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Review

Pulsed Electric Field Treatment in Extracting Proteins from Legumes: A Review

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Abstract: A healthy diet rich in plant proteins can help in preventing chronic degenerative diseases. Plant-based protein consists of derivatives from algae, fungi (like mushrooms) and other plant products including stems, leaves, fruits, vegetables, grains, seeds, legumes and nuts. These sources are not only rich in protein, but also contain a high percentage of iron, calcium, folates, fiber, carbohydrates, fats etc. Hence, it is essential to explore plant-based protein sources and their other nutritional components to address existing food insecurity issues. Nowadays, the impact of food processing has produced promising results in extracting valuable bio-compounds including proteins from the plant matrix. In this view, PEF technology has secured an exceptional place in solving food quality issues through minimized thermal effects in the samples, improved extraction capabilities at a shorter time, higher extraction levels, high nutritional content of extracted samples, greater shelf-life extension and increased microbial killing efficiency. It is an energy efficient process which is used as a pre-treatment to increase selective extraction of intracellular compounds through electroporation technique. Here, the processing parameters play a significant role in obtaining enhanced extraction levels. These parameters have also considerably influenced the protein digestibility and amino acid modification. So far, PEF has been producing remarkable results in plant protein extraction research. Among various plant sources mentioned above, there is a limited literature available on the use of PEF-assisted protein extraction from legumes. In this review, the authors have discussed essential legumes and their nutritional components and have highlighted how PEF can be beneficial in extracting the protein levels from these sources. Further research should focus on PEF-assisted protein extraction from legumes, specifically analyzing the properties of protein quality and quantity.

Keywords: pulsed electric field; legume protein; pulsed electric field assisted protein extraction from legumes

Citation: Ramaswamy, R.; Bala Krishnan, S. Pulsed Electric Field Treatment in Extracting Proteins from Legumes: A Review. *Processes* **2024**, *12*, 2667. https://doi.org/10.3390/pr12122667

Academic Editor: Dariusz Dziki

Received: 8 October 2024
Revised: 18 November 2024
Accepted: 22 November 2024
Published: 26 November 2024

1. Introduction

Food insecurity impacts the lives of people globally, and research is ongoing to explore the correlation between food insecurity and several health concerns, like blood pressure and glycated hemoglobin levels in individuals with diabetes and hypertension issues [1]. Also, studies have looked at how food insecurity affects cancer survivors' chances of dying in the US [2]. Undernourishment is one of the primary indicators of food insecurity, which hinders access to sufficient and safe nutritious food for a healthy life. Consuming a well-balanced diet rich in proteins and other essential nutrients protects against a variety of chronic and noncommunicable illnesses including cancer, diabetes and heart disease. Elderly individuals are more prone to health ailments and weaknesses if they do not meet everyday nutritional needs, especially proteins. It is found that higher socioeconomic status was linked to an increased intake of animal and total proteins in Korean senior citizens [3]. Since the intake of sufficient protein is related to socioeconomic status, global feasibility and affordability should be considered in the first place to address food insecurity issues. Animal proteins are expensive and can have adverse health effects, especially in relation to cardiovascular disease and type 2 diabetes [4]. Elevated branched-chain amino acid (BCAA) levels due to high animal protein consumption are considered as one of the significant predictors of these diseases [5,6]. This has driven the increasing demand for more affordable alternative protein sources. When compared to animal proteins, plant-based protein sources offer several advantages including lower production costs, easy availability, comfortable accessibility, minimal maintenance and greater environmental sustainability. Some plant proteins lack essential amino acids, but this can be compensated by combining them with a cereal blend. In many developing countries, plant proteins are preferred due to their cost effectiveness when compared to animal-based proteins [7]. Western countries, on the other hand, have a higher rate of animal protein consumption than plant proteins [8]. This is depicted in Figure 1, where overall plant protein contribution is less compared to animal proteins and the contribution of plant protein consumption from legumes is found to be the least [8]. It is, hence, understood that legumes often do not receive as much attention as other plant and animal proteins, despite being an excellent nutritional option. These are basically rich in proteins, vitamins, complex carbohydrates and fiber, which, when extracted, can then be utilized for the production of several value-added products.

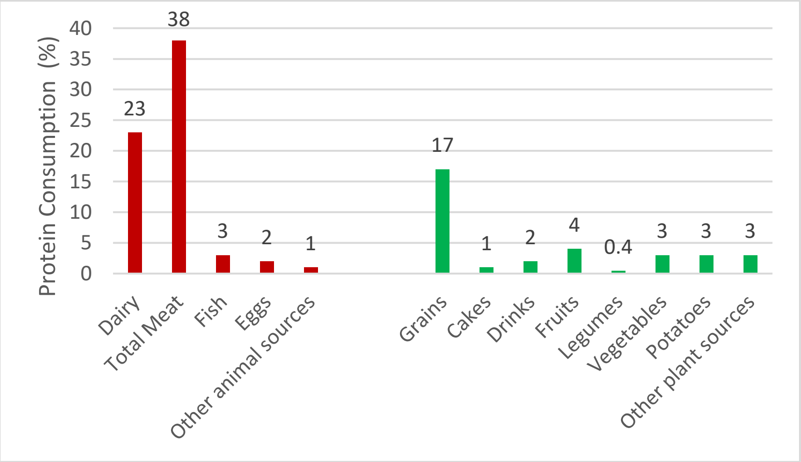


Figure 1. Proportion of total protein intake from animal and plant sources [Reproduced with permission from Elsevier. License number: 5871670317304] [8].

Degrees of food insecurity can be substantially reduced by availing the benefits of underutilized crops, and particularly, legumes, due to their innate characteristics including their high nutritional content, capacity to withstand drought and high potential for fixing nitrogen, which can boost plant growth, crop yield and overall food production [9]. Legumes are widely available, affordably priced and safe to eat [10]. Legumes must be dried to remove the moisture content so that they can be conveniently stored for a long time. For proper consumption, these legumes will be soaked in water before they are cooked, germinated, mashed, extracted or malted. Legumes including lentils, beans, peas, chickpeas and soybeans are rich sources of plant-based proteins that have gained considerable attention for their nutritional value and potential health benefits. These proteins are considered as an important alternative to animal-based proteins for vegetarians and individuals with dietary restrictions. According to a recent study, the growing popularity of the vegetarian lifestyle is driven by the increasing awareness on sustainable food production and healthy nutrition along with the rise in the global population, which is anticipated to increase the demand and income for legumes [11]. In 2021, the global legume production has reached 551,125,349.85 tons and the global plant-based protein market is expected to grow from USD 12.2 billion in 2022 to USD 17.4 billion by 2027 [11]. As a result, awareness of the health benefits of legumes is expected to increase in the coming years, thereby promoting healthier dietary choices.

