to quantify sustainability of mildly refined ingredients. Exergy analysis bases resource efficiency on the first and second laws of

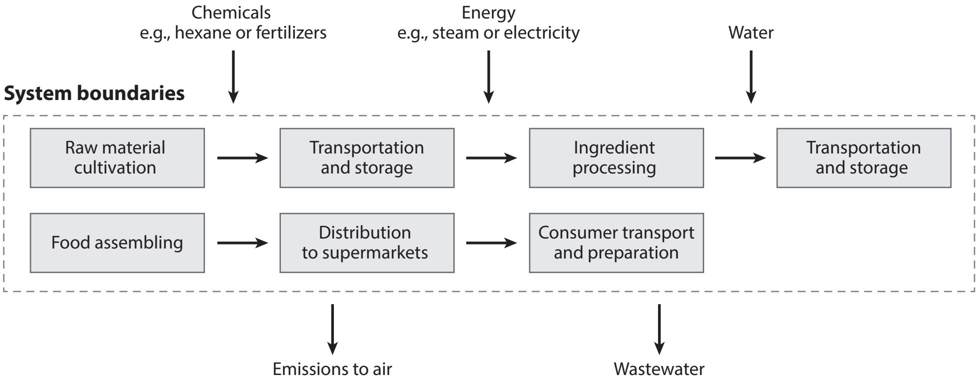


Figure 3

The production chain from food ingredients to consumer.

thermodynamics (Zisopoulos et al. 2017), whereas an LCA focuses on the multidimensional impact of a process, ranging from the global warming potential to water usage (Guinée 2002). Both consider the input of raw materials, energy, chemicals, and water. To quantify sustainability, the impact of each step in the production chain should be assessed (**Figure 3**). In general, plant-based ingredients have a similar supply chain, including cultivation of the crop, processing to final ingredient, and transportation. Next are food assembly and consumer usage, which are not discussed, as the scope of this review is on sustainable ingredient production.

The impact of processing of the final product depends on the target component to be isolated. For example, the conventional wet extraction of starch and fiber from yellow pea (Geerts et al. 2018) or potato (Grommers & van der Krogt 2009) is relatively efficient, as it solely requires the suspension of the milled or ground crop with subsequent decanting, sieving, centrifugation, and hydrocyclones to separate the components. Drying of these fractions contributes significantly to the final resource use. The impact of drying is reflected in their share of the cumulative exergy loss, i.e., destroyed useful energy, of 15% for the (pneumatic) drying of starches and 26% for the evaporator to concentrate fibers in the extensive wet fractionation method. This percentage is even larger in mild wet methods (Geerts et al. 2017a, Pelgrom et al. 2015a), which emphasizes the importance of the impact of drying. Dry fractionation using air classification omits this, as drying is not required (Schutyser et al. 2015). The impact of the omission of drying is projected in **Figure 4**, which shows that the extensive and mild wet production of a fiber- and starch-rich fraction have a higher impact compared to dry fractionation (Lie-Piang et al. 2021).

On the other hand, wet extensive and mild isolations of protein have a higher global warming potential compared to the other components, due to protein extraction and drying (**Figure 4**). The latter contributes significantly to the overall impact and is responsible for 40% of the total cumulative exergy loss (Geerts et al. 2018). Because mainly the insoluble protein at the isoelectric point is extracted, there are many losses of soluble proteins. While isolates that are produced by isoelectric precipitation typically have a protein content of at least 79%, mild fractionation methods produce concentrates that are substantially lower in protein content. Mild wet fractionation yields approximately 54% protein, whereas dry fractionation yields 43% protein (Lie-Piang et al. 2021). Nonetheless, the same study showed that the impact expressed per kilogram of protein instead of total ingredients is still lower for the more mildly refined ingredients than the extensively refined ingredients. More specifically, dry and mild aqueous fractionation of yellow pea yield a protein concentrate of 1.6 and 4.9 (combined for soluble and insoluble fraction) kg CO_2-eq/kg protein,

