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Review

Developments in the Dry Fractionation of Plant Components: A Review

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Abstract: Over the years, pulses and cereals have been identified as promising sources of plant proteins. The intensive production of these crops and concerns about food security and malnutrition worldwide have intensified research into their separation. While wet extraction remains the standard protein isolation method, the search for more sustainable extraction methods is still ongoing. Two dry fractionation techniques, air classification and tribo-electrostatic separation, have been discussed in this review. This review highlights the design aspects of air classifiers including the cut-off point and flow rate, and for electrostatic separators, factors such as charger materials, the nature of the flow in charger tubes, and the strength of the electric field potential have been discussed in detail. Our analysis revealed that cascading the two techniques should help enhance the concentration and purity of the separated fractions. While limitations such as low purity and low yield exist, current research studies are focused on overcoming such drawbacks. Dry fractionation exhibits potential as a sustainable processing method while also preserving the native functionality of the proteins, making it easier to incorporate the fractions in commercial scale processes.

Keywords: dry fractionation; air classification; tribo-electrostatic separation; plant proteins

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1. Introduction

Protein sources have been a point of high interest in the food industry for a long time. Beyond being a macronutrient required for the normal metabolism of the human body, proteins possess a variety of functional properties. Emulsification, water solubility, gelling, and film-forming capacities are among the highly valued functionalities in proteins [1]. Throughout history, various sources of proteins have been explored to achieve the desired functionality. Ingredients with good functional properties are sought after in the development of food products. Animal proteins, such as milk and egg proteins, have been the primary source of proteins in the past and due to their excellent functional properties, have been used as functional ingredients as well [2].

However, alternatives for animal proteins are being explored to substitute a portion of the global protein utilization [3–5]. Plant-based protein sources are excellent alternatives to animal proteins due to several reasons. Some of the notable ones include increase in productivity, lower pollution levels, higher quality of proteins, and value addition. In addition, rapid urbanization and increased nutritional awareness among the consumers are also causes for the rise in protein demand [2,6]. Among the plant sources, pulses, cereals, and pseudocereals have shown excellent potential in terms of protein content, quality and bioavailability [7,8], while oilseeds on account of their nutritional, functional and bioactive properties could be a potential alternative protein source [9]. With the rising population and the demand for nutritious foods, food security has become a prominent issue.

Malnutrition remains a major issue, with a considerable proportion of our population suffering from protein malnutrition. In the post-covid pandemic era, the progress towards

the omission of malnutrition and food security remains stagnant. Three-quarters of all countries are behind the targets [10]. This raises serious concerns of food security as some regions have prevalent problems of undernutrition and some parts have concerns of overweight and obesity.

Growers are looking for ways to increase their profits while researchers are interested in environmentally sustainable crops. Pulses are effective in satisfying these two conditions and are an excellent source of nutrients in food products [11]. Pulses have started to become an important part of diets around the world since their per capita consumption started to decline in the 1960s. The world trade of pulses is expected to reach 23 Mt by 2032. India is the largest producer of pulses while Canada is the largest exporter (35% of the global trade) [12].

However, while there has been a slight fluctuation in lentil production in Canada, there has been a remarkable increase in pea production at over 10,000 tonnes in 2023 alone [13]. Pulses, owing to their ability to fix nitrogen, do not require nitrogen fertilizers. This has led to a low carbon footprint status, stimulating research interests in them [11]. With the high productivity in Canadian prairies, the export potential of these crops could prove valuable to the economy of the country. Not only the export of raw materials, but also the value addition of these sources could prove to be a boost to the economy because of the recent surge in demand for protein products [6,14]. The last decade saw a massive 275% increase in the utilization of pulses as ingredients in food products and it is still growing significantly [15]. With an increasing price signal, the seeded acreage of the pulse crops is also expected to grow [16]. A trend of the pulse acreage over the years is depicted in Figure 1. The increase in seeded acreage shows that the demand for pulses and the grain output are increasing rapidly.

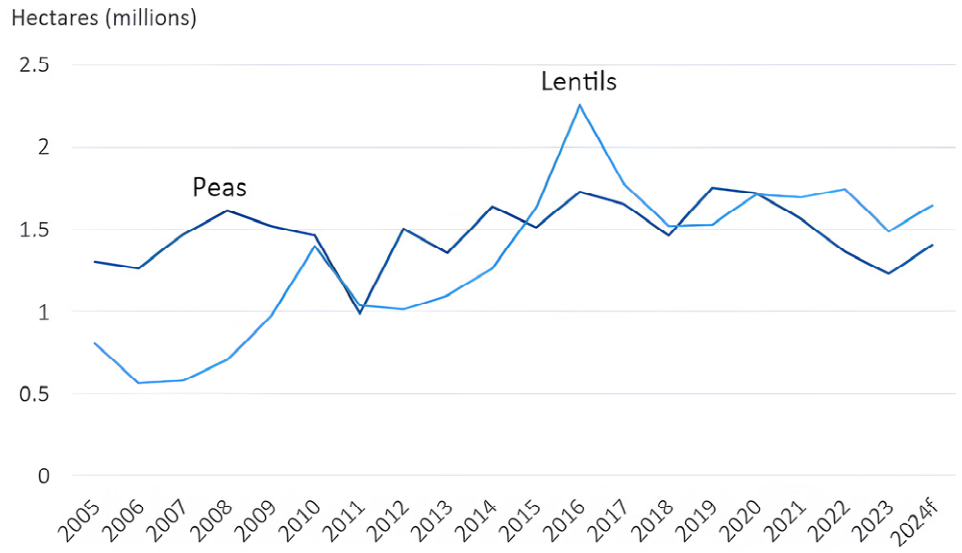

Figure 1. Seeded acreage of peas and lentils over the years [16].

In order to fully understand and have a holistic view of the plant protein production process, feasibility studies and a techno-economic analysis of protein production from plant sources need to be evaluated along with their techno-functional performance. Structural properties, physio-chemical properties, amino acid profiles of the materials and bioavailability of the components need to be evaluated before further processing. This enables the proteins to be suitable for commercial applications. Thus, it is within the scope of this review to touch on the functional properties of plant proteins. The composition and performance of plant sources in relation to these properties limit their use in a wide range of applications. Owing to the relatively low protein content of the ingredients in plant-based meat analogues, it has been quite challenging to create a complete alternative, which has led to further research into food ingredients with high plant protein in recent years [17,18].

Plant proteins usually vary based on their solubility as albumins, globulins, prolamines and gluteins, as per the Osborne system of classification [19]. The structural difference between the proteins is a major factor in their differing solubilities. The modification of the proteins' structure during the extraction process leads to a subsequent change in their techno-functional properties. There have been studies that investigated the extent to which the techno-functional properties were affected [20,21]. These studies also concluded that some protein applications are more effective in their native form [2,22].

Although modern farming innovations have ensured the extensive availability of raw materials, their utilization is limited due to technical and commercial limitations. For example, the extraction process differs based on the type and structure of the material in question and can be tedious for separation. Developing sustainable protein extraction procedures for the locally abundant raw materials would reduce the logistics cost while improving consumer acceptability [2]. Optimization of the extraction process, such as the time duration of extraction, specific machine parameters, temperature, pH, and other factors that are both intrinsic as well as extrinsic to the plant materials, is the primary influencing factor on the quality of the extracted proteins [14,23–25]. The cost for the effective separation of particles is also expensive and may act as a barrier for commercial upscaling of the process [26].

Current data available on protein extraction from plant sources indicate that the most widely used sources for commercial applications are soybeans and peas. Other pulses and legumes like yellow pea, chickpea, faba beans, lentils, lupin, some cereals, and millets also have the potential for fractionation [27]. The structure of the materials and the strength of bonding between the proteins and other components play a major role in the selection of the source material. Seeds that have a higher oil content usually undergo some kind of prior processing to prevent rancidity during fractionation [28] and to prevent low dispersibility and low separation efficiency [29]. This defatting step also helps in preventing agglomeration due to the oil content [30]. Whatever the proteins are, the initial step is to mill or grind them to obtain a powder to allow for the easier separation and extraction of proteins [27,31].

Generally, protein extraction is accomplished through the wet fractionation technique which involves the use of chemicals in the extraction process. The product obtained is usually either a concentrate, isolate or hydrolysates of the protein [22]. This process is capable of resulting in a higher yield of proteins with a purity of ~95% [26]. Wet extraction involves solubilizing the protein in an appropriate solvent, followed by separation through pH adjustments and/or differences in solubilities [6,21,32]. The use of alkaline or acidic solvents may have detrimental effects on the quality of proteins obtained. They lead to a lot of chemical side streams that pollute the environment and are difficult to discard [26,32]. These limitations mask the high yield and quality obtained through wet extraction. Commercial isolates and concentrates of soy proteins usually contain ~90% and >60% proteins, respectively [18,33].