Suitable food-processing techniques produce desirable biocompound extraction levels from different plant products. Thermally assisted extraction methods increase the temperature of the product, leading to detrimental effects. Among the existing non-thermal methods, pulsed electric field (PEF) technology stands out as the emerging and energy efficient technique for improving the quality and quantity of extracted proteins from the plant matrices. It involves ultrashort high-voltage pulse application to samples placed between two electrodes in the treatment chamber. The electric field thus generated in the treatment area increases the permeability of the plant cell membrane, leading to electroporation. The intensity of electroporation relies primarily on the electric field strength variation with subsequent leakage of intracellular compounds, leading to enhanced extraction levels. Thus, the electric field and other process parameters such as the pulse width, number of pulses and pulse waveshape assist in obtaining desirable protein levels. Recently, several studies have begun utilizing PEF treatment to assist the extraction processes of plant-based proteins. However, focusing on PEF-assisted protein extraction in underutilized crops such as legumes could significantly aid to improve the production cycle of legumes and economically generate value-added products.

The article has discussed selective and affordable legume species and their nutritional information. The study has also analyzed the potential benefits of pulsed electric field application to legumes for the extraction of valuable proteins.

2. Plant Protein Sources—Legumes

Protein helps with regulating body functions including the creation and repairing of cells. While animal-based protein products such as meat, fish and eggs are considered to be the strong sources of protein, it is still possible to get adequate protein from the variety of vegan and vegetarian choices. Out of the vegetarian options, mushrooms are high-protein sources, and act as a good alternative to animal proteins. Ramaswamy et al. (2024) presented a detailed list of commercially cultivated mushroom varieties, their protein content and their nutritional and medicinal properties [12]. In this paper, the author has continued to provide a detailed discussion on legumes.

The terms “legumes” and “pulses” are related but not interchangeable. All pulses are classified as legumes, but not all legumes qualify as pulses. Pulses are the essential source of nourishment with worldwide usage, and can play a significant role in human nutrition. They are an excellent source of protein, carbohydrates, dietary fiber, vitamins, minerals and phytochemicals, with approximately 21–25% of protein content [13]. While the amount of global pulse production has remained stable at about 40 million tones, western consumption rates are still lower when compared to Asian and African consumption rates [14]. However, a huge population consume pulses as a staple food with a cereal blend for their everyday protein requirement. Utilizing the nutritional benefits of pulses can aid in overcoming ‘global malnutrition’ issues. Pulses have a special place in developing countries due to their high protein content and rising consumption rate. Incorporating pulses, beans and legumes into the diet can help in improving health conditions including cardiovascular disease and type 2 diabetes [15]. Despite the availability of a wide range of high protein legumes, only few studies are available that examine the extraction of proteins from them. The World Health Organization recommends an increased consumption of legumes to overcome overweight and obesity issues as well as lower the risk for non-communicable diseases [16]. Table 1 refers to the multiple health benefits of most commonly used legumes. While some legumes belong to the same family, they share common characteristics.

Versatile availability and affordability are the two essential factors to be considered when choosing a suitable food product for extraction studies, which would aid in the growth of environmental and economic sustainability. Based on the above two factors, the author has discussed the essential and affordable legume protein sources below.

Table 1. Multiple health benefits of most commonly used Legumes.

S.No.	Legume/Pulse	Nutritional Profile	Health Benefits	References
1.	Chickpea *Cicer arietinum* L.	• Contains phytic acid, sterols, tannins, carotenoids and other polyphenols such as isoflavones. • Consumers of chickpea have shown to have higher intake of dietary fiber, polyunsaturated fatty acids, vitamin A, vitamin C, vitamin E, folate, potassium, magnesium and iron as compared to non-consumers.	• Promotes weight control • Lowers blood glucose levels • Improves cardiovascular health • Reduces the risk of certain cancers • Improves gastrointestinal tract health	[17]
2.	Pigeon Pea *Cajanus cajan* L.	• Rich in protein, carbohydrates, minerals, vitamins and essential amino acids for both mature and immature pigeon pea seeds. • Economical source of high-quality and -quantity protein food and feed for tropical and subtropical livelihoods.	• Hypocholesterolemic effect • Antimicrobial effect • Hypoglycemic activity • Hepatoprotective effect • Cancer prevention • Anti-inflammatory effect • Antihyperglycemic activity • Antidyslipidemic activity	[18,19]
3.	Lentils *Lens culinaris*	• Rich in boron, calcium copper, iron, magnesium, manganese, molybdenum, phosphorus, potassium, selenium, vitamin B1 (thiamin), vitamin B2 (riboflavin), vitamin B3 (niacin), vitamin B5 (pantothenic acid), vitamin B6 (pyridoxine), vitamin B9 (folate), vitamin E (tocopherols), vitamin K1 (phylloquinone) and zinc.	• Weight management • Blood sugar regulation • Supports overall gut health • Immunity development • Anti-inflammatory properties • Antioxidant properties • Antimicrobial properties	[20]
4.	Dry Pea *Pisum sativum* L.	• Rich in proteins, starches, dietary fiber, non-starch polysaccharides, polyphenols including flavonoids and phenolic acids. • Good resource of minerals (e.g., calcium, iron and zinc) and vitamins (e.g., carotenoids and folic acid).	• Antioxidant • Anti-inflammatory • Antimicrobial • Anti-renal fibrosis • Regulation of metabolic syndrome effects • Antidiabetic • Antihypertensive	[21]
5.	Cow Pea *Vigna unguiculata* L.	• Presence of soluble and insoluble dietary fiber, phytochemicals, proteins and peptides.	• Anti-diabetic • Anti-hyperlipidemic • Anti-hypertensive properties	[22]
6.	Black Bean *Phaseolus vulgaris* L.	• Good source of proteins containing the most essential amino acids, especially lysine. • Rich in carbohydrates, protein, fibres, vitamins, minerals, phytochemicals, such as saponins, anthocyanins, flavonols, and phenolic acids.	• Antioxidant • Antidiabetic • Anticancer • Anti cardiovascular • Anti-inflammatory properties	[23]
7.	Lima Bean *Phaseolus lunatus* L.	• Excellent source of proteins, amino acids (AA), minerals, dietary fiber and B-complex vitamins (folate, B6 and niacin). • Rich in lysine, phenylalanine, leucine, valine, threonine, isoleucine and histidine.	• High mineral content assists with muscle movement, healthy nervous system and strong bones and teeth	[24]
8.	Pinto Bean *Phaseolus vulgaris* L.	• Rich in protein, fiber, prebiotics and vitamin B. • Contains high levels of chemically diverse components including phenols, resistance starch, vitamins and fructooligosaccharides.	• Antioxidant activity • Protects against oxidative stress, cardiovascular disease, diabetes, metabolic syndrome and many types of cancer	[25,26]
9.	Red Kidney Bean *Phaseolus vulgaris* L.	• Rich in amino acids, red kidney beans offer a cost-effective source of dietary protein. • Isolation of hydrolysate exhibits antimicrobial effects.	• Antimicrobial, Antibiofilm, and Quorum sensing inhibitory effects	[27]

