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Review

Pulsed Electric Field Technology for Recovery of Proteins from Waste Plant Resources and Deformed Mushrooms: A Review

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Abstract: Proteins are complex molecules, which play a vital role in our body's function, the building of tissues, and the regulation of metabolic activity. They are crucial to children's growth and serve as a key component in the body's process of distributing oxygen. Proteins fuel the body by supplying the required nutrition and energy. Currently, there is an increasing demand for proteins on large scales with no detrimental effects. The adverse health effects of animal proteins have resulted in a growing preference for plant-based proteins, which offer a healthier daily dosage. Valuable proteins can be extracted from various parts of the plant, including stems, leaves, seeds, fruits, vegetables, and roots. Notably, protein extraction from waste plant and mushroom parts minimizes the product wastage and improves the overall production to support economic sustainability. There are several protein extraction techniques available, where the replacement of non-thermal methods with thermal ones is promising nowadays due to the appreciable retainment of protein quality. Pulsed Electric Field (PEF) technology is one of the most efficient non-thermal tools used to assist with extracting these proteins at the minimum processing time and energy consumption when compared with thermal techniques. It relies on the application of a high-voltage pulse between two electrodes to treat samples inside the treatment chamber. While electrode shapes and treatment chamber designs primarily govern the electric field's application, optimizing process parameters such as electric field strength, pulse width, number of pulses, and pulse waveshape assists in obtaining a desirable enhancement in the protein yield. The primary objective of this review is to explain the PEF-assisted protein extraction process applicable to waste plant parts and deformed mushrooms. While PEF is not a novel concept, utilizing it as a pre-extraction treatment to the aforementioned waste resources would aid in improving the production of value-added protein products economically. So far, PEF has shown immense promise in assisting with protein extraction studies, but requires further research in order to establish this area for large-scale industrial applications.

Keywords: pulsed electric field; protein extraction; waste plant resources; deformed mushroom parts

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1. Introduction

Based on the Food and Agriculture Organization, around one third of the food produced for human consumption is wasted globally, i.e., 1.3 billion tons per year [1]. According to the United Nations Environment Programme's Food Waste Index Report, 17 percent of our food ends up being wasted in retail and by consumers, particularly in households [2]. For instance, India is among the countries that remains high in the production of fruits and vegetables. However, it suffers from 40% wastage of total food production. In that, fruits and vegetables contribute to 18% wastage of total food production [3]. These wastes comprise leaves, peels, stems, roots, and seeds, which have been found to have high nutritional value and contain a substantial amount of valuable bio-compounds, including proteins [4]. Upon extraction, the byproducts of these wastes can

be efficiently utilized in food processing and pharma industries to produce nutritional and pharmaceutical supplements.

Several research studies have observed the significance of the protein requirement to the body [5,6]. This requirement is increasing globally due to the high population growth and the awareness of protein's positive impact on the body. Research studies indicate the Recommended Dietary Allowance of protein to be 0.8 g per kg body weight (BW) per day since insufficient protein intake can lead to anemia, physical weakness, stunting, edema, vascular dysfunction, and impaired immunity [7]. Dietary recommendations from other countries, including the joint FAO/WHO committee, do not significantly deviate from the US recommendations for macronutrient intake [8,9]. In the case of insufficient protein intake, protein supplements can provide dietary compensation. Hence, it is necessary to consume a protein-rich diet every day since protein's nutritional benefits are decided by both the quality and quantity. It has been reported that sufficient consumption of plant proteins may reduce the risk of all-cause and cardiovascular disease mortality and may increase longevity [10]. Thus, proteins play a significant role in providing nutritional value to foods and act as thickening, stabilizing, emulsifying, foaming, gelling, and binding agents.