Dry fractionation, on the other hand, uses the structure and arrangement of the components inside the cell to separate the proteins. It may use the difference in size and density in the case of air fractionation [20,23,26] or the charging behavior of the individual particles inside the cell [34,35]. In either case, the protein must be sufficiently separated from starch and fibers prior to separation. Dry fractionation techniques have been shown to reduce the energy consumption required for the isolation [8,32,36]. The absence of any chemicals during fractionation also ensures that the protein's functional properties and integrity remain unchanged [30]. Due to the ease in scalability, air classification has been used in ingredient manufacture lately [37]. However, these advantages are countered by the lower yield and purity of the proteins obtained compared with those derived via wet fractionation. Inefficiency in the separation may lead to an increase in the cost required for purification and the high demand for raw materials [37]. Consequently, research on dry fractionation focuses on increasing purity and yield while research on wet fractionation focuses on sustainability [31]. Dry fractionation has been applied to various

pulses including peas [38], lupine [34], mung bean [39,40] and cereals such as wheat [25], oats [22], and quinoa [41]. Schlangen et al. [39] performed air classification on mung bean, yellow pea and cow pea and reported that the protein content increases with the air classifier wheel speed. This enrichment was dependent on the size of the milled flour and the source utilized for fractionation. Wang et al. [34] applied electrostatic separation on lupine flour and found that the airflow rate had a proportional relationship with the protein content. This relationship between protein content and the airflow rate arose because of the direct influence that airflow rate had on the imparted charges on the components.

The use of tribo-electrostatic separation (TES) techniques to isolate the proteins could prove as a valuable alternative to the traditional processes. Tribo-charging occurs when surface charges are acquired by the particles upon contact with each other, other particles, or the walls of the containing vessel or transporting material [8,42,43]. The resultant tribo-charged particles are then separated in a high-strength electric field. Particle–particle and particle–wall collisions during feeding and charging is considered the primary mode by which the particles acquire surface charges. Several factors, including room temperature and humidity, could influence the extent of charging and separation [44].

Proteins extracted through the TES process may exhibit better functionalities than the wet-extracted proteins, although the extent to which the functionalities and performance of the protein concentrates are better than the wet-extracted counterparts is still unclear. A knowledge gap on the factors that influence the extraction and the behavior of particles during fluidization and transit makes further inquiry into the topic a necessity. The influence of the material used for charging, the length of the material and the subsequent time of contact during the fractionation are also significant. However, contradicting results found in the existing literature make it challenging to understand the influence of individual parameters. These parameters include environmental conditions such as the relative humidity, temperature, and configuration of the charging tubes (spiral vs. straight), and the length of the charger tubes [35,44,45].

This review is focused on providing a good understanding of the principles, limitations, and previous findings related to dry fractionation techniques. Two dry fractionation techniques, namely the air classification and tribo-electrostatic separation, are focused on primarily. Combining these two techniques may have extraordinary potential in the field of protein extraction and has been looked at in this review. As a clear understanding of the particle behavior during charging using different materials is not yet present, compilation of the proposed ideas from different researchers has been attempted. An important aspect of this literature review is to present the current state of the technology, possibility of commercial scale-up, influence of the process on the final protein and to find areas for further research.

2. Sources of Plant Proteins

Plant proteins can be separated from a range of sources including pulses, cereals and oilseeds [7]. Plant proteins can be classified based on their structure and their solubility. The proportions of these different protein types vary among crops, thereby affecting the functionality of the extracted proteins. Legume proteins are typically divided into legumin and vicilin, while cereal proteins are largely globulins [46]. Legumes have been consumed by humans for over 10,000 years as part of the balanced diet requirement of the human body [47]. Direct consumption of pulses is known to fulfil the daily requirement of proteins and essential amino acids, complementing cereal intake. Pulse flours have been used as enrichment ingredients in the development of food products [31]. Other than flour, the protein isolates from peas and soybeans have gained attention as substitutes for animal proteins, owing to the rise in veganism and the search for alternatives to functional ingredients.

Other pulses and legumes are also being explored for protein extraction, such as beans, lentils, and lupine, along with cereals like oats and wheat. The intensity in research towards the extraction of protein from these products is due to the variability in their protein

quality, quantity and the structure of the seed [25,30]. The structure of pulse cotyledons are arranged such that the starch granules are embedded in a protein matrix, which, in turn, is surrounded by the fiber-rich cell wall [29,45]. The presence of starch and fiber influences the extraction of proteins from them. For instance, a chickpea has smaller starch granules than other pulses, thereby interfering with the separation of finer proteins [30]. Similarly, oats and other cereals include the germ, endosperm and bran with similar size distributions. The fluidization is affected due to the presence of bran particles in the mixture [48]. All these factors affect the separation efficiency of the process.

Wet fractionation overcomes this drawback by dissolving the protein in specific solvents, making it easier to precipitate the proteins. In dry fractionation, the interference of both starch and fiber can only be overcome by proper milling and operational conditions during separation. Although the protein content of the final product is affected by the initial protein content of the grains, it should be noted that the initial protein content is mainly affected by the cultivating conditions of the crop [49].

In meat analogues and plant-based protein isolates, soybean has been the most utilized source of plant protein [27]. This is due to certain properties such as the excellent gelatinization, emulsification and the associated binding capacity with fat and water that soybean possesses. It also has about 36.49% of proteins in the seed form with relatively higher extractability [36]. For the above reasons, soy protein isolates have been used as the reference to compare the functional properties of proteins from other plant sources.

Following soy protein, pea protein has been the most utilized for protein separation owing to its protein content. Pea protein also has sufficient binding and emulsifying properties. The protein and starch content in peas range from 21.2 to 25.9% and 36% to 40%, respectively [30,50]. A list of the sources and the amount of protein, starch and fiber content is compiled in Table 1. Pea protein has found acceptance among consumers because of its low allergenicity and low estrogen activity which, on the contrary, has been a major issue with soy proteins [40,51,52].

Table 1. Composition of various plant-based protein sources [53].

Source	Protein (% WB)	Starch (%)	Fiber (%)	Fat (%)	Ash (%)
Soybean	36.5	20.9	9.3	19.9	4.8
Wheat	13.7	58.9	13.2	2.4	1.7
Navy Bean	22.3	45.5	15.3	1.5	3.3
Oat	13.5	53.8	10.5	5.8	1.7
Yellow Pea Flour *	22.3	59.1	14.8	1.4	3.5
Faba bean	26.1	33.3	25.0	1.5	3.0
Lentil Seeds	24.6	52.7	10.7	1.0	2.7
Mung bean	23.9	46.3	16.3	1.1	3.3

* Obtained from [54].

Researchers have started to explore the possibilities of protein separation from cereals, millets and pseudocereals. Oats and quinoa show excellent promise because of their high protein content [48]. Teterycz et al. [55] carried out experiments for the enrichment of pasta with wheat germ protein isolates and was able to enrich the protein content by 20% with the addition of 8% wheat germ protein isolate. The combination of cereals and pulses in the formulation of high protein products is also common. Guo et al. [56] utilized corn flour, buckwheat flour and chickpea protein isolates in the manufacture of a 3D-printed dysphagia diet. Several such formulations are being utilized by commercial manufacturers in the extrusion process to produce meat analogues. This popularity could be due to the complementing nature of the functional components like protein, starch and fiber. Although the potential of cereals has been identified, there is a lack of research in the dry fractionation of cereals because of their invariable protein and carbohydrate sizes, as well

as low dispersibility [40]. The development of a sustainable and efficient methodology to produce protein isolates from these materials is the first hurdle in the process. Various plant sources that have been explored to produce protein isolates are presented in Figure 2.

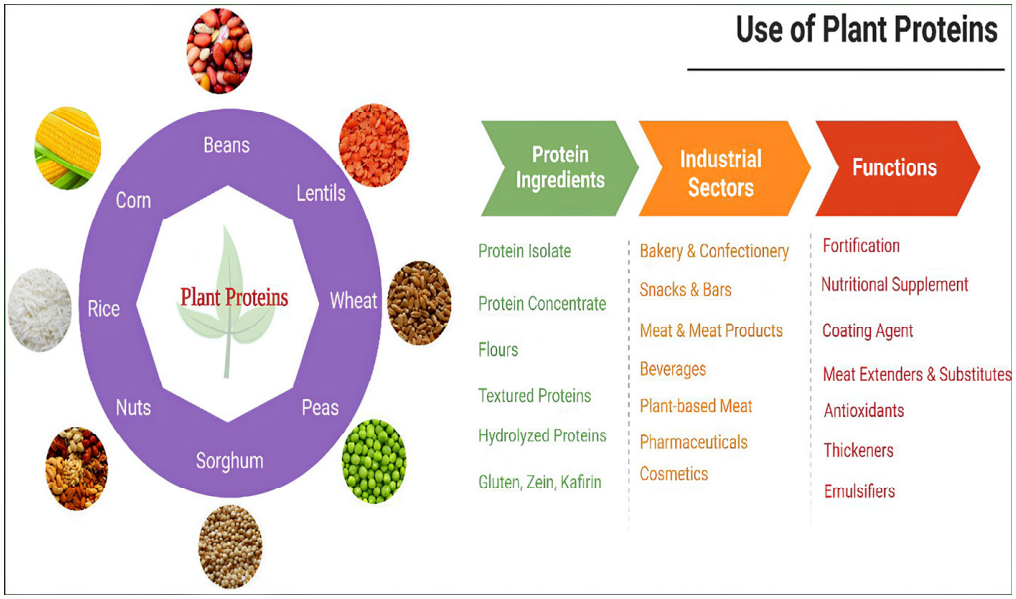

Figure 2. Some of the common sources of plant proteins and their uses [26].

3. Fractionation Methods

The isolation of proteins from plant sources involves either dry or wet fractionation. Extensive studies have been conducted on the wet fractionation of proteins from plant materials and have been applied commercially [18,22,40]. Dry fractionation, on the other hand, has only recently been adapted to a large-scale level. A graphical illustration of the wet and dry fractionation methods is provided in Figure 3. This section will deal with a detailed review of the two processes.