Table 1. *Cont.*

S.No.	Legume/Pulse	Nutritional Profile	Health Benefits	References
10.	Soybean *Glycine max* Black (BS) & Yellow (YS)	• Contain a high amount of protein, carbohydrates, dietary fiber, vitamins and minerals and phytochemicals, including isofavones (i.e., daidzein, genistein and glycitein), saponins, phenolic acids and antho-cyanin. • Rich source of proteins, carbohydrates, dietary fibers, lipids, vitamins (such as vitamins B, C and E) and minerals such as sodium, potassium, phosphorus and iron, as well as bioactive compounds including saponins, phenolic acids, isoflavones and anthocyanin. • Higher contents of polyphenols such as anthocyanin, soyasaponin and isofavones than YS.	• Excellent antioxidant effects • Anti-obesity and hypolipidemic, anti-inflammatory, and anti-cancer effects and cardiovascular protective activities	[28,29]

i. Lentils

Lentils (*Lens culinaris*) are a versatile legume with great potential for direct consumption and food-processing applications. Depending on variations in the growing conditions, the nutritional, chemical and antioxidant capacity of lentils can change [30]. The demand for lentils is spread worldwide, most of which are not very price sensitive. Lentils are low in fat and high in protein, fiber and minerals [31,32]. They contain essential bioactive compounds including flavonoids, phenolics and saponins [33]. Lentils have potential health benefit in preventing cancer, diabetes, hypertension and cardiovascular diseases [32]. Since 2001, the production of lentils has increased more than two folds, leading to 6.58 million metric tons in 2020, with Canada being the primary producer [33]. Certain processing conditions including milling, splitting and dehulling can modify the nutritional value of lentils to produce value-added products [33]. Thus, Lentils can meet growing consumer demands towards plant-based proteins and can act as sustainable food sources [32,33]. They can be added to a variety of food items to help fight malnutrition, particularly in underdeveloped countries [34].

With regard to protein content, lentils have a high percentage of proteins and nutrients that can act as a good substitute for animal and soybean proteins in food processing [35]. Lentil proteins contain around 16% of albumins, 70% of globulins, 11% of glutelins and 3% of prolamins [36,37]. Additionally, it has been found that the rhizosphere of lentils contains rhizobacteria, which would have a favorable effect on plant growth [38]. Furthermore, lentil extract has the potential to be used as a fertilizer for the growth and production of other crops, such as beets [39]. With aprotein content ranging from 32.2 to 35.6% in seeds and up to 85% in concentrates, lentils are a promising plant-based sources of protein [40,41]. Lentil proteins demonstrate good functional properties, including emulsifying activity, water and oil absorption capacity and gelling capacity, making them suitable for various food applications [40,41]. The amino acid composition of lentil proteins can positively impact human health by maintaining amino acid balance and preventing protein-energy malnutrition [34]. To enhance lentil protein quality, genetic biofortification through conventional breeding and molecular technologies is being explored [34]. As the most rapidly expanding crop for direct human consumption, lentils have significant potential for a greater impact in the plant-based market [35,41].

Finally, the submerged fermentation of lentil protein isolate has been explored as a method to enhance the protein functionality, nutrition and quality of lentil-based products [42]. Overall, the diverse research on lentils highlights their importance as a nutritious food source and their potential for further agricultural and food processing innovations. Figure 2 illustrates the general range of the essential nutritional composition in lentils, which may vary broadly among different lentil varieties [20].

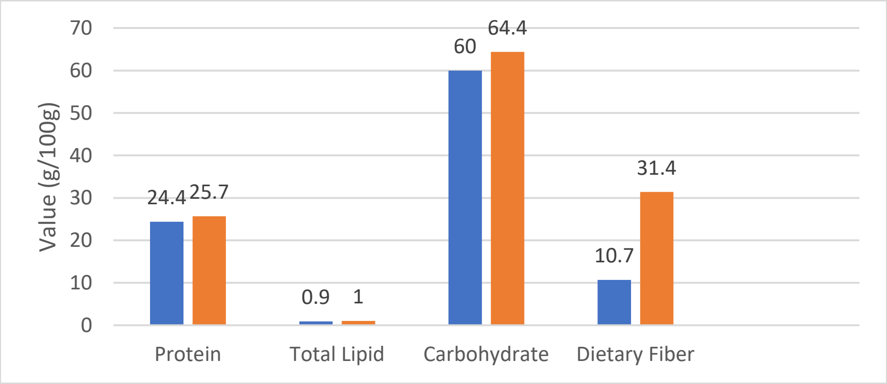


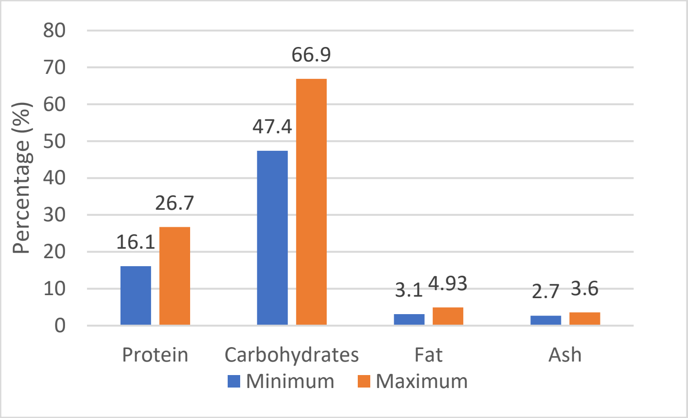
Figure 2. Nutritional composition of lentils [20].