Over the past few decades, a consistent increase in the demand for proteins has been observed due to multiple health benefits. With a global population of 7.3 billion, the demand for protein consumption has increased to approximately 202 million tons [11]. This demand may exceed the protein supply in future decades if the available resources are not efficiently utilized. This has led to the search for plant-based protein resources since the perception of the negative health effects of animal proteins has escalated. This extensive search is carried out with the objective of producing value-added products from the plant protein isolates in order to meet the ever-increasing protein demand. In order to improve the socioeconomic viability and to minimize product wastage, research is currently in the phase of extracting proteins from waste plant resources such as vegetable peels, fruit peels, seeds, stems, and roots from the processing industries. When compared with animal-derived waste, plant-derived waste makes up 63% of the overall food supply chain [12]. Globally, huge quantities of mushroom and plant pieces are discarded from packaging industries since they do not meet the required standards for size and shape regulations. These rejected or wasted plant parts, which are excellent sources of proteins and other necessary bioactive compounds, will typically be disposed of or utilized using conventional practices [13]. Here, valorization can play a major role in the efficient utilization of these resources to produce value-added products and minimize the overall wastage. Figure 1 illustrates the valorization of waste plant resources [14].

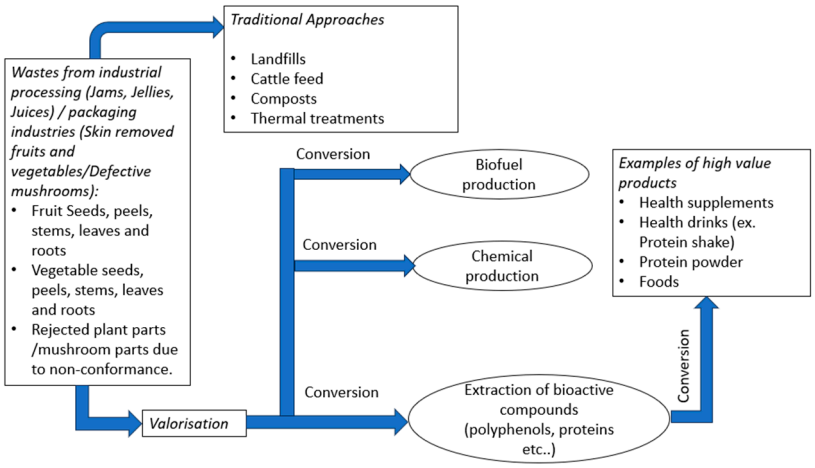

Figure 1. Valorization of waste plant/mushroom resources (reproduced with permission from Elsevier, license no. 5670740416741).

Plant waste valorization is the process of transforming plant wastes into higher-value products by converting them into chemicals or fuels or extracting them to produce

nutrient-rich byproducts or supplements. The significant advantages of plant wastes include (i) ease of access, (ii) sustainability, and (iii) biodegradability [15]. Pertaining to the global protein requirement, efficient protein extraction techniques are prioritized to meet the rising demand for high-quality proteins. The food industry has constantly explored pathways to the extraction of plant-based proteins using several conventional processing methods, such as Soxhlet extraction, thermosonication, heat reflux, maceration, bead milling, mechanical grinding, ultrasonic treatment, osmotic treatments, and acid and alkaline pretreatments [16–19], which involve solvent usage, chemicals, heat, and the heavy mechanical disruption of plant cells, resulting in a requirement for additional processing due to purification and the low extraction efficiency. These poor results depend on the size of the testing particle, the usage of solvents, the pH value, the extraction time, and the temperature, which eventually degrade the quality and affect the functional properties of extracted proteins. Emerging non-thermal methods are gaining recognition due to their eco-friendly nature, consumption of less energy, and generation of minimal to no thermal effects on the extracted bio-compounds. These methods do not have an environmental impact and preserve the overall quality and nutrition of the resulting proteins. The application of a Pulsed Electric Field (PEF) is supposed to be a good alternative to thermal treatments in order to overcome the undesirable degradation in the protein quality. This non-thermal technique has been used for several years to inactivate food-borne pathogens in order to extend the shelf life and preserve the quality of foods [20–22] throughout their storage time [23,24]. When compared with other thermal food processing methods, PEF preserves the nutritive value of foods with the minimum degree of damage [25–27]. Initial PEF studies were reviewed by multiple authors [28–36], which aided in the development of PEF into having a global reach. The gradual scaling-up of lab-scale to industrial systems has led to developments in the design of high-voltage pulse generators that provide higher-end performance to suit PEF industrial processing [37,38]. Also, the optimization of electrode shapes and chamber designs have overcome the existing drawbacks of electric field enhancement and dielectric breakdown in foods [39]. Due to the myriad advantages of PEF, recent research has focused on using the technique in protein extraction procedures, where the finally derived protein isolates would be used for producing value-added products including health supplements and nutraceutical and pharmaceutical products. Though PEF enables physiological, functional, and enzymatic changes in proteins, the area still requires further insights into the factors causing these changes in order to promote them for industrial processing.