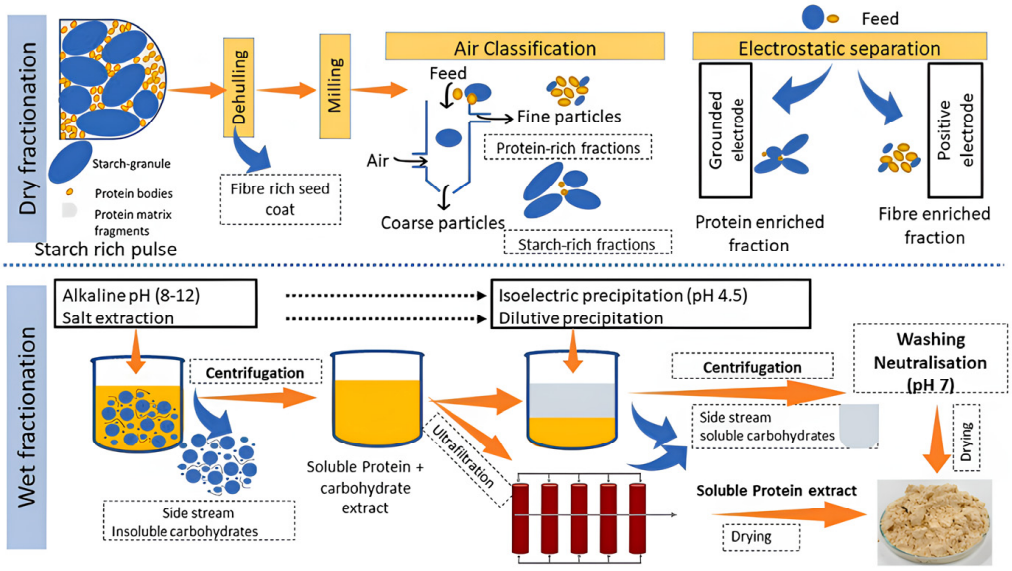

Figure 3. An overview of wet and dry fractionation techniques [40].

3.1. Wet Fractionation

The large-scale production of proteins is commonly performed through the wet fractionation process. The wet fractionation of proteins involves various solvents at different

stages of the separation of proteins [40]. The modification of type of solvent and other intrinsic factors may be necessary based on the nature of proteins present in the plant material and the composition of amino acids in the proteins. Alkaline solvent systems are usually preferred for the separation of protein because as the pH nears the isoelectric point (pI), the proteins' solubility gets reduced, thus making precipitation possible [19]. The protein yield after extraction is dependent on the initial protein content of the source crop and the nature of subsequent extraction procedures. Higher yield usually comes along with harsher chemical treatments, with increased chemical residues in the protein concentrates [31,40].

Jarpa-Parra et al. [57] studied the isolation parameters for the preparation of lentil protein isolates. They obtained a 14.5% yield from the sources through alkaline extraction at pH 9 and a solid–liquid ratio of 1:10 (w/v). It was then precipitated at pH 4.2 overnight. Although adjusting the pH to near the isoelectric point of the protein is sufficient, bringing the pH down to the exact isoelectric point can improve the yield. This was demonstrated by Wang et al. [58] where after solubilizing the proteins in an alkaline solution of pH 9, isoelectric precipitation of proteins was carried out to adjust the pH to the isoelectric point of 4.4. The study reported a yield of 77% through this method.

Coupling different methods in the extraction process also helps in increasing the yield and purity [40]. Boye et al. [4] also carried out isoelectric precipitation and ultrafiltration after neutralizing the precipitates. Ultrafiltration is a popular downstream processing technology in protein processing to obtain highly pure proteins. Since it does not rely on thermal or chemical applications, it does not affect the native state or the functionality of the proteins.

Another wet fractionation technique that utilizes aqueous solvents is salt extraction. A salt solution that has a high enough concentration and ionic strength can easily solubilize the proteins. The resulting protein can be precipitated through the process of salting out by increasing the ionic strength post-solubilization [40]. It is usually performed on the salt soluble proteins, which are globulins.

Several factors such as the concentration, time of centrifugation, speed of centrifugation, as well as the precipitation method and conditions influence the purity, functional properties, and yield of the protein isolates [31,40]. The presence of chemical residues in the protein isolates has been a major drawback of the wet fractionation process. The removal of chemical residues must be ensured after every batch of extraction through the necessary washing procedures [59]. The use of chemicals in different stages of the separation process increases the chemical residues in the sample and the environment as well as increases the cost of production [36,60].

Protein loss is also common in the salt extraction method where the proteins must be washed for salt removal after precipitation [19,61]. While washing, soluble proteins might get lost with water [32]. The extraction of salt-soluble proteins also poses the possibility of the proteins being lost in the salt as the complete salting out may not be possible for proteins if conditions are left unmonitored. Safety issues due to the volume and concentration of the solvents, such as NaOH and acids, also need to be addressed.

Moreover, the volume of used chemicals disposed from this process is alarmingly high, raising water quality and environmental sustainability issues [62]. Thus, transforming to a more sustainable approach that lowers the energy requirement while simultaneously being more environmentally friendly becomes necessary.

3.2. Dry Fractionation

Dry fractionation is the process of separation of components under dry conditions, i.e., without using solvents/chemicals. The mechanical detachment of proteins from other components, such as starch and fiber, is essential for the separation of proteins [8,20,24,26]. Milling is the initial step, followed by either air classification or electrostatic separation as the actual separation step. The efficiency of separation in the case of dry fractionation

is also influenced by the size and amount of starch granules present in the feed material initially and the extent to which they are comminuted in the milling step [30].

Variables such as protein content, yield, protein recovery and protein separation efficiency are used to check the effectiveness of the fractionation process. Protein content is defined as the concentration of protein in the original flour or the fractionated sample, expressed as % of the total weight. Yield is the ratio of the mass of the fractions obtained to the weight of flour fed to the separator, and protein yield is the amount of protein in the specific mass of fractions obtained [29]. Protein separation efficiency represents the total protein in the fraction compared to the total protein in the flour. It is calculated as the mass of the dry weight of the fraction multiplied by the protein content of the fraction divided by the product of the mass and protein content of flour [63]. The terms are all described mathematically by the Equations (1)–(4)

$$Protein\ Enrichment = \frac{Protein\ purity_{target\ fraction} - Protein\ purity_{starting\ material}}{Protein\ purity_{starting\ material}} * 100 \quad (1)$$

$$Yield = \frac{Mass\ (g) of\ each\ fraction}{Mass\ (g) of\ starting\ flour} * 100 \quad (2)$$

$$Protein\ Recovery = \frac{Protein\ content_{target\ fraction}}{Protein\ content_{starting\ material}} * 100 \quad (3)$$

$$Protein\ Separation\ Efficiency = \frac{Yield\ of\ target\ fraction * Protein\ content\ of\ target\ fraction}{Mass\ of\ feed * Protein\ content\ of\ feed} * 100 \quad (4)$$

4. Milling

Milling is carried out primarily to reduce the average particle size of the raw materials and to separate the components. As the protein and fiber molecules are present as fine particles while starch remains as larger granules, further steps in the separation becomes easier [64]. Coarse milling of the materials may result in improper separation between the starch and protein, as the components might still be bound together [60]. However, fine milling, when carried out, can result in the starch molecules being reduced in particle size down to the level of proteins. This phenomenon results in the starch particles mixing with protein fractions, reducing the protein separation efficiency [45,63]. Fine starch particles can also be dragged along with the proteins in the air stream and may mix with the protein fractions.

During milling, the speed of the milling equipment, such as the hammer in the hammer mill, pins in the case of pin mills, is influential on the subsequent separation [2,6,24,38]. The separation steps depend on the particle size distribution of the individual components and the extent to which they are detached [29,38,45]. Very high speeds in the mills may lead to fine powders being produced, which affects the detachment of the components of interest, as discussed above.

Wang et al. [65] tried cryogenic milling to reduce the particle size to about 50 µm. It was found that the further reduction did not enhance the separation efficiency but rather increased the energy expenditure of the material. It was observed that the agglomeration of the fine materials resulting from the high surface area created by the cryogenic milling reduced the protein separation efficiency. The selection of milling techniques should be based on achieving the necessary size that allows for highest charging without particle agglomeration, while keeping the cost in mind.

In milling, it is desirable to use successive stages of milling with two different mills to obtain the required uniform particle size distribution. For instance, Schlangen et al. [39] used a pin mill followed by an impact mill, resulting in a better separation of starch granules and protein particles as observed through Scanning-Electron Microscopy (SEM). The usage of a particular mill is determined based on its ability to achieve the desired particle size for a given plant material.

The nature of particles in the source also affects the choice of milling equipment. A knife mill was utilized in the coarse reduction of biomass by Basset et al. [66], followed by fine milling using an impact mill. Opazo-Navarrete et al. [41] utilized a lab-scale pulveriser for the size reduction of quinoa. The milling was performed with a 2 mm screen following which the fractions were jet sieved to obtain different size fractions. All these previous works represent the use of mills, based on the particle size of raw materials as well as the desired size of feed, in the dry fractionation process.

5. Air Classification

Air classification refers to the separation technique wherein the fractionation is achieved through a continuous stream of air. This technique has been reportedly used for the separation of plant materials from as early as 1978 [67]. It utilizes the difference in physical characteristics between the different components such as size, density and flowability. Proteins, starch, and fiber vary in their size and density. Proteins, which are small and have lower density than the starch and fiber fraction, get lifted by the air to the highest elevation while the denser fractions do not go as far in elevation. By changing the parameters of the air classifier (classifier wheel speed and airflow rate), it is possible to produce protein-rich fractions of different compositions [60].