ii. Chickpea

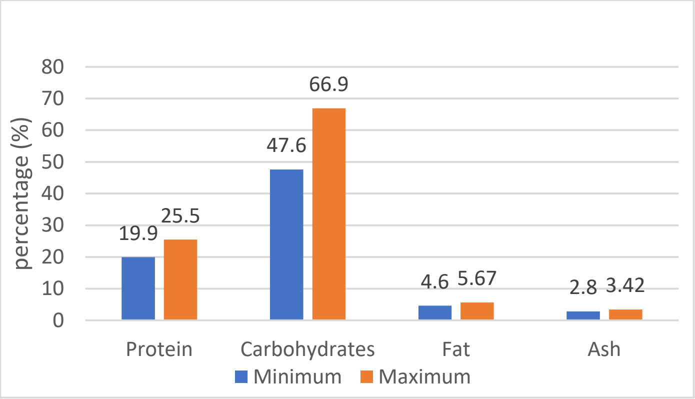
Chickpea (*Cicer arietinum*), otherwise known as garbanzo beans, is a nutrient rich legume with a high percentage of proteins and packed with several health benefits. There are many research studies available on this legume highlighting their protein content and nutritional characteristics [17]. Chickpea can be added to a variety of dishes to meet protein needs and is the most preferred option in the vegetarian diet. Canada, Australia and India make up to more than 40% of global chickpea exports in 2022 [43]. Functionally, chickpeas demonstrate good emulsification capability, better solubility, excellent water and oil absorption capacity and superior foaming and gelling properties [44]. In addition to initial processing, the functionality of chickpea proteins can be well increased during protein-enrichment processes [44]. Being a good source of proteins and carbohydrates, it is also an important source of essential minerals and vitamins. Chickpeas are the most affordable and easily available for developing countries without compromising on nutritional value [45]. When consumed on regular basis, chickpea not only makes up for nutritional deficiencies, but also acts as a functional food for the management of several diseases [46]. The bioactive compounds obtained from chickpea demonstrate anti-hypertensive properties [47]. The proteins derived from chickpea contain a balanced source of key amino acids with a high degree of bioavailability [46]. The protein content of chickpea varies significantly depending on the type and processing conditions. The overall nutritional content of two different types of chickpeas is illustrated in Figure 3, where each kind of chickpea is based on different growing and storage conditions [44].

The impact of dehulling the chickpeas creates a significant change in the protein content. On a dry mass basis, the percentage of protein of Kabuli chickpea was 17 to 22% and 25 to 29% before and after dehulling, respectively [46]. Different studies reveal different percentages of protein and other nutritional contents with little variations [48,49]. Based on Hall's study, the protein composition of dried chickpea ranges between 19 to 27%, with 53 to 60% of Globulins, 8 to 12% of albumins, 3 to 7% of promaline and 18 to 24% of glutelins, respectively [50].

Protein digestibility plays a crucial role in assessing the nutritional characteristic of chickpea protein. Protein digestibility refers to the proportion of protein ingestion and utilization by the human body after consumption. Chickpeas exhibit good protein digestibility, with different research studies showing variations between 48 and 89% [46,51]. Generally, pulses contain rich source of water-soluble vitamins, particularly, vitamin B. Chickpea is an excellent source of tocopherol, folic acid and other water-soluble vitamins including riboflavin, pyridoxine and pantothenic acid and the concentration of these vitamins was found to be higher in chickpeas than in other pulses [46]. Mesfin et al. studied the effect of germination, roasting and variety on the physicochemical, techno-functional and antioxidant properties of chickpea protein isolate powder. It was observed that these treatments had the potential to significantly enhance these properties in the samples with optimized temperature levels [52]. Though there are many research studies exhibiting different processing treatments on chickpeas, further research is still required on the various approaches for the extraction process and other food processing treatments. Overall, the literature indicates a promising interest in understanding and enhancing the protein content of chickpeas through different food-processing techniques. Figure 3a,b represent the nutritional composition of Desi and Kabuli chickpea varieties [44].



(**a**)



(**b**)

Figure 3. Nutritional composition of (**a**) Desi and (**b**) Kabuli chickpea varieties [44].

iii. Dry Pea

Dry pea (*Pisum sativum* L.) is a highly nutritious legume from the *Fabaceae* family containing high levels of protein (20–25%), carbohydrates, vitamins and essential minerals, including 1.04% of potassium, 0.39% of phosphorous (P), 0.10% of magnesium (Mg) and 0.08% of calcium (Ca) [53]. It also contains phytic acid, selenium, micronutrients, saponins, polyphenols, oxalates and water-soluble B vitamins [54–56]. It is considered an excellent meat alternative with low-fat contents in the matured seeds and has low allergenic properties [53]. Dry pea has been cultivated in Europe for thousands of years and is now grown in 84 countries including Australia, Canada, China and the United States [54].

With 14.2 million tons harvested in 2022, dry peas are the third most widely harvested legume worldwide, with an increased global consumption due to their cost effectiveness, sustainability, resourceful efficiency and high protein content [53]. Dry pea is not only useful for human consumption; its empty pods can be used fresh or dried for animal feed stock. Dry pea protein contains a high percentage of significant amino acids such as lysine and threonine but low quantities of sulfur-containing amino acids including methionine and cysteine [55,56]. Albumins and globulins are the two principal components present in the dry pea, while legumin is predominantly present in the matured seeds. On the other hand, albumins account for 15% to 25% of the total protein content in dry peas [53,57]. From a global perspective, the availability of enhanced protein levels through biofortification would aid in satisfying the exceeding protein needs. Through biofortification and breeding processes, dry peas can be transformed into a highly nutritive legume with readily available and digestible protein. Though pea protein exhibits good functional properties, it has some disadvantages including the exhibition of weaker and less elastic gels when compared to soybean protein under food-processing conditions [58]. Therefore, further research is required to improve formulations and applications in food technology and product development. Figure 4 illustrates the nutritional composition of dry pea [59].

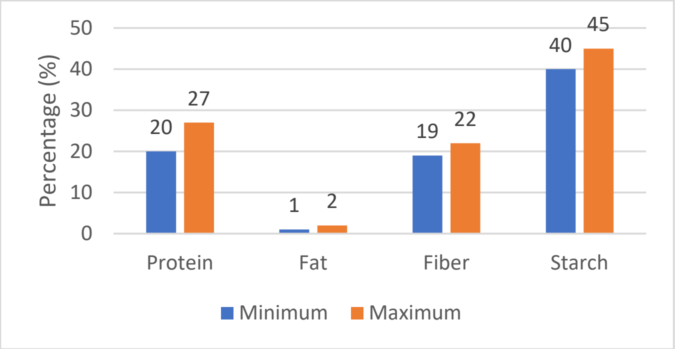


Figure 4. Nutritional composition of dry pea [59].

iv. Black bean

Black beans (*Phaseolus vulgaris*) are a nutritionally rich food source with significant health benefits [21]. Black beans are also known as black turtle beans, belonging to the Fabaceae family, which are most the essential legumes in Latin America and Africa due to their high nutritional components, starch, proteins, fibers, minerals, vitamins and bioactive compounds, including phenolics [60]. They are excellent sources of protein, containing most of the amino acids, particularly, lysine. Black beans have a higher protein content than soybeans, milk, eggs and other similar foods [61]. They are also rich in phytochemicals, which have excellent health beneficial properties, including antioxidant, antidiabetic, anti-inflammatory, antimutagenic activities, anti-obesity, anticancer and hypercholesterolemia effects, and reduce the risk of cardiovascular diseases [23]. The anthocyanins present in the black bean lower the risk of cardiovascular ailments [23]. This high concentration of anthocyanin in the black bean seed coat boosts antioxidant activity and hence, it is consumed as medicinal food in Asian countries including Korea and Japan [23].