This review discusses protein extraction from waste plant resources and deformed mushrooms using PEF treatments. This paper covers waste resources, protein extraction methods, the Pulsed Electric Field technique, PEF-assisted extraction of proteins, optimization of the PEF-assisted extraction process, industrial processing, existing challenges, and future directions.

2. Waste Resources

2.1. Plant Wastes

Protein is the most significant macronutrient and is essential to our daily survival needs. Though animal proteins, including meat, fish, milk, and eggs, are rich in essential amino acids, minerals, and vitamins, they use extensive land and feed resources. Also, animal proteins cause adverse health effects that nowadays necessitate low-cost alternative protein sources [40]. Plant protein sources have several advantages, such as a lower cost of production, ease of access, minimal maintenance, and better environmental sustainability. Developing countries rely on plant proteins as they are more economical than animal proteins [9]. Specifically, when the proteins are extracted from waste plant resources, such as seeds, stems, peels, and roots, the extraction of proteins can be made more economical. Vegetable and fruit processing industries generate a huge amount of plant waste (about 90 to 92%) [41]. This leads to a high percentage of wastage at a global level. Plant wastes can be recycled for value-added purposes, thereby reducing environmental pollution and

enabling sustainable green development. Some examples of nutrient-rich industrial plant wastes include papaya seeds, sesame press cake, olive pomace, flaxseed hulls, tomato residues, and brewer spent grains. These wastes will be dumped in the garbage, landfilled, or used as cattle feed. Selva Ganesh et al. reviewed the downsides of excessive vegetable and fruit waste and discussed the emerging opportunities to generate value-added products from these wastes [42]. They have also indicated the ever-increasing level of global food wastage and have recommended that we evaluate efficient waste management techniques to valorize and minimize wastage in the long term [42]. In this regard, the accurate monitoring of plant waste is necessary in order to minimize overall wastage. Although plant wastes are produced in all stages of the fruit and vegetable manufacturing chain, recovery and recycling are essential factors to be considered to improve sustainability. The recycling of plant wastes for the recovery of bioactive compounds to produce value-added products minimizes the environmental stress in the global scenario. Table 1 refers to examples of protein-rich plant wastes that can be used for ongoing protein extraction studies.

Table 1. Protein contents of plant wastes.

S.No	Plant Wastes	Protein (%) *	Protein (g/100 g) **	Ref.
1	Sesame Press Cake	35–50	X	[43]
2	Papaya Seeds	27.3–28.3	X	[44]
3	Olive pomace	4.51 (Crude)	X	[45]
4	Flaxseed hulls	17.45–19.14	X	[46]
5	Tomato wastes (i) Pomace (ii) Peels (iii) Seeds	 15.1–22.7 5.7–20 16.6–39.3	 X X X	[47]
6	Brewer's spent grains	26–30	X	[48]
7	Cabbage leaf residues	17.0–17.2 (Crude protein on DM*)	X	[49]
8	Potato peels	13.02 (dry powder)	X	[50]
9	Apple pomace	4	X	[51]
10	Orange peel waste	7.7	X	[52]
11	Rapeseed meal	36	X	[53]
12	Pumpkin peel	1.65	X	[54]
13	Ripe Banana peel	5.5–7.87 (Crude)	X	[55]
14	Pomegranate peel	4.90–8.97	X	[56]
15	Inodorus melon peel	34.90	X	[57]
16	Sweet potato peel (i) Primary peel (ii) Blanched peel	 6.40–6.49 8.11–8.20	 X X	[58]
17	Pumpkin seeds	~35 (Crude)	X	[59]
18	Wheat Bran	13.2–18.4	X	[60]
19	Rice bran	10–16	X	[61]
20	Outer scales of onion	2.64	X	[62]
21	Almond Husk	X	3.27 (Crude)	[63]
22	Brewer dry grain	X	19.96 (Crude)	[63]

* Representation of protein quantity in %. ** Representation of protein quantity in g/100 g. X, not applicable. DM*, Dry Matter.