The general process flow for fractionation during air classification involves an initial milling step, followed by an optional sieving step, and finally air classification. Sieving is carried out for the particles to obtain a uniform particle size distribution which ensures that similar-sized protein molecules are present in a single fraction [2,47]. The milled powders are air classified to separate the proteins from other components based on their aero-dynamic properties [68]. Factors such as the speed of the air classifier and the nature of plant materials affect the extent of separation from the components in the process [39].

By setting a cut-off point, the air classification of particles can be better controlled based on the set threshold. The cut-off point of the particles is usually set just below the size of starch granules, which is usually about 10 µm [62,69]. The extent of protein separation in an air classifier depends on the aerodynamic properties of the particles suspended in the air. The variation of these parameters in different plant seeds, such as cereals or legumes, affect their ability to be separated. For example, an inherently lower size of the grains, such as in the case of oats, can inhibit the separation characteristics [48].

High speeds of air in the classifier may cause the starch to come out at the highest outlets while too low an airflow rate in the classifier may not elevate the proteins to the higher outlets. The optimal velocity and the flow rate of air are influenced by factors such as the nature of the material, the amount of centrifugal force, drag force and draft due to the air classifier fan [63]. These parameters affect the distribution of the particles in the airstream due to their physical differences. Apart from the size distribution of particles, the initial protein content also reflects the protein content of the fractions after separation.

Design of Air Classifiers

Air classifiers have a common design of having an air supply or blower at the bottom with a minimum of two outlets, one at the bottom and another at the top. As the air is being supplied, the protein molecules, being finer, gets laden with the air to the top-most outlet, the starch molecules, due to their bigger particle size, get carried to the lower outlets [20,24,30,68]. All these air classifiers have an adjustable feed rate and a controllable classifier wheel speed on them. These adjustable settings are helpful for the optimization process. Most of these operate based on the centrifugal effects of the classifier wheel, with either a concurrent or counterflow of air [26].

Xing et al. [30] used an ATP50 multi-wheel type air classifier for the fractionation of pea, chickpea, and lentil. They followed a two-step fractionation process where electrostatic separation was performed after the air classification. The protein and starch fractions get separated due to the size of the openings present near the wheel of the air classifier and because of the density difference between the particles. An airflow rate of 52 m^3/h and a

wheel speed of 10,000 RPM enabled them to obtain a protein enrichment of 81.5% through air classification alone. But the higher wheel speed of 10,000 RPM reduced the yield to about 20%. So, 8000 RPM was determined as the optimized condition for peas, considering the yield along with the separation efficiency.

Pelgrom et al. [24] utilized the same air classifier (ATP50 multi-wheel) for the fractionation of peas and lupine after pre-treating them by soaking or defatting them. Schematics of the air classifier used by the authors are presented in Figure 4. The study utilized a classifier speed of 3400 RPM at 60 m^3/h airflow rate for peas, and 1200 RPM and 80 m^3/h for the lupine seeds. The results showed that the pretreatment of the pulses prior to air classification improved the separation efficiency.

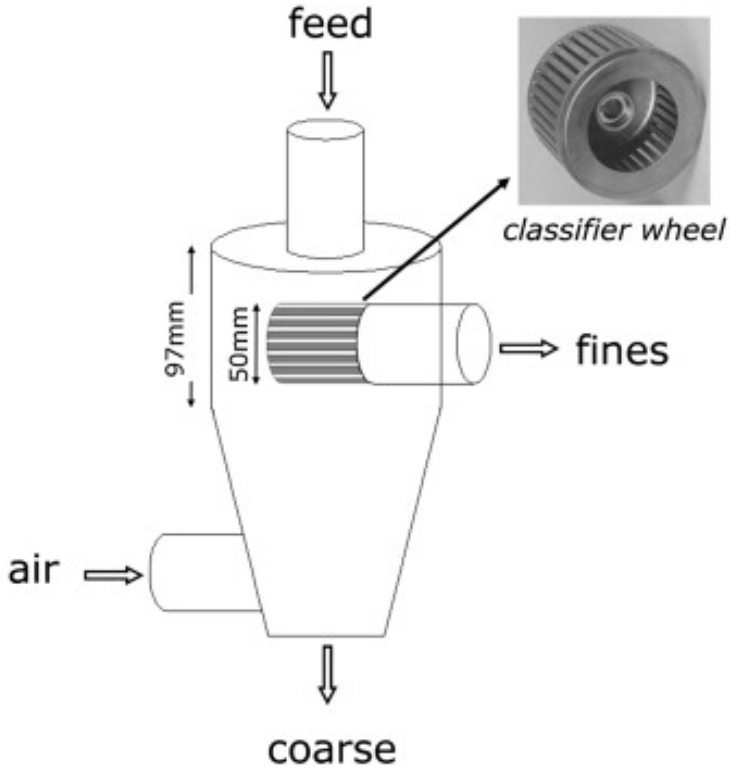

Figure 4. Schematics of an air classifier that fractionates using a classifier wheel with labels [38].

In their work, Pelgrom et al. [38] used a ZPS 50 air classifier which consists of a classifier wheel capable of separating the fine and coarse fractions in the first step. Coarse particles beyond the required size limit are sent back to the milling stage by passing them through a gap in the classifier wheel. From the observed disparity in airflow rates and classifier wheel speed for the two materials, it can be understood that different materials demand a wide range of speeds in the air classifier wheel for effective separation.

Angelis et al. [23] mentioned the use of an air classifier that operates via cyclone-based separation. As the air is supplied via a turbocharger, the particles of starch and protein separate based on their density difference in the cyclone and are collected via two outlets. An air classifier utilizing centrifugal force has been used for the separation by Reichert [69] in their study and the schematics are presented in Figure 5. Various speeds of the air classifier and the airflow rate used in the previous literature are compiled in Table 2.

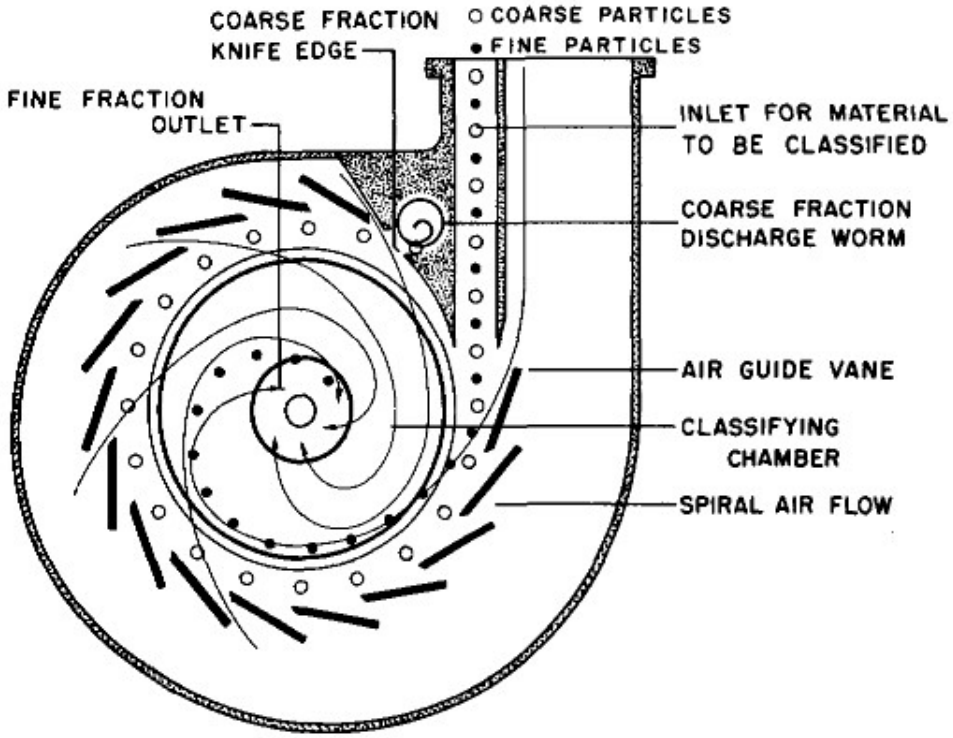

Figure 5. Schematics of a centrifugal force-based air classifier [70].

Table 2. Air classification parameters of some of the plant sources.

Source	Initial Contents			Protein Content in Fine Fraction	Air Classifier Speed	Reference
	Protein	Starch	Fiber			
Pea	23	44	NA	55%	12,000	[45]
Lentils	24.63	42	10.7	58.5	10,000	
Mung bean	25	47–58	18.8	58	12,000	[39]
Yellow pea	23	NA	NA	51–55	12,000	[38]
Faba bean	30.1	38.23	15.67	60	15,000	[71]
Yellow pea	~24	~50	NA	~53	12,000	[39]
Cowpea	~22	~70	NA	~49	12,000	

Air classification, as a dry fractionation technology, is quite beneficial in the manufacturing of functional ingredients. This is because the native state of the proteins are preserved during air fractionation, and the functionality of the proteins remains unaffected [29,60]. However, the yield of air classification is quite low compared to that of wet fractionation. Instances of clumping between the protein molecules is high because of the fine nature of the components. The interference of starch molecules in the protein fraction further reduces the protein separation efficiency of the whole process [40,59,63]. Hence, air classification becomes inefficient when the difference in particle size and density is small [29]. To overcome this limitation, another technique where the size and density affect the separation to a lesser extent, called tribo-electrostatic separation, has been recently explored for protein separation.