Due to their high protein content, between 20% to 25%, black beans are considered an excellent plant protein source [62]. They are packed with well-balanced essential amino acids, with leucine and lysine being the major ones [23]. Studies have demonstrated that a variety of techniques can be employed to produce black bean protein isolates and concentrates, which may enhance antioxidant activity via enzymatic hydrolysis [62,63]. In animal testing, the feeding of black bean protein concentrate to rats exhibited beneficial effects on body composition, glucose metabolism and energy expenditure [64]. According to Li’s study, ultrasound treatment has been found to induce changes in the structural and functional properties of black bean protein isolates, thereby enhancing solubility, foaming and emulsifying properties at low- to medium-power levels [65]. Earlier studies on food processing applications improved the yield and enhanced the techno-functional properties of black bean protein concentrates [66]. Also, polyphenolic black bean extracts demonstrated excellent antioxidant and antiaging characteristics for cosmeceutical applications [67]. These findings suggest that black bean has the potential to have varied applications in different areas; however, further research is needed for its full utilization to produce value-added products. The essential nutritional composition of black beans is depicted in Figure 5 [62].

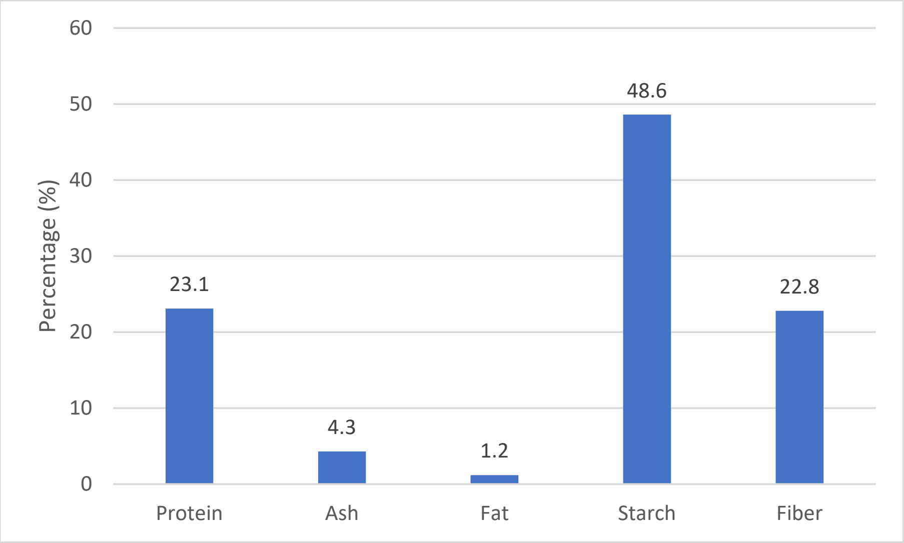


Figure 5. Nutritional composition of black bean [62].

v. Mung bean

Mung beans (*Vigna radiata*) are a nutritious legume widely consumed in Asia, rich in protein, carbohydrates, vitamins and minerals [68]. They are a staple food in many vegetarian diets around the world, particularly in Asia. Known for their versatility and nutritional benefits, mung beans have recently gained attention for their nutritive value, and especially, for their protein content. Mung beans are not only nutritionally beneficial but also environmentally friendly. They require less water and energy to cultivate compared to animal proteins, making them a more sustainable choice. Economically, mung beans are relatively affordable and widely available in many regions, making mung bean protein a cost-effective option for both consumers and manufacturers [68].

Mung bean protein (MBP) has emerged as a promising plant-based protein source, exhibiting high nutritional characteristics and functional properties. Mung bean protein is emerging as a notable alternative to traditional animal-based proteins and other plant-based sources [69]. Mung bean protein has a well-balanced amino acid profile, which contains a variety of essential amino acids including leucine, lysine and valine, making it a complete protein source. The protein content in mung beans typically ranges from 20.97 to 31.32% [70]. Mung beans contain several valuable bioactive compounds such as polyphenols and other phytochemicals including alkaloids, saponins, flavonoids, phenols, glycosides and bioactive peptides, which demonstrate a wide range of medicinally significant properties including lipid metabolism modulation, antioxidant, anti-inflammatory, antinociceptive, antibacterial and anticancer effects [68]. Recent studies highlight the potential of mung beans as an emerging functional food with anticancer properties, requiring further research into their bioactive compounds to establish their functionality [71]. Earlier studies have shown that mung bean protein has a high protein digestibility corrected amino acid score (PDCAAS), indicating that it is well-absorbed and utilized by the body. This is crucial for individuals seeking to maximize the nutritional benefits of their protein intake [72].

Mung bean protein can be extracted through dry and wet extraction methods to derive protein concentrates/isolates and flours respectively [73]. Though mung bean protein has desirable techno-functional properties, its applications in food technology are limited due to poor solubility [73], which have been addressed through physical, biological and chemical methods. This has extended its potential applications in traditional foods and emerging fields, such as microencapsulation, three-dimensional printing, meat analogs and protein-based films [73]. Future research should focus on different processing methods to investigate the full potential of mung bean protein to enhance its commercial and large-scale applications. The essential nutritional composition of mung bean is furnished in Figure 6 [74].