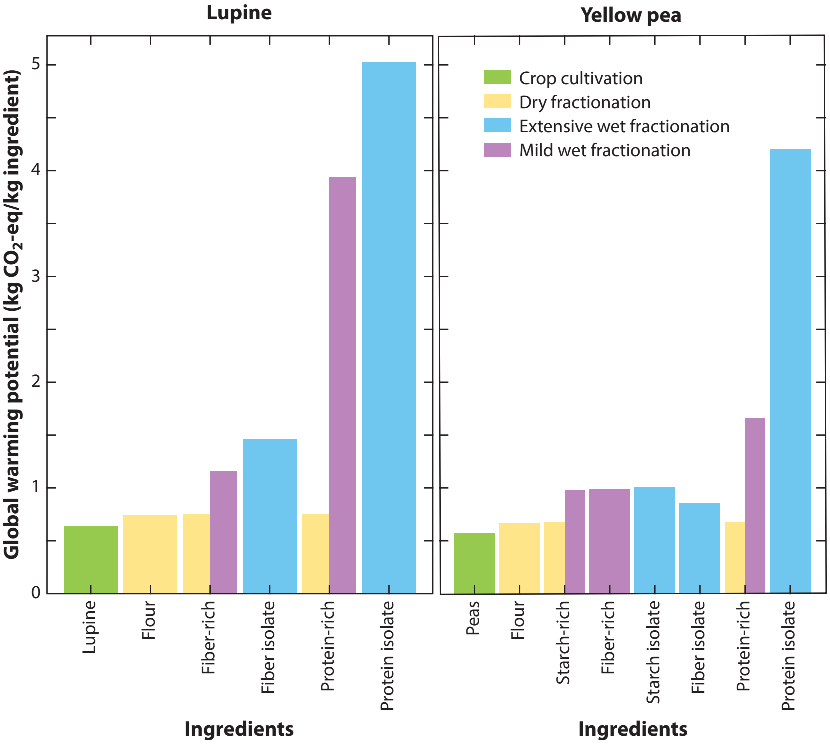


Figure 4

Global warming potential (system boundaries from cultivation to processing gate) of yellow pea and lupine isolates produced with extensive wet fractionation and enriched ingredients produced with mild wet and dry fractionation. Data adapted with permission from Lie-Piang et al. (2021).

respectively, and the extensive wet process yields a protein isolate of 5.3 kg CO_2-eq/kg protein. Vogelsang-O'Dwyer et al. (2020) also calculated in an LCA that a faba bean protein isolate had a threefold higher global warming potential per kilogram protein compared to an air-classified protein concentrate.

When hybrid fractionation, a combination of dry and mild wet fractionation, is used to produce a fraction with just the soluble protein originating from the fine fraction, it has a protein delivery efficiency (ratio of protein to invested life cycle energy) of 29.1 compared to 14.6 and 55.8 g protein per MJ for conventional extensive wet and dry processing, respectively (Schutyser et al. 2015). The same difference was found for the soluble protein fraction from hybrid fractionation in an LCA (Lie-Piang et al. 2021). Yet the sidestream after centrifugation containing all insoluble components such as protein, fiber, and starch granules can also be considered as an ingredient. The same LCA study found that when considering the insoluble as well as the soluble fraction, the global warming potential per unit of protein comes closer again to the impact of a conventionally produced protein isolate. Nevertheless, hybrid fractionation is a relatively new concept and can still benefit from the optimization of water usage, as is done for mild wet fractionation separately (Möller et al. 2022).