6. Electrostatic Separation

Electrostatic separation is a popular approach to segregate materials based on their surface charge. Traditionally, the process has been in place to separate impurities in mineral enrichment [44], coal refinement [72], recycling of plastic wastes [73] and separating pharmaceutical impurities [74]. Recently, it has been explored for the separation of food materials based on their chargeability and positive results have been produced as an

additional step in air classification [30]. Three possible methods of charging have been identified for charging the biomaterials, namely corona charging, inductive charging and tribo-charging.

Induction and corona charging are dependent on a moving conveyor and electrode for charging. A corona gun that supplies the current is the basis of corona charging while induction through a rotational drum imparts the charge in induction charging [43,75]. Contact charging between the walls and the particles of the same material in a high-speed stream of air is known as tribo-charging. During contact, the transfer of electrons from one surface to another results in the particles acquiring a surface charge. Plant proteins, acquire a charge different from those acquired by starch and fiber [68]. For food systems, the ability of the material to acquire charge through their physical properties enable them to be subjected to tribo-electrostatic separation.

Although the setups used for the separation procedure may vary between literatures, the principle of TES is the same. By applying a strong electric field between two electrode plates, charged particles in the airflow can be separated. Obtaining a maximum protein enrichment is dependent on the feed rate, the electric field strength, the charge to mass ratio of the particles which in turn depend on the number of collisions, residence time inside the charger tube, and the material of the charger [48,65,68].

The versatility of TES has been demonstrated by various researchers since it is based on the surface properties and the extent of charge acquisition by the particles in the charging step. The use of TES for the fractionation of arabinoxylan from wheat bran was carried out by Wang et al. [65] in wheat gluten starch mixtures [25] and in biomass [66]. One of the major drawbacks of the air classification method is the low purity obtained in the separation of the components. TES enhances the purity of proteins as a successive step after air classification systems. Since it is in the developmental stage, its low yield prevents it from being an individual separation process [30,45]. Setup and design aspects of the TES process are discussed in the following subsection.

6.1. Design of Electrostatic Separation

6.1.1. Feed System

The setup for the tribo-electrostatic separation can be looked at as three basic components: a feed system, a charging tube, and a separation system. The extent of purity obtained in the final fractions at an industrial scale is dependent on efficient feeding [76]. Fluidized bed feeding systems owing to the capability of increased particle–particle collisions [29] have been explored in most literatures. Suspending the particles in the air by supplying a stream of air at positive pressures is the base of a fluidized bed system [77]. This suspension disperses the particles in the air and the free-flowing characteristics in the fluidized bed promotes the particle–particle collisions [78]. Tribo-charging refers to the charges acquired by the particles due to the collisions between particles as well as particle–wall collisions [25,48]. This initial frequency of collisions enhances the charging behavior that will follow in the charging tube.

Other than fluidized bed systems, vibratory feeder, air vacuum pump [35], screw feeder [25], pneumatic systems [66] and particle feeding systems [76] have been applied. The screw feeders and vibratory feeders can be adjusted to have a specific feed rate to the charging system. While this is helpful, the dispersibility of the flour in the other systems gives them an edge in the charging and separation efficiency of the process. Zhu et al. [75] noted that the dilute phase in the feeding section would be beneficial compared to the dense phase conveying, since the latter could lead to excessive electrification. Particle feeders had an excellent control over the angle as well as the feed rate and have been in place for the mineral processing and recycling industries. The application of TES at an industrial level has led to the realization of the importance of the feeding systems [76]. Hence, for the development of a commercial scale TES system for foods, feeding should be given extensive consideration for optimal results.

Fluidized bed feeding has the advantage of being an anchor for automation in such processes. As long as the bed has a reservoir of particles, the fluidization will happen consistently. Without sufficient airflow rate at minimum velocity for fluidization, this might lead to agglomeration and clumping [77]. The initial particle–particle contact in fluidized bed feeders, as discussed above, will also be helpful in the next step, which is charging.

6.1.2. Tribo-Charging

The charging tube is the next and most critical part of the TES setup as the extent to which the particles get charged determines the degree to which the particles get separated [44]. The assessment of chargeability, type (positive or negative) and magnitude of the charge usually precedes the actual separation experiments [48,79]. A Faraday cup is usually employed for the preliminary chargeability assessments. Pre-assessment of the charging characteristics of the materials would give an idea of the separation characteristics as to the type of plates to be used and the collection of fines and coarse materials on the specific electrodes. The type and extent of charges acquired by the plant components depend on a range of factors. A broader view of these factors would result in three main factors: (1) nature of components, (2) nature of charging materials, and (3) particle behavior during their flow through the system.

Nature of Components

The charging of particles upon contact with the wall or other particles is said to be achieved by a difference in the work function. As a general electrostatic principle, charges are acquired due to electron transfer. The minimum energy required to remove electrons from a material is termed as the work function of that material [8,62,80]. Materials with higher work function acquire a negative charge while the ones with a lower work function acquire a positive charge [62,81].

Protein and starch molecules are known to exhibit different charging characteristics if obtained from different sources [24,30,62]. In their study, Tabtabaei et al. [62] mentioned that the starch from faba beans acquired a negative charge while proteins acquired a positive charge. The acquisition of opposite charges by the protein and starch leads to their agglomeration because of coulombic attraction. As the particles get finer, this effect becomes more prominent and the agglomerates become larger, leading to a reduction in the separation efficiency [65].

The type of functional groups influences the charging behavior of the particles. Proteins have amino and carboxyl groups in their structure, which are highly ionizable and hence, more easily chargeable. Starches, on the other hand, are difficult to charge because of their chemical structure. Fibers have a limited chargeability and, due to the nature of their functional groups (mostly being aldehydes), acquire an opposite charge of a small magnitude [8,14,82]. The extent of charging, in the case of proteins, depends on the isoelectric point of the protein, as well as the material of the charger [43,62].

The dielectric nature of the particles in the stream has an influence over the charging capacity of the materials. Chen et al. [83] reported that in the separation of bran, the aleurone layer's high permittivity allowed it to obtain more charges while the pericarp layer, owing to its high resistivity, only obtained low charges. Such differences between proteins and fibers could lead to a significant enhancement in their separation.

Moisture is another factor that could affect the separation efficiency of the TES process by altering the capacitance of the material. Capacitance is the property of a material that determines the duration a particle can hold a charge on its surface without losing it in the further path of flow. An increase in the moisture content would increase the capacitance of the material, leading to a higher number of charges. Conversely, a high moisture content could also lead to low flowability inside the charging tube which may reduce the separation efficiency. Thus, a higher capacitance in the protein molecules could become a deciding factor for their successful separation from other components.

Nature of Charging Material

Other factors, like the conditions of the experimental environment, also affect the separation process. Chen et al. [83] reported the use of a freeze dryer for the control of relative humidity in their tribo-charging experiments to ensure the same conditions. The freeze dryer was connected to the air compressor to regulate the relative humidity at the desired level $(37 \pm 2)\%$. The effect of temperature and relative humidity on separation efficiency has been discussed by Wang et al. [44]. As different materials have different affinity levels to water, it is expected that the separation characteristics would vary with changing relative humidity. However, their results showed that there was no significant difference in the separation of plant components at RH values between 0 and 100%.

The charging system can either be based on a tube or a slit system. Wang et al. [25] used an aluminum-based slit to charge the particles in a custom lab-scale electrostatic separator. A slit, which had four stripes of channels, was made to extend from the feeding system. Particles get charged due to particle–particle and particle–wall collisions. Air was used as a carrier in the setup. The slit system was compared with the tube system by Xing et al. [28] and they found that the tube-based system was better for food applications.

Various factors affect the efficacy of the charger tube, such as the material of the tube, the type of flow inside the tube, the length of the tube, the configuration of the tube and the nature of charge of the tube [44,76,83]. Tubes with various materials have the potential to be tribo-chargers including copper, stainless steel, nylon and other plastic materials like PTFE and PVC [83]. The tubes can be shielded with an insulation layer to avoid any external interference on the charging phenomenon [44]. The chances of tubes reacting with external charges should be minimized, as this interaction could affect the surface charges obtained by the components of interest.

The conducting nature of charging materials can influence the separation characteristics. Furthermore, the number of cycles of tribo-charging would also affect the charge on the particles. For example, the use of stainless steel was reported to have a constant amount of charge in the particles in multiple runs while the charge imparted due to Teflon has been reported to have changed from positive to negative [83]. Stainless steel being an excellent conductor allows continuous flow of charges without any accumulation. Teflon on the other hand, is a better insulator which causes the accumulation of negative charges such that when the particles bombard the walls of the tube, the tube acquires a negative charge of equal magnitude as the particles. So, in the first run, plant particles acquired a net positive charge, and the pipe wall acquired a net negative charge. However, the accumulated negative charges on the pipe wall is picked up by the flowing stream of particles. Thus, the overall charge in the particle stream becomes uneven between positive and negative. The charging mechanism during the flow of the particle stream inside a tube can be understood using Figure 6. On a multiple run trial, as conducted by the authors, it becomes detrimental as the stream is positively charged on the first run and is oppositely charged on the second run. As the separation is based on the overall charge of the particle stream, it resulted in reduced separation. This proves that there is a potential in increasing the overall protein enrichment using multiple separation steps, but the materials used must be carefully considered.