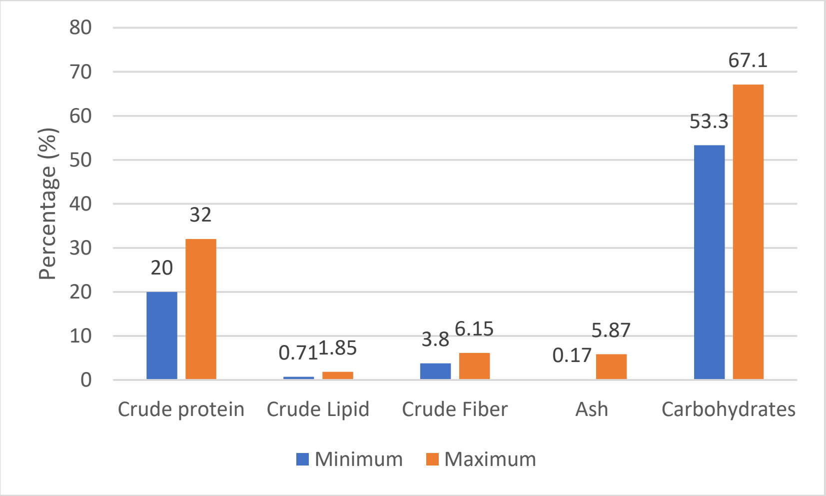


Figure 6. Nutritional composition of mung bean [74].

vi. Soybean

The edible seed of the soybean plant, *Glycine max*, is an annual legume in the *Fabaceae* family of peas. It is a high-quality plant-based protein, which has been used in various food products and supplements due to its rich amino acid profile and nutritional advantages. According to USDA, the global production of soybean for 2023/2024 is estimated as 394.73 million metric tons [75]. Recently, the universal demand for soybean is growing due to the increasing interest towards plant-based protein products [76]. Soybean has a low glycemic index and is high in protein, vitamins, minerals, dietary fiber and other bioactive compounds [76]. Though soy lacks sulfur-containing amino acids such as methionine and cysteine SAA, the amino acid profile is similar to that of milk and whey proteins [76,77], and the quality of soy protein is equivalent to that of egg protein [77]. Recent advancements in processing technologies have significantly influenced the conformational and functional properties of soybean proteins, with potential benefits for both food and industrial applications. Future research should focus on optimizing these technologies and exploring new applications [78]. Figure 7 illustrates the essential nutritional composition of soybeans [76,79,80].

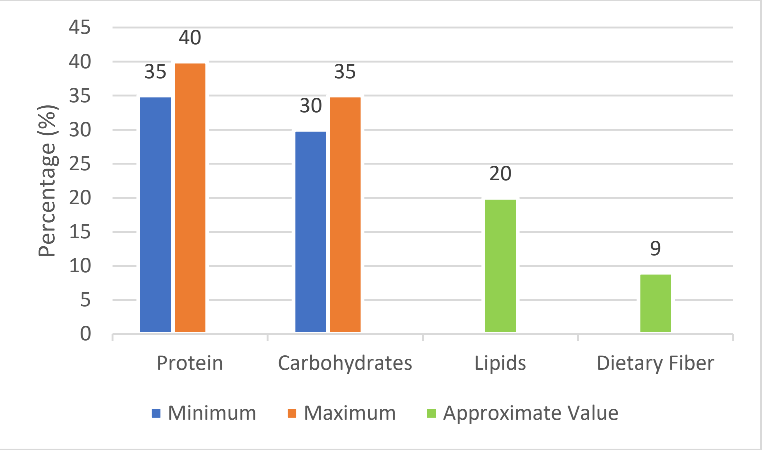


Figure 7. Nutritional composition of soybean [76,79,80].

3. PEF Technology

Pulsed electric field (PEF) technology is a food-processing technique used to apply high-voltage electrical pulses to a substance, typically for industrial, scientific or medical applications. The concept involves applying short bursts of high voltage to create an electric field strong enough to cause changes in the electrical properties of the sample being treated. In biological systems such as microbial cells or plant tissues, the electric field can induce pore formation in cell membranes, a process known as electroporation, to make cells more permeable, thereby allowing substances to pass through the pores or facilitating other biological processes. Some of the specific applications in different areas include wastewater treatment, shelf-life extension of foods through microbial inactivation, PEF-assisted extraction of valuable bio compounds from plant/animal cell membranes, texture and sensory improvement in foods, drug delivery systems, tumor ablation, improvement of germination and seedling growth rate etc. Conventional food-processing techniques including thermal processing methods can degrade several heat-sensitive nutrients in the food samples, whereas PEF can cause negligible thermal effects on these nutrients, thereby potentially preserving the food's original nutritional qualities.

The operation of PEF involves high-voltage pulse application to samples positioned between two electrodes, with one electrode charged to a high voltage and the other will be grounded. It comprises the necessary components including the high-voltage pulse generator, a treatment chamber with two electrodes, an oscilloscope for pulse display, a pump for circulating the liquid food samples (in the case of continuous treatment) and a control unit to optimize and monitor the pulse parameters. Nowadays, all the PEF components are integrated into a single unit and commercialized for food-processing industries as well as for advanced research applications in educational institutions. Figure 8 presents a simplified block diagram illustrating the operation of a pulsed electric field unit where T_1 and T_2 represent variable temperature levels before and after PEF treatment. The untreated sample will be placed inside the PEF treatment chamber prior to the application of high-voltage pulses. In batch treatment mode, the samples will be subjected to high voltage in batches, or they will be continuously pumped and circulated into the chamber for a continuous mode of operation.

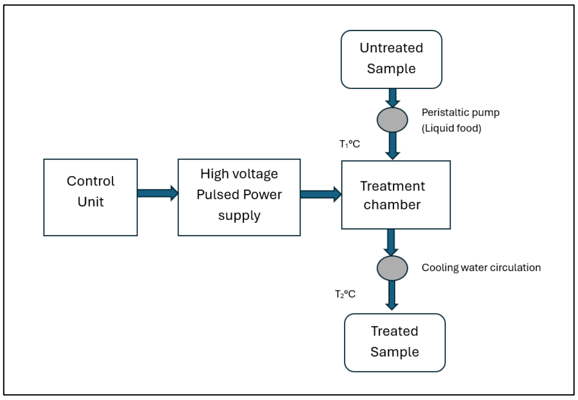


Figure 8. Block diagram of PEF setup (continuous mode).

The pulsed power supply has a pulse generator unit with a pulse-forming network, which delivers the required pulse waveshape to the testing medium within the PEF treatment chamber. Square, exponential and oscillatory pulses are the most commonly used waveshapes while delivering the high voltage pulses. Parallel plate, coaxial and collinear electrode shapes are frequently used in PEF treatment, and the shape can be further optimized for different modes of operation. Parallel plate electrodes generate a uniform electric field in the treatment area, while the coaxial design ensures a consistent flow of the testing sample. The collinear electrodes, on the other hand, allow for the easy passage of the sample through the chamber.

a. Pulsed Electric Field-Assisted Extraction

Efficient extraction of bioactive compounds is a significant factor for the food processing applications. Various extraction techniques have been recently proposed to extract the bioactive compounds present inside the plant cell matrix. Among the existing efficient techniques, pulsed electric field-assisted extraction is gaining greater recognition due to its potential to provide enhanced extraction levels of bioactive compounds at less extraction time, a higher extraction yield and a mild processing temperature. This technique has the potential to be used in variety of plant tissues for prospective industrial applications. Some of the critical parameters influencing the extraction efficiency include the electric field strength, pulse width, number of pulses and frequency of operation. The operation of PEF has been studied elsewhere for more than a decade [81–85]. Ramaswamy et al. reviewed the construction, working principle and operation of PEF in detail with applications in waste plant resources and mushrooms for efficient protein extraction [12]. From the earlier studies, PEF has now become a non-novel technique, since several industrial units have started using PEF for food-processing applications [12,86]. However, further research is still required in optimizing the input parameters and expansion of food varieties to be tested.