For a protein source to be considered valuable, it must have substantial protein content with an essential amino acid composition [64]. From Table 1, plant wastes such as sesame cake, papaya seeds, brewer spent grains, rapeseed meal, and inodorus melon peel contain a higher percentage of proteins than other resources that, if extracted, would be beneficial in producing value-added products in an economical manner. Effective valorization of these plant wastes can improve food safety, security, and sustainability in food production.

2.2. Deformed Mushroom Wastes

Mushrooms have the potential to expel large quantities of proteins when extracted. Mushrooms belong to the fungi family, which has a 'plant-like' structure consisting of stems and heads lacking chlorophyll. Edible mushrooms are low-calorie, no-fat, and no-cholesterol foods that are rich in protein, fibers, and antioxidants. The protein quality of mushrooms has been found to be superior to that of plant sources. The nutritional value of edible mushrooms is primarily due to their protein content, consisting of all essential amino acids required to meet each day's dietary requirements, and they are more economical than plant and animal sources [65]. Generally, edible mushrooms have a protein level ranging from 6.60 to 36.87 g/100 g dry weight, with an average of 23.80 g/100 g [66]. The amino acid composition of the proteins in mushrooms is equivalent to that of animal proteins and, hence, they are considered to be more valuable than plant proteins [67,68]. However, different mushroom species will differ in terms of the overall amino acid composition [69]. Based on earlier studies, the protein digestibility of mushrooms ranges from 60% to 70% [70], which can be further improved by eliminating the components that prevent proteins from being hydrolyzed in the gastrointestinal system [70]. Additionally, mushrooms have a significant role in preventing and suppressing disease-causing factors [71].

Mushrooms are consumed widely due to their excellent organoleptic properties and are considered to be novel protein alternatives [65]. Due to their flavor-enhancing and nutritional characteristics, the demand for mushroom consumption is increasing every year. Mushrooms possess antiviral, anti-inflammatory, antifungal, antithrombotic, and antimutagenic functional properties, which are used extensively in foods and medicinal formulations [72–75]. Around 2000 species of mushrooms exist in our environment but only a few species can be used for edible purposes [76]. The most widely grown edible mushrooms are *Agaricus bisporus*, *Lentinula edodes*, and *Pleurotus* species, which are produced primarily in China [77]. The consumption of edible mushrooms on a per-person basis increased by approximately fourfold between 1997 and 2013 [78]. In addition, it is anticipated that the global production of mushrooms will increase to 20.84 million tons by 2026 [79]. On the other hand, the edible mushroom industry's accelerating growth and increasing production has led in parallel to a regrettable level of wastage (60 million tons of mushrooms per year) [80].

In the overall wastage, large quantities of misshapen parts are produced, whose size and shape do not comply with commercial standards. Since these misshapen mushroom parts are still edible, they are used as cattle feed to overcome wastage. It is noted that these misshapen parts are rich sources of carbohydrates, proteins, vitamins, minerals, and healthy fats. Unfortunately, there are few studies on mushroom-extracted bio-valuable compounds [80].

It is a known fact that proteins play a significant role in providing adequate nutrition to the body, and mushrooms are considered to be highly valuable sources of proteins in the human diet. Hence, in order to not neglect these valuable nutritional sources and to overcome food and nutrient wastage, extraction is a wise approach to addressing food sustainability and nutritional quality issues. Due to the extensive demand for alternate protein sources these days, rejected mushroom parts have been found to be an economic resource to boost protein production. Thus, the functional properties of rejected mushroom parts can be improved by extracting valuable proteins from them and processing them to produce food, medicinal, and health supplements. Numerous studies have projected the health benefits of mushrooms due to their medicinal properties. Table 2 lists the

commercially cultivated mushroom varieties that have been recommended for protein extraction studies. When these mushrooms are transported to packaging and processing industries, large quantities of rejected waste would be generated, which are not only edible but also have numerous medicinal uses.

Table 2. Properties, health benefits, and protein content of commercially cultivated mushrooms.