The triboelectric sequence is a series of materials arranged by the order and the nature of the triboelectric properties a material holds. This is presented in Figure 7, where a material located at the positive end of the series would gain a positive charge upon impact, whereas the particle that comes in contact with that material would be negatively charged [75,83]. Studies conducted previously on the characteristics of particles indicate that proteins usually acquire a positive charge while fibers acquire a negative charge [8,21,42]. As the plant materials usually get positively charged by losing electrons, materials at the negative end of the triboelectric series are preferred for plant protein isolation.

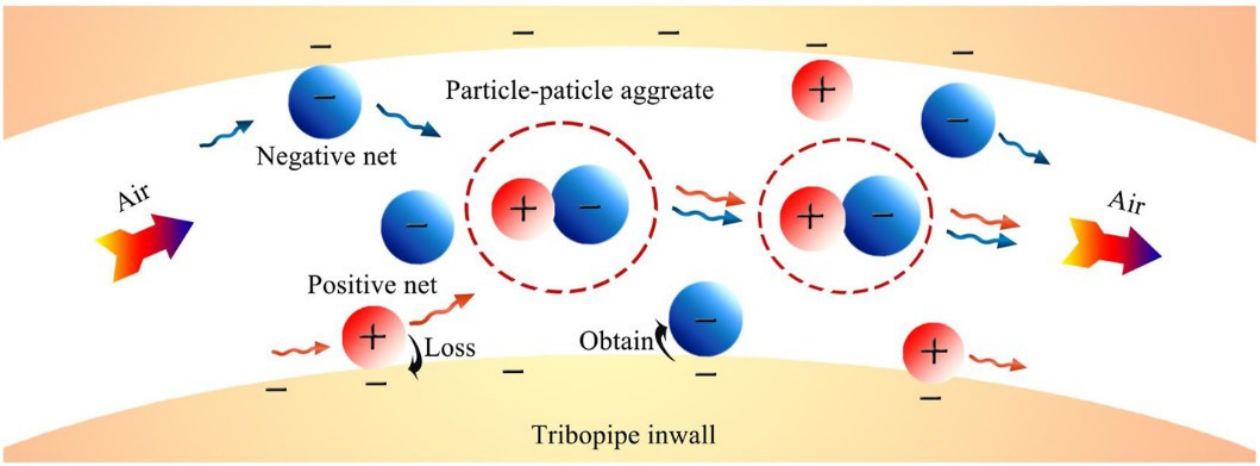

Figure 6. Mechanism of the charging of particles inside a tribo-charger [75].

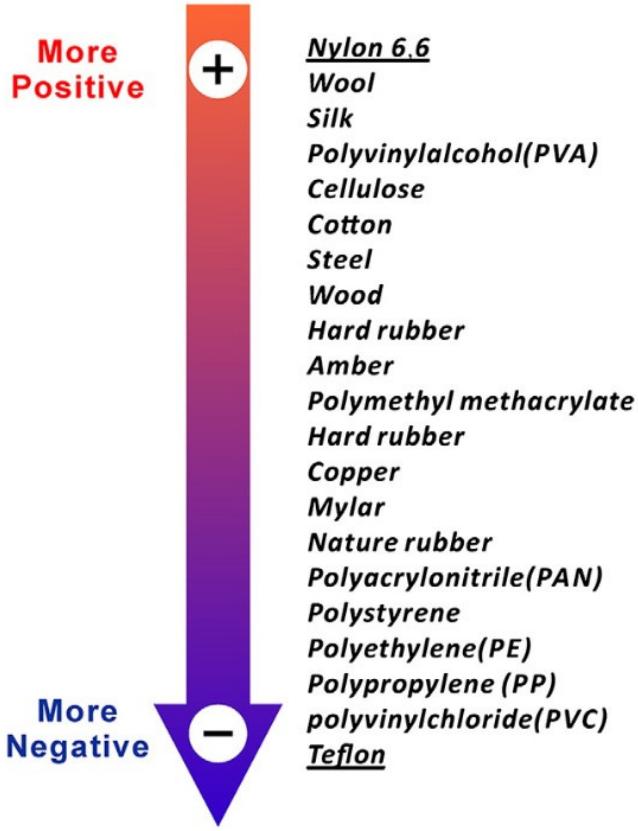

Figure 7. Triboelectric series of various materials applied in the separation process [83].

The tubes used for charging can either be configured in a straight or spiral manner. Spiral tubes were thought to have better separation efficiency, and this was tested by both Tabtabaei et al. and Xing et al. [28,62] with both kind of tubes. They reported contradicting results as the spiral tube for soybean resulted in an increased protein separation efficiency than the slit system [28]. The spiral tube for navy bean overcharged the particles due to their extended residence in the charger tube and having a poorer protein separation than the straight tubes [62]. It was mentioned in their findings that increasing the diameter of both the spiral and straight tubes increased the total protein content by 39.6% and 43.5%, respectively. This increase is likely due to the enhanced particle–particle collision leading to a higher charge exchange and a better separation.

Particle Behavior During Flow

The residence time of particles inside the tube is decided by the length of the tribo-charger tube. This time of residence, in turn, affects the extent of charging and the stability of the charged particle prior to being introduced into the separation chamber. As a standard, 1 m has been utilized as a starting point and optimized further based on the source material and the type of tribo-charger material used for the process [28,83]. A spiral tribo-charging tube will generally require a longer length than straight tubes. The longer tube length creates a longer residence time for the particles and a more efficient separation process [62]. However, increased contact charging as a result of a longer tube may cause the agglomeration of particles due to coulombic attraction, as mentioned before [30,65]. Apart from the agglomeration, the air resistance in longer tubes would lead to a backflow of air, resulting in reduced charges on the surface. Hence, a tube length which allows sufficient residence time and necessary charging but prevents agglomeration would be optimal.

The particle concentration and the gas flow rate are important factors for the efficiency of the process [25]. As the materials are charged, it is important to maintain the speed of air inside the tubes at a constant rate. This is because too high of an airflow rate will cause the effect of gravity to be larger and make the flour particles go straight down, neglecting the influence of the electric field that is present in the separation chamber [25].

The velocity of air during charging was found to affect the number of charges acquired in a positive manner [44]. When the air velocity is higher than 28 m/s, the change in charge was found to be unproportional. Konakbayeva et al. [48] experimented different airflow rates in the laminar, transient, and turbulent regime for oat fractions and found that a laminar flow at 7 l/min was the most beneficial in terms of yield and protein separation efficiency. At turbulent flow regimes, the yield and separation efficiency were reduced. This was reportedly due to the increased levels of agglomeration between the protein and starch molecules in the turbulent flow. This was supported by Landauer and Foerst and Tabtabaei et al. [29,42], where the increased turbulence did not observe any significant positive impact on the separation.

Chargeability can also be affected by the nature of flow inside the tube which can be either turbulent or laminar. Although it may seem that the turbulent flow would have a higher separation efficiency because of the random collisions caused by the nature of the flow, Tabtabaei et al. [29] found, during the separation of navy bean flour, that the laminar flow had a lower protein content with higher yield due to the effect of gravity experienced by the larger particles settling at the bottom of the plate. Konakbayeva et al. [48] compared the protein content of enriched fractions obtained through TES under laminar, transient and turbulent conditions. The study revealed that the protein content of protein-rich fractions obtained under laminar conditions was higher than those separated under transient and turbulent conditions. Such conditions could increase particle–wall collisions, thereby increasing the charge on the starch molecules. This leads to the attraction of starch granules to the protein fractions [48].

Contrarily, Wang et al. [25] observed that the increase in the airflow rates in a gluten–starch mixture led to improved separation efficiencies. A proper airflow rate becomes essential and needs to be optimized for various materials due to the difference in flow characteristics of these materials. In industrial conditions, fine particles pose an explosion hazard when in contact with the air, hence the need for the use of nitrogen or other inert gases to prevent explosions during such processes [65].

6.1.3. Separation

Separation is the step whereby the materials are collected into different fractions based on the charges obtained by them in the tribo-charging step. As the starch molecules are bigger than that of protein molecules as seen from the Scanning Electron Microscopy (SEM), finer particle fractions obtained from the collection step are usually higher in proteins than the coarse fraction. This is supported by Opazo-Navarrete et al., Pelgrom et al., and

Tabtabaei et al. [29,41,84], where the particle size distribution analysis shows the higher protein content in fractions that have a size of less than <0.6 mm.

Usually, a two-electrode system with a ground and a positive electrode is used for the fractionation of components. These electrodes attract the positively charged particles and the negatively charged particles, respectively [25,30]. But there have been instances in the literature where a three electrode system, with the middle electrode grounded, is seen [83]. Wang et al. [25] reported that a larger gap between electrodes is beneficial as it reduces the chance of particles landing on the opposite electrode after bouncing off the surface. The use of a single electrode system that is negatively charged to attract the protein molecules while the uncharged starch molecules get collected in the collection bags at the bottom has been reported by Tabtabaei et al. [62]. The plate used in their study was placed at an angle that was controllable to prevent the starch and fiber portions from depositing on the plate due to the flow from the air stream. Various setups that have been reported in previous literatures have been listed, with the important parts of the setups, in Table 3.

Table 3. Design of the previous setups that were used for the tribo-electrostatic separation of plant materials.