In PEF-assisted extraction, the strength of the electric field plays a primary role in enhancing electroporation effects in plant cells, which subsequently increases the extraction efficiency through mass transport mechanism, where the solvent diffuses into the intracellular environment while the solute dissolves out of the cell matrix. In addition to electric field strength, the capacity of the electroporation of the plant cells also depends on other supporting process parameters including the pulse number, pulse frequency and treatment time. When the electric field increases beyond the critical value, transmembrane potential (tmp) will be induced across the cell membrane, which causes the cell to undergo electromechanical stress, leading to the creation of pores. The radius and number of pores will continue to increase depending on the increase in electric field past the critical value. The electroporation thus depends primarily on the electric field application, which has been extensively studied in earlier research discussions [87–89]. Earlier studies that reported enhanced protein extraction based on PEF application include the following. Buchmann et al. observed a significant increase in the protein yield with an increase in electric field in *Chlorella vulgaris* cells, with additional yield based on incubation period after PEF application [90]. Similarly, in *H. Pluvalis*, an increase in extraction rate was contingent upon increasing the electric field strength within a single pulse duration shot [91]. In *Agaricus bisporus* mushrooms, a synergistic effect between the electric field and temperature was observed at the same frequency values, leading to a 49% increase in protein extraction [88]. In another study by Gomez, an increase of 11.29% of the extracted protein was observed at 2.5 kV/cm and 50 kJ/kg at an extraction time of 6 h for this species [92]. In sesame press cake, a significant increase in protein extraction was observed at an optimum temperature of 40 °C, an electric field strength of 13.3 kV/cm and a pulse duration of 10 µS [93]. In papaya seeds, a higher disintegration was observed at 13.3 kV/cm and 2000 pulses, when combined with supplementary aqueous extraction. When compared to HVED (High Voltage Electrical Discharges), PEF + SAE (supplementary aqueous extraction) provided enhanced extraction of proteins [94]. An increase in protein extraction levels was observed in rapeseed stems and leaves, and was influenced by the electric field strength and the plant's growth phase. The electric field caused tissue damage at 800 V/cm, which led to an increase in protein extraction of up to 80% at 20 kV/cm [95]. A higher protein yield of up to 10.58% was observed in the PEF-assisted extraction of perilla seed meal using an electric field strength of 4 kV/cm and a pulse width of 120 µs [96].

Based on the above observations, the impact of PEF process parameters differs for each plant product and the extraction rate is highly dependent on the plant cell type, cell size, rigidity of the cell wall, cell concentration, properties of the growth medium (as applicable to microalgal cells) and environmental conditions.

b. Pulsed Electric Field treatment on Pulses and Legumes

So far, pulsed electric field has been used in different food products, including solid and liquid foods, for different food-processing studies. Several earlier studies have reported the usage of PEF for food-preservation applications intended for the inactivation of microorganisms [97–101], the inactivation of spores and enzymes [102], extraction studies including the extraction of proteins [91,103–105], polyphenols [106–110], carbohydrates [110], lipids [111,112], anthocyanins [113,114], carotenoids [115–117] and flavonoids [118,119], drying applications [120–122] and in combination with other food processing techniques including ultrasound, HHP, Thermal treatments, ohmic heating, microwave treatments, etc. [93,123–126].

Various food products were tested for PEF extraction studies that reported a higher yield of bioactive compounds. The author provides a non-exhaustive list of food products, that were tested in earlier studies including sesame seed cake, papaya seeds, grape seeds, date palm fruits, coco bean shell, peach byproducts, brown rice, plum, grapes, grape peels, papaya peels, apple peels, potatoes, tomatoes, carrot, blackcurrant, pomegranate peels, custard apple leaves, blueberry fruits, brewers' spent grains, etc. [93,94,127–140]. Though the establishment of PEF technology has a vast array of lab-scale and industrial applications, there are many unknown parameters and food products to be analyzed, tested and understood to gain deeper insights. Pertaining to the above sentence, pulses and legumes are the least explored area with limited PEF studies when compared to fruits and vegetables.

PEF technology has been widely studied in the food industry for its potential to improve food processing and preservation methods. Though studies are limited, PEF has shown appreciable reports in extraction levels and demonstrated improvement in the overall nutritional quality of legumes and legume-based products. Lavaraj Devkota et al. studied PEF-assisted hydration in dried bean processing of *Phaseolus vulgaris* and analyzed the hydration behavior, mass loss and leaching of bioactive compounds. The beans were PEF-treated at an electric field strength of 4 kV/cm, frequency of 2 Hz, pulse width of 15 µs and pulse numbers from 200 to 1000 and further hydrated until equilibrium moisture content was reached. PEF treatment resulted in higher water intake with the larger number of pulse application, reached higher equilibrium moisture content and was faster than thermal hydration method. This may be attributed to the strong electroporation effect on the seed coat enabling changes in the textural properties of bean. Additionally, it was understood that the suitable optimization of parameters will lead to modification in leaching of bioactive compounds [141]. Andreou et al. investigated the impact of pulsed electric field (PEF) pretreatment on the rehydration kinetics, firmness and release of the intracellular components of dried chickpeas from 35 to 65 °C. Chickpeas were subjected to PEF treatment at 2.5 and 3.3 kV/cm, an energy range from 0.2 to 12.0 kJ/kg, a 15 µs pulse width and 20 Hz frequency. PEF treatment resulted in up to 70% higher rehydration rates of dried chickpeas and decreased firmness by 30% during the rehydration process, when compared to untreated chickpea samples. This was achieved at a very short rehydration time of up to 30 min compared to 300 min for untreated samples. After PEF-assisted rehydration, more than 47.7%, 76.1% and 86.6% of total raffinose, stachyose and verbascose have been extracted. However, only 0.03% of proteins were extracted from PEF-treated chickpeas [142]. Anne K. Baier et al. studied the potential of high isostatic pressure and pulsed electric fields to improve mass transport in pea tissue. Both treatments resulted in changing the cell-wall structures and improving mass transport in comparison to the control samples, thereby preserving more than 99.9% of the nutritionally valuable protein. The drying and rehydration rates of whole peas were increased to a higher extent by HP and PEF than by conventional thermal treatments [143]. Nguyen studied the impact of pulse electric field on the total phenolic, isothiocyanate and radicle elongation of mung bean sprout. The parameters, including pulse electric field strength and pulse number, resulted in increased isothiocyanate and total phenolic contents while retarding the root length extension. On the other hand, the pulse width reduced the total phenolic and isothiocyanate contents while extending the root length. Hence, it was understood that the suitable parameter combination should be maintained to increase phenolic and isothiocyanate contents while keeping the radicle length reasonable [144]. With reference to soybeans, PEF induced a denaturation effect in soy protein isolate at >30 kV/cm [145]. The purpose of inhibiting the enzyme reaction is to prevent product deterioration, which would have a significant impact on the quality and shelf life of the product. In soymilk processing, elimination of enzymic off-flavor is crucial, and these off-flavors are caused by soybean lipoxygenases. According to Li's study, the application of PEF at a 42 kV/cm electric field strength for 1036 µs, 400 Hz of pulse frequency and 2 µs of pulse width at 25 °C resulted in the maximum inactivation of soybean lipoxygenase by 88% [146]. From all the above studies on legumes and pulses, it is understood that though there is a high availability of these economic protein resources, only a few analyses have been made on bioactive compound extraction from these legumes, and there is a lack of complete understanding of the legumes' extraction potential. It is a known fact that legumes play a significant role in terms of having high nutritional value and easy availability. This necessitates further research on utilizing these economic resources for efficient protein extraction to promote environmental and economic sustainability and to generate value-added products.