Lastly, the use of hexane and heat to distill the solvents during extraction of oil from crops leads to large chemical and physical exergy losses, which were shown in lupine (Berghout et al. 2015a). One could omit the oil extraction step, as it was found to have minimal impact on the functional property of lupine fractions (Berghout et al. 2014). The mild wet oleosome/protein extraction method discussed previously is another alternative. An exergetic comparison was performed on a final product containing oil, i.e., emulsions (Romero-Guzmán 2020). As the final application of these oleosomes is an emulsion, the exergy loss was compared between an oleosome- and conventional oil–based mayonnaise. The study showed that although the oil recovery efficiency is high in conventional extraction, there is a high chemical exergy loss due to the cake, which is only suitable for livestock feed. Mild extraction, however, requires a lot of water to process, but the efficiency, defined as the percentage of input exergy that remains useful after processing, of the mayonnaise based on oleosomes was 90% compared to 47% for conventional mayonnaise. Hetherington (2014) calculated that an oleosome-based mayonnaise has the potential to be more environmentally friendly compared to conventional mayonnaise. Notably, both of these analyses are based on lab-scale experimentation; hence, yield or energy uses may be overestimated (Hetherington 2014).

Another essential element in the production chain of sustainable ingredients is the cultivation of crops and transportation to processing facilities. Comparing the impact of crops can be challenging because of the spread of production worldwide; hence, different distances exist between crop cultivation locations and the ingredient processing facilities. Nevertheless, the impact of cultivation can be compared to the impact of processing. For example, Heusala et al. (2020) showed in an LCA that processing an oat protein concentrate contributes to a larger share of the total impact (75%) than cultivation (19%), attributable to the high energy costs. In contrast, for two faba bean concentrates, cultivation was responsible for a larger part of the total carbon footprint. Lie-Piang et al. (2021) showed that cultivation is mostly responsible for the global warming potential of the production of dry-fractionated ingredients from yellow pea. However, for mild wet fractionation, the impact of processing for yellow pea and lupine is similar to or larger than that of cultivation. Both of these studies do not take the impact of food assembly into account, which can only mean that the impact of ingredient processing on the final consumer would be larger. This comparison shows that it is necessary to consider both cultivation and processing.

Earlier, we mentioned that drying is the largest contributor to all wet fractionation processes. However, it also reduces the environmental impact and costs of logistics and improves the shelf life. If the distance between the production and processing facilities is considered constant, the state of the product (wet versus dry) greatly influences environmental impact. Therefore, it is not desirable to transport wet products over long distances. However, for shorter distances under chilled conditions, transporting wet products might require fewer resources than drying the product first and transporting it in a dry state, depending on the final concentration of the product. For example, transportation of concentrated milk with 35% dry matter up to approximately 1,000 km still required less energy compared to the spray-dried variant (Depping et al. 2017). However, there is a limit to the concentration of an ingredient for it to remain processable, which is probably relatively low (10–20 w/w%) (Kornet et al. 2020) and thus requires a lot of energy for transportation and reduces its advantage. Another option to remove the drying step from fractionation methods is to produce ingredients in-house, just before their use in consumer foods. This latter option is made more realistic with the mild processing methods now developed, as they are relatively more suitable for lower-scale production.

So far, the exergy and LCA studies presented in this review use either total ingredient or total protein to express the exergy efficiencies or impact categories. Even though both of these units are of importance, others might also be of interest. For example, the functionality that an ingredient will add to a product can also be expressed in environmental impact. This was presented in the functionality-based exergy assessment of Geerts et al. (2018), who indicated a lower cumulative exergy consumption for the mildly fractionated ingredients per unit of functional property, in this case, viscosity compared to the conventional starch isolate.

4.2. Functionality-Based Fractionation and Formulation

The final functional properties of ingredients depend on both the composition and type of processing. The latter relates to the structure that ingredients retain from their raw materials and also the degree of damage [e.g., thermal load or (de)hydration]. One can adapt the processing routes to achieve the required functional properties instead of obtaining an ingredient of high purity. A previous review by van der Goot et al. (2016) introduced this topic and concluded that it is not always necessary to fractionate the crops into pure components and functional ingredients produced with mild fractionation have been used for decades, for example, with the use of flour in bread. In other words, the type and degree of intensity of a fractionation method can be adapted to what is required for the final application.