Reference	Charger System	Separation System	Feed Type	Dimension of Charging System (mm)	Collection Method
[44]	Squared Aluminum tube	-	Feed hopper	Inner dimension—1.8 × 1.8	-
[25]	Aluminum Slit	Two electrodes (positive and ground)	Screw feeder	2.5 × 3 × 220	Electrodes and filter bags beneath them.
[85]	NA	Two electrodes (positive and negative)	Controlled funnel feeder	NA	Cyclone systems leading to collecting jars
[62]	Spiral/straight tubes (PTFE, PVC, nylon and copper)	One copper electrode with adjustable angle	Fluidized bed feeding system with a magnetic stir bar	200 × 10 (length × diameter)	Collection bins (4) at the bottom of the chamber
[65]	Squared Aluminum	Two electrodes (positive and ground)	Funnel	1.8 side	Surface of electrodes
[66]	Teflon	Two electrodes (positive and negative)	NA	NA	Particle recovery system with cyclone separators
[76]	Fluidized bed charging made of polypropylene	Two electrodes (positive and negative) inclined at 5°	Fluidized bed connected to an input feeder	NA	Faraday cups at bottom (to check charges alone)

NA—Not Available.

Variations in the amino acid composition between proteins could lead to a difference in the charge accumulated on the surface of the molecules [34]. Usually, proteins are charged positively and deposit on the ground or on the negative electrode, while the positive electrode collects fractions rich in fibers and starch [8,78,85]. As mentioned before, the difference in the charging characteristics leads to a drawback, which is the inability to use a universal process parameter for all the materials.

An electric field (in the order of kV) is applied between two electrodes in an enclosed space. Electric field strengths, measured in kilovolts per unit length, ranging from negative voltages of −7.5 kV [29] to about 109 kV/m [42], have been reported in the literature. While the authors of [42] have applied 109 kV/m, it was found that no significant increase in the protein content was visible after 66 kV/m. This could be due to the sensitivity of the surface charge not being affected after certain electric field levels. Thus, it becomes clear that it is

necessary to have an electric field strength at a cut-off point to ensure the separation of proteins from the starch matrix. Beyond the cut-off point, there is seemingly no influence of the electric field on the separation characteristics.

When the particles are made to fall between the electrodes, separation takes place because of the influence of electric potential in the space and the surface charge of the fine powders in the stream [30]. A high electric field strength does not mean a high separation efficiency as too much deflection in the particle stream would lead to a mix up of the protein, starch, and fiber fractions in the flow [82]. One important advantage of the TES process over air classification is that the mixing of the fine and coarse fractions is less frequent as they are separated based on the surface charge of the materials. The process parameters used for the TES for various materials have been presented in Table 4.

Table 4. Details of tribo-electrostatic separation of plant materials in cited literatures along with their yield.

Source	Milling System	Voltage	Particle Size Distribution of Fine Fraction	Protein Content	Reference
Lupine	Pin milling and impact milling	20 kV	<25 µm	57%	[34]
Soybean flour	-	20 kV	-	63%	[28]
Pea, lentil and chickpea	2 step (air classification and electrostatic separation)	20 kV (Air classification + TES)	8–10 µm with 10 to 50 µm for chickpea	58%, 62%, 33%, respectively	[30]
Pea	2 step (air classification (8000RPM) and electrostatic separation)	20 kV (Air classification + TES)	8–10 µm	~70%	[30]
Navy bean flour	-	(−1 to −3 kV)	NA	21 to 47%	[62]
Rapeseed Oil Cakes	Knife milling followed by impact milling	10 kV	89.3 µm	51%	[66]
Barley Starch and Whey Protein Mixture	Agitator bed mill to obtain uniform particles	22 to 109 kV/m	~ 6 µm	~75%	[42]
Wheat bran	Impact mill followed by cryogenic grinding	15 kV	<50 µm	23.8 to 38.4%	[85]
Wheat bran	Pin milling followed by cryogenic milling	20 kV	NA	20%	[65]
Wheat gluten–starch mixture	-	10 to 25 kV	NA	Up to 95% of initial concentration	[25]

It is common to separate the milled flour into four different fractions based on the location of the particle settling. Major fractions are collected on the surface of the electrodes which make up the protein-enriched and starch-enriched fractions. These fractions are to be manually collected from the surface of the electrodes. Xing et al. [28] used a brush cleaning system that was attached to a conveyor belt. The study reported that the removal of particles at a regular interval increased the separation efficiency. This was because of the reduction in the charge shielding effect due to the previously deposited particles. Beneath the two electrodes, it is preferable to place the collection bags/chambers to collect the particles that were not settled on the surface of the electrodes. Electrodes where the protein-rich fractions deposit also have a gradient where the protein content of the fractions could differ. Konakbayeva et al. and Tabtabaei et al. [48,62] reported the top portions of the plate having a higher protein content than the bottom portions. This could be due to the

larger particles of lower charge density not being attracted to the top portions and moving near the bottom portions.

6.2. Multiple Stages of Separation

There have been instances in the literature where multiple stages of separation were employed to increase the protein content as well as the protein separation efficiency of the process. After tribo-charging, the protein and fiber molecules obtain opposite polarity while the starch might sometimes obtain the same polarity as the proteins. This can be rectified by adjusting the electric field based on the difference in the magnitude of the charges in the protein molecules and the starches, or by multiple steps of electrostatic separation [30].

A second stage that involves re-introducing the fractions from the collection chambers beneath the electrodes into the separation chamber has shown promising results as indicated by Tabtabaei et al. and Wang et al. [29,65]. Another method of application of multiple stages is where the protein-rich fraction collected at the ground electrode can be subjected to the electric field again for the further enrichment of proteins obtained from the first stage of separation [34]. The latter method resulted in an increase in the protein content by a factor of 1.3 after two additional steps of separation while the former technique can help recover the protein present in the coarse fractions. The recovery led to a protein content of 40% in the recovered fraction. Milling the coarse fractions before introducing them with the oncoming feed is another way to increase the protein content. Although protein content can be boosted in these multiple stages of separation, the yield reduces with each step.

Other multiple fractionation techniques that combined different dry fractionation techniques in successive steps have been employed. The use of two dry fractionation approaches in succession can increase the purity of the protein fractions with improved yield. Fractionating the flour with air classification can help in the removal of starch and the electrostatic separation can further purify proteins from the fibers [30]. For example, Wockenfuss et al. and Xing et al. [30,68] utilized air classification as the first step, followed by electrostatic separation as an aid to increase the protein content, with both obtaining promising results. The initial air classification step removes the starch while the electrostatic separation helps in removing the fiber from the protein-rich fraction.

Wockenfuss et al. [68] explored the combination of air classification and electrostatic separation in both orders for improved protein yield, recovery, and enrichment. They found that the use of a protein-depleted fraction from air classification in electrostatic separation resulted in a higher protein separation efficiency and yield (38% after the second step). The enrichment of fiber takes place when the protein-enriched fraction is reseparated using TES after air classification. The protein separation efficiency was the lowest among all the combinations (19%) while using electrostatic separation as the first step in the fractionation of rapeseed oil cake, compared to the methodology with air classification as the first step (35%) or air classification alone (47%).

Dietary fiber enrichment in the protein-depleted fractions on the electrodes were reported while the fractions collected in the collection bags were depleted of the fibers [48]. Wang et al. [65] mentioned the enhanced separation of fibers from wheat bran by employing sieving after the electrostatic separation.

Care must be taken to ensure the cleaning of electrodes after every trial. This helps to reduce the interference of the particles, by the space charge effect, from previous runs with the separation, [25,29]. The space charge effect refers to the phenomenon where the settled particles on the electrode prevent other particles from getting attracted to the electrodes. The shield created by the already settled particles results in a lowered electric field strength. The presence of particles from the previous runs also reduces the force of attraction by the electrodes, which also limits the separation characteristics.

To summarize the setup of the tribo-electrostatic separation procedure, it consists of a feeding zone followed by a tribo-charging tube, leading to the separator setup. Fluidization of the particles in the feeding zone will lead to a minimal surface charge in the particles due to particle–particle contact between the flour particles. Tribo-charging is a critical part

of the TES flow as the charging is a crucial step for the separation of the particles. The behavior of different charger materials needs thorough study as the mechanism, and hence the extent of charging, changes based on the material. The separator is composed of a set of electrodes arranged in a specific manner. The electrode arrangement can range from one to three within the chamber. All these parameters need sufficient optimization for different materials to be effective in fractionation.

7. Pretreatments

Various pretreatment methods have been explored by researchers for better separation of the protein and starch matrix. Moisture control helps to loosen the cotyledon, aiding in easier separation in the milling process. Drying, conditioning, soaking, dehulling and defatting [28] are some of the common pretreatments that are applied [26]. Drying, soaking, and conditioning have an impact on the milling step. As mentioned earlier, proper separation of starch granules and protein molecules is vital in the subsequent step. These pretreatments help the materials attain the suitable moisture content that can prevent agglomeration and achieve a uniform particle size after milling [14,31]. During the transfer in the charger tube, presence of excess moisture may lead to the agglomeration of particles and inhibit the separation of the fractions.

Conditioning and soaking tend to increase the moisture levels to 12–18% (wet basis) and 45%, respectively. This helps in the hardening of the hulls and brans and helps in their effective separation during milling. Drying reduces moisture, leading to the reduction in the size of the cotyledons, facilitating their easy removal in the dehulling process. All three of these pretreatments are for grains, while drying is performed on milled flour as well as grains [26].