4. Long Term Challenges in PEF

Scaling up the existing lab-scale systems to industrial scale is one of the significant challenges as it requires extensive design efforts, an industrial setup and heavy cost commitments. Due to these drawbacks, the availability of industrial PEF units is limited worldwide. However, PEF manufacturers including Pulsemaster from The Netherlands and Elea Technology GmbH from Germany have achieved this huge transformation and successfully commercialized the PEF systems and exported them to several countries including Canada (Potatopro), Italy (Amica Chips) and New Zealand (McCain Foods). Though the capital cost of the PEF system is high, subsequent processing costs would be lower, which would render PEF research a valuable contribution to society. However, the initial investment would be the greatest challenge in moving the research establishment to an advanced stage. Though PEF is an energy efficient process, it can consume substantial amounts of electrical energy when operated on a continuous large-scale manner. Hence, managing energy consumption and optimizing the overall process to minimize costs is an essential consideration here.

Another major challenge in PEF treatment is the occurrence of electrochemical reactions, leading to electrolysis. This drawback may be possible after the heavy usage of electrodes while delivering the high voltage electric pulses, leading to the degradation of electrode material. The negative impact of electrolysis includes corrosion on the electrode surface and the migration of metallic ions from the electrode, which gets mixed with the food samples during the treatment, causing chemical changes in the food. Some of the earlier studies suggested ways of minimizing the electrolysis effects in PEF, including the usage of bipolar pulses, the maintenance of minimum peak voltage, a decrease in the pulse duration and non-invasive PEF application [147–150]. Another disadvantage is the presence of gas bubbles during PEF application, which would disrupt the uniformity of the electric field in the treatment area, thereby causing operational concerns [151]. Temperature fluctuations may be a contributing factor for gas bubble formation. The presence of gas bubbles causes the electric field intensity to decrease near the bubble edge, leading to significant perturbation in the electric field uniformity when the bubble formation increases, thereby increasing the possibility of dielectric breakdown in treatment chambers [152].

Process parameter optimization is a significant challenge for the allied research groups in extracting proteins from different plant products. Each plant product would exhibit different physical and chemical properties which would alter plant tissue characteristics when subjected to PEF treatment. Hence, different optimization levels could be adopted for each type of plant matrix to obtain desirable protein extraction levels. While PEF can enhance certain beneficial properties of food products, it may also have contradictory results, including changes in the texture or nutritional content if each parameter change is not in line with desired product characteristics, and hence, careful optimization is needed to achieve the best outcomes. Moreover, developing this research area to a larger scale requires prior validated research reports based on PEF applications specific to each protein source and sufficient expertise in the area to ensure the safe operation of PEF.

5. Conclusions and Future Research Direction

Proteins are essential biomolecules composed of amino acids linked together by peptide bonds. Proteins are crucial for the growth, repair and maintenance of tissues and organs. Recently, consuming animal proteins has led to adverse health effects, resulting in an escalated interest towards plant protein consumption. Many plant protein sources have an abundant protein composition equivalent to that of animal proteins. When these protein molecules are extracted, they can be used for several health applications in the form of value-added products. While conventional extraction techniques involved the high usage of chemicals and heat, emerging extraction techniques developed as an alternative to improve the quality of food products and to enhance the extraction potential in a shorter treatment time. Pulsed electric field is one of the techniques to assist the extraction process to enhance the expulsion of intracellular components including proteins from the plant cells. Pulsed electric field (PEF) treatment can help to improve the extraction, solubility and functionality of protein from various plant sources. For the past few decades, PEF has been applied to various plant sources including vegetables and fruits. Though there are many promising results available from PEF treatment, very few extraction studies have been performed on legumes. Since legumes are nutritionally rich sources and economically affordable, there is a need to perform extensive research in future to extract valuable proteins from them. In this way, the PEF-assisted protein extraction research can possibly aid to meet the ever-rising protein demand in an economical manner for long-term prospects.

Based on the above review, the author has listed the challenges and suggested future developments as follows:

1. As only limited research is available on PEF-assisted protein extraction in legumes, further studies are highly encouraged to explore these protein sources in order to promote this research for large-scale processing.
2. Dry legumes have a thick seed coat, which should be soaked in water before it is processed. Hence, process parameters should be carefully optimized to improve the extraction of valuable biological compounds, in a way that does not induce thermal effects in the product.
3. In order to gain deeper insights into PEF-assisted protein extraction from legumes, structural and functional characteristics and the complex composition of each kind of legume should be studied for efficient PEF application to obtain desirable protein extraction levels.
4. Research should also be executed by combining other green-processing techniques with PEF to obtain a higher yield.
5. Research on electrolysis is at an amateur stage in extraction studies and can be further deepened to minimize the effects in the final byproducts.

Author Contributions: Conceptualization, R.R. and S.B.K.; formal analysis, R.R.; investigation, R.R. and S.B.K.; writing—Original draft preparation, R.R.; writing—review and editing, S.B.K.; supervision and funding acquisition, S.B.K. All authors have read and agreed to the published version of the manuscript.

Funding: This work is supported by Singapore Institute of Technology (SIT) under the Food Extraction Programme.

Data Availability Statement: No new data were created or analyzed in this study. Data sharing is not applicable to this article.

Conflicts of Interest: The authors declare no conflicts of interest.

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