Möller et al. (2022) showed that with additional washing steps in a mild aqueous fractionation, the purity of the protein fractions can be increased according to the final functionality in terms of protein content required. The isolation and extraction processes of yellow pea protein can be adapted to obtain fractions with excellent functional properties (Kornet et al. 2020, 2021b) while reducing the degree of processing. For oil-bearing crops, the extraction of oleosomes is an example of shifting the perspectives from extracting pure oil to attaining the oil bodies already as an emulsion (Romero-Guzmán et al. 2020b). Moreover, a more mildly derived protein mixture from sunflower seeds was also proven to efficiently stabilize oil/water emulsions and had similar functional properties to harsher refined sunflower protein isolate (Karefyllakis et al. 2019). Another study found that a milder purification process of rapeseed proteins created more functional fractions, resulting in a protein extract with both napins and cruciferins, which are complementary in emulsion-filled gels (Ntone et al. 2022). The pH of mildly fractionated soy protein fractions can also be steered to obtain desired functional properties, which can result in, for example, a different water-holding capacity, protein solubility, or viscosity (Peng et al. 2020). All these studies show that the fractionation methods can be adapted to achieve functional fractions and that the degree of fractionation should focus on the target functional properties required for the final food application.

Besides adapting fractionation processes of ingredients to attain specific functional properties, the focus in food formulation can also be on functional properties rather than purity. Jonkman et al. (2020) proposed to create food formulations with a process systems engineering approach by blending different ingredients based on the target composition to increase the applicability of more complex and multicomponent ingredients. It was proposed that with a focus on functional properties, these blends could be made even more resource use efficient. Manzocco & Nicoli (2002) formulated low-calorie syrups based on the functional contribution of each ingredient using mathematical equations. In this way, food formulations could be optimized for caloric content while controlling functionality such as viscosity or color in the final application. The mathematical equations were fitted to the functional properties of the individual ingredients, which was also the limitation of this study, as interactions between ingredients in formulations were not taken into account.

Lie-Piang et al. (2022) extended this by creating ingredient blends, or formulations, based on functional properties. For example, the final viscosity of extensively and mildly refined ingredients was quantified using multiple linear regression. The advantage of using these types of mathematical models is that relations are quantified based on the data from ingredient blends, which means that possible interactions are covered. This allows the application of a broad range of ingredients with different functional properties and purities. For example, the regression model was used to generate the formulation window of components that achieved a similar viscosity of 1,500 mPa.s at a constant shear rate of 160 rpm (**Figure 5*a***). The formulation window can be constrained

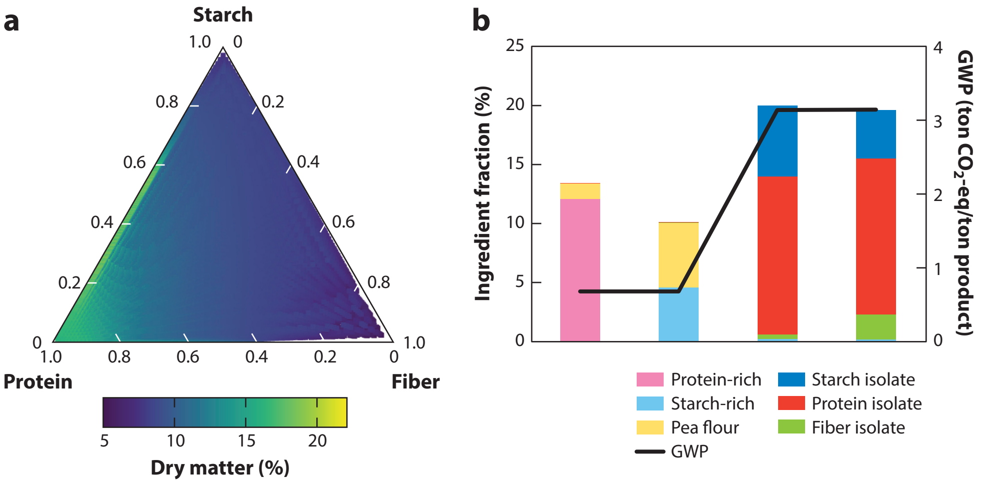


Figure 5

(*a*) Formulation window of compositions that achieve 1,500 mPa.s viscosity at a shear rate of 160 rpm. (*b*) Possible ingredient formulations to achieve the same viscosity, including the respective global warming potential (GWP). Data adapted with permission from Lie-Piang et al. (2022).