Carmo et al. [71] assessed the effect of the dehulling step on the protein separation efficiency in peas and faba beans. Due to its aid in the flow rate inside the tube, the presence of hulls, in the case of faba beans, can have a slight positive effect in protein separation efficiency. This is not the case for the peas as the hulls interfere with the milling characteristics and reduce protein enrichments. Moreover, dehulling can result in a reduction in the antinutritional factors in the flour [86]. The use of microwave and infrared for effective dehulling could reduce the antinutritional factors in legumes [26].

Defatting is mainly applied to oil seeds to reduce fat content, which otherwise interferes with the protein yield and promotes agglomeration and oxidation in the material [28]. Pulses like chickpea which have a high fat content must be defatted to achieve better flow properties. Defatting is usually performed through the solvent extraction procedures, which goes against the sustainability goals associated with the dry fractionation process [24]. Novel techniques like supercritical fluid extraction have the potential to serve as an alternative to the solvent extraction process. Defatting can separate the protein bodies from the fat molecules in the flour, thus improving the separation efficiency. As defatting can improve the protein yield and efficiency of the process, it would be a valuable pretreatment procedure by matching the cost for the low separation yield if whole grains were used [28].

Additionally, microwave pretreatment can help in the efficient milling of the grains due to the induced thermal effect. Apart from the prevention of agglomeration and efficient milling, pretreatment of the grains helps to increase the flow properties of the flour as a high mass flow of the flour typically results in better protein yield and separation efficiency.

8. Quality of Proteins

By applying TES in the separation of proteins, it is possible to obtain them in their native structure. As the TES is a dry fractionation technique which avoids the use of chemicals in the process of extraction, any harsh reactions on the structure of proteins are avoided [21,40]. Conventional techniques use alkaline and acid solutions for the solubilization and precipitation of the proteins [23,87]. These reactions, apart from fractionating the proteins and starches, contribute to the chemical modification of the proteins by break-

ing the hydrogen bonds through condensation and substitution reactions that change the functional groups in the protein molecules, thereby altering the protein structure [3].

Changes in the chemical structure and the native state with respect to the hydrophobicity and the reactive group in the proteins would affect the functionality of the proteins such as solubility, foaming capacity, emulsion capacity, water and oil holding capacity [29,37,88]. All these parameters decide the effectiveness of the end-product uses which changes with the source of the proteins. Another important factor to be considered during the formulation is the bioavailability of the proteins, which is the ability of the human digestive system to absorb the proteins. Studies that focused on the comparison of wet and dry fractionated proteins found that the bioavailability of wet fractionated proteins are better than that of the dry fractionated counterparts [88]. Saldanho do Carmo et al. [7] reported that the presence of bioactive components, including antinutritional factors, in dry fractionated proteins can interfere with the bioavailability of the protein-rich flours. Hence, more research on the antinutritional factors and ways for their reduction in dry fractionated fractions techniques are needed.

Unlike wet fractionation where drying is the final step needed to obtain the protein isolates or concentrates, TES does not involve any high temperatures that would denature the proteins. The absence of both chemicals and high temperature in the fractionation process ensures a protein of high functionality and quality. Although the functionality of the proteins is unaltered, the inevitable change in functionality due to the damage in structures in the milling step still lingers. Only a limited number of literatures discuss the effect of milling on protein functionality, so the extent to which the functionalities are affected is unclear [63,71].

9. Limitations of Electrostatic Separation

Real life application of a process would primarily depend on the ease with which the methodology can be implemented in a commercial setting and the feasibility of the process with respect to the available resources. One major limitation of the TES method of extraction is the limited yield in the protein fractions [38,45,63]. Optimization of the process is difficult in this case because when the protein enrichment increases, the yield decreases, and vice versa.

The longer time taken for the process and the low yield are also the current obstacles in the technique. Improvements to the yield and the time of operation can be made through the optimization of the process flow, along with the improvements in the design of the separator system. Milling, as mentioned before, is a crucial step in the process. This can influence the cost of the overall process on a commercial scale based on the material hardness and the mill's attributes. As most of the studies were conducted on lab-scale setups, further studies that deal with commercial milling, cost and techno-economic analyses are needed [26].

Moreover, the achievable purity of the protein-rich fraction is limited in most plant proteins due to their structural characteristics and the capabilities of mills [68]. So, the ability to obtain a high yield of pure proteins is difficult. The number of particles collected in the coarse fraction is always higher than the fines and contains some amount of unrecoverable proteins. This is due to the minimum separable levels of the protein and also due to starch particles acquiring higher charges than the protein molecules [42].

10. Other Methods

Apart from the tribo-electrostatic separation of the particles, similar methods that differ in one or two aspects of processing have been explored by researchers. One such method is the tribo-electromagnetic separation of proteins, where the use of a magnetic field along with the electrodes was utilized by Zhu et al. [50]. As the particles enter the electric field, they experience the Lorentz force due to the magnets which affect their path and cause a deflection in the flow. By a combination of these two forces, the separation of protein molecules is made possible.

Another method is known as the tribo-aero-electrostatic separation, where the electrodes are made to oscillate in the vertical direction in a rock and forth motion and subsequently introduced in a fluidized chamber after a given amount of time before separation. The sudden introduction of the electrodes causes a flash of electric surge, causing the charged particles to be attracted to the electrodes, resulting in the enrichment of the flours [89].

11. Commercialization Potential

Tribo-electrostatic processing of the pulses and cereals is an effective step to produce protein-rich products. Dry separation techniques can reduce the energy consumption by five-fold, making it a sustainable option for the protein separation process [26]. The purity of the proteins, in comparison to the wet fractionated protein samples, is less and it is difficult to obtain the protein isolates and concentrates of the highest quality, but it can be improved. Design considerations and processing considerations are the key to achieve the required purity of the proteins in the concentrates [30].

Protein-rich flours are utilized in the development of ready-to-eat foods, functional foods and as functional ingredients [15], where the application of TES proteins is highly suitable. The preservation of the native structure of the proteins, in contrast to that of conventional isolation techniques, help in the use of protein-rich functional flours in the product development. E-numbers are given to additives that contain chemical formulations. Proteins that are obtained through dry separation processes do not require E-numbers, enabling the production of clean label foods [31,87]

Moreover, the use of protein isolates would require the addition of other ingredients such as starch and fiber, which would mean increased cost and ineffective product utilization [14]. Starch and fiber are needed because of their nutritional and functional benefits in certain products [31,87]. In these applications, the functionality of the ingredient is more important than the purity [37]. Opting for an ingredient that is aimed at functionality needs further study [14]. Dry fractionated protein and starch fractions would be an ideal source for such an application.

The electrostatic separation technique is still in the research stage and requires further development for its successful application in the production of protein isolates and other protein-rich products. Initially, it was thought that the application of TES is infeasible for grains with high starch content [30,45]. Studies from recent years have enabled us to develop a working system of tribo-electrostatic separators. Findings, such as the overlapping particle size distribution of flour and starch granules, towards the better recovery of protein have been significant contributions in the development of methodologies [45].

Focusing on the protein-rich fractions, their utilization and application is much needed, but the starch-rich fractions obtained from the dry fractionated processes are also quite valuable. In their review, the authors of [5] talked about the gelatinization, retrogradation, swelling and pasting properties of starches. Dry fractionated starches show excellent potential as functional ingredients in applications requiring gelling and pasting [90]. Such properties demonstrate the potential of starch-rich fractions from the dry separation process, implying the complete utilization of the crops.

Cascading the air classification and electrostatic separation of particles shows promising potential to be a viable option. Optimizing operating conditions so that the agglomeration of the components are avoided, or designs that can counteract such conditions, will have the potential to revolutionize the process [25]. Along with the increased purity and yield, reduction in antinutritional factors should be a point of focus during the optimization procedures [60]. The effects of air classification on the chargeability and surface properties of the particles, and how it is affected in further stages, needs to be studied. A clear understanding of the order of dry fractionation techniques and the effect it has on the yield and protein content will have a major impact on its applicability.

12. Conclusions and Future Works

Growing food demand in the world needs a corresponding supply of proteins to prevent and counter malnutrition. The use of animal proteins alone, which can negatively affect the environment, is not a good resource management strategy. Plant proteins provide an excellent way of overcoming the problem, but the use of chemicals in the process of extraction is not favorable to the protein structure and function. A review of two dry fractionation techniques, namely air classification and tribo-electrostatic separation, their principles, applications and industrial relevance has been presented in this paper. Tribo-electrostatic separation as a dry fractionation method could help in the sustainable extraction of proteins from plants. In combination with air classification, it presents an opportunity to obtain increased yield and purity.

There has been a lack of research in the resistivity and permittivity of plant materials in terms of the particle composition, particle size after milling, etc. These areas can be a focal point for upcoming research. As the particles are charged, it is necessary to confirm their chargeability, which is conducted only prior to the actual experiments in all the literatures. A measuring system capable of measuring the charges while being transferred in the tribo-charger tubes could improve the separation characteristics [48]. Contradicting results in airflow rates [25,29,48] and charging behavior [28,82] indicate that the process needs optimization for different materials.

Contradicting results in the charging behavior, flow type and the electric field strength indicate that the process needs to be devised for each grain. This could be the focus of upcoming research to develop a working methodology for specific plant materials. Such a development would prove useful in the development of separators that can accommodate the large-scale production of protein-rich concentrates. The application of tribo-electrostatic separation in plant protein separation would pave way for a green and sustainable technological development.

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