to the composition of available ingredients with a different processing history and, hence, global warming potential (**Figure 5*b***). The same viscosity could be obtained with significantly less environmental impact when ingredients were used using mild fractionation compared with more extensively produced isolates. To apply this method to other (nonlinear) properties and consider process conditions as well, more complex machine learning algorithms can be used, such as neural networks (Batista et al. 2021).

The combination of being able to adapt fractionation processes and ingredient formulations to achieve specific functionality allows us to minimize the environmental impact of the food products. At the same time, the functionality that is needed to create (healthy) foods is retained.

5. CONCLUSIONS AND OUTLOOK

Although dry and other mild refining fractionation processes have been explored for many decades, they were originally considered to be cost-effective ways to enrich feed for livestock. The emergence in recent times of pulses, grains, and other plant materials as promising materials for plant-based foods for humans has led to a surge in the development of milder fractionation processes. The need for more sustainable ingredients is amplified because current methods for isolation are quite intensive in energy, water, and auxiliary chemicals and have relatively low yields.

There are two classes of mild processes, each with its advantages: dry processes and wet processes. The most important dry processes are air classification and electrostatic separation. The first separates based on particle size and density; the second—when based on tribo-electric charging—separates based on the surface properties of flour particles. They can be cascaded to enhance yield and recovery and obtain better separation between starch, bran, and protein. Dry separation at this moment is not suitable for crops that bear large amounts of oil (>35–40%) and thus first have to be defatted. The second class is mild wet separation, in which conditions are less extreme regarding the use of solvents, temperature, pH, and ionic strengths compared to the existing processes. The yields are generally somewhat lower compared to conventional alkaline extraction routes. Avoidance of a precipitation step leaves proteins in a more native state and improves their properties. In addition, mild wet separation can also help recover the albumins, which often do not precipitate at the isoelectric point and are conventionally lost. These albumins have been found to have quite good technical functionality. The two classes can also be combined in hybrid processes, in which the first dry separation leads to an enriched fine fraction. Only this enriched fraction is then further purified, for example, by using a simple dissolution and sedimentation step in plain water. This leads to yields and purities of a protein fraction that are comparable to conventional wet isolation but with only a fraction of the energy and water used.

For oil-bearing crops, the mild wet routes seem promising, as they allow the recovery of oils as oleosomes, proteins, and other components. However, they still require significant amounts of water and either need dehydration, which is energy-intensive and compromises the quality of many fractions, or result in aqueous concentrates that probably need to be chilled for storage and transportation.

As we are transitioning into an era in which raw materials, energy, and water will all be scarce, it is crucial to make the best possible use of the complete raw materials; we should therefore aim at using all different fractions. At the same time, environmental impacts of processes should be assessed to ensure that the most sustainable ingredients are used. This is especially important because the impact of processing in some cases exceeds the impact of crop cultivation. A focus on functional properties when designing fractionation processes or formulating ingredients will facilitate the use of more mildly refined ingredients.

Milder fractionation methods are very attractive for the intermediate future because they (*a*) can be cost-effective, as they are simpler than current methods; (*b*) deliver good quality ingredients; (*c*) are clearly more resource efficient and sustainable; and (*d*) fit the wishes of consumers for less processed foods. These milder processes are however still very much in development. The understanding and adaptation of technical and nutritional quality are only in their initial phases. In addition, the removal of antinutritional factors and other hazards is still an issue, even though several methods have already been developed for specific antinutritional factors.

DISCLOSURE STATEMENT

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Contents

Errata

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