Mushroom Species	Protein Content (%)	Nutritional Properties	Medicinal Properties	Reference
Agaricus Bisporus	29.64–39.84	• Rich in carbohydrates, amino acids, fats, and minerals	• Used in the treatment of cancer, bacterial and fungal infections, diabetes, heart disorders, and skin problems	[65,81]
Pleurotus: *Pleurotus ostreatus* *Pleurotus eryngii* *Pleurotus sajor-caju* *Pleurotus giganteus*	 7 11 37.4 17.7	• Rich in proteins and fiber, low in carbohydrates, fats, and sodium	• Used to treat atherosclerosis. Other properties: • Antimicrobial • Antiviral • Anticancer • Antioxidant • Hypolipidemic • Hypocholesterolemic • Antihyperglycemic • Immunomodulatory effects	[82–84]
Morchella esculenta	32.7 (DW)*	• Rich in protein, fiber, and necessary vitamins and minerals. Low in calories and fat	• Antioxidative • Immune protective • Anti-inflammatory • Anti-colon cancer effects	[85,86]
Lentinula edodes	15–23	• Low lipid contents • Rich in protein, vitamins, and minerals. • High concentrations of calcium, iron, phosphorus, potassium, zinc, manganese, phenolic compounds, and glucans	Medicinally used for the following diseases • Depressed immune function (including AIDS) • Cancer • Environmental allergies • Fungal infection • Frequent flu and colds • Bronchial inflammation • Heart disease • Hyperlipidemia (including high blood cholesterol) • Hypertension • Infectious diseases • Diabetes • Hepatitis • Regulating urinary inconsistencies.	[87–89]
Auricularia heimuer	10.62	• Plentiful in several amino acids, especially leucine and lysine. • High in protein, trace element, vitamin, and carbohydrate content • Low in fat	• Anticancer • Detoxifying Anticoagulant • Hypoglycemic • Cholesterol-lowering capacity	[90]

Table 2. *Cont.*

Mushroom Species	Protein Content (%)	Nutritional Properties	Medicinal Properties	Reference
Volvariella bombycina Fruit Body Mycelia	 28.30 25.50	• Rich in proteins, carbohydrates, vitamins, and minerals	• Resistance to ovarian, lung, breast, and prostate cancer	[91]
Enoki mushrooms: *Flammulina filiformis*, *Flammulina velutipes*	5	• Rich in carbohydrates, proteins, unsaturated fatty acids, many valuable micronutrients, and dietary fiber • Highly comparable to vegetables	• Anti-inflammatory activities	[92,93]
Tremella fuciformis	9.63	• Contains large quantities of vitamin D, proteins, vitamins, minerals, immune-boosting polysaccharides, trace minerals, and carbohydrates • Low in fat	• Used medicinally for cough, sore throat, stethalgia, constipation, and paramenia	[94–96]
Hypsizygus tessellatus,	33.9	• Rich in proteins, fiber, carbohydrates, potassium, calcium and phosphorous	• Has organoleptic and pharmacological properties, • Anticancer properties	[97,98]
Stropharia rugosoannulata,	30−50	• Rich in proteins, peptides, and amino acids	Essential Properties: • Antidiabetic • Antimicrobial • Antioxidant • Antiproliferative • Antitumor • Immunomodulatory and osteoclast-formation-suppressing properties	[99,100]
Cyclocybe aegerita	37.6	• Rich in carbohydrates, ash, and proteins	Essential Properties: • Antitumor, antioxidant, and antifungal • Hypocholesterolemic and hypolipidemic effects	[101–103]

Table 2. *Cont.*

Mushroom Species	Protein Content (%)	Nutritional Properties	Medicinal Properties	Reference
Hericium erinaceus	Dried powder, 20; Mycelia, 42	• Enriched protein and dietary fiber content and low in calories and fat	Essential Properties: • Antibiotic • Anticarcinogenic • Antidiabetic • Antifatigue • Antihypertensive • Anti-hyperlipodemic • Antisenescence • Cardioprotective • Hepatoprotective • Nephroprotective • Neuroprotective properties • Improvements in anxiety, cognitive function, and depression.	[104,105]
Phallus Indusiatus	4.813	• Contains very high water, crude fiber, protein, fat, carbohydrate, ash, and vitamin C content.	• Improves cardiovascular health • Reduces the risk of cancer • Promotes immune function • Prevents viruses, bacteria, and fungi • Reduces inflammation • Combats allergies • Helps to balance blood sugar levels • Supports the body's detoxification mechanism	[106]

DW*, Dry Weight.

Mushrooms are a great source of nutritionally valuable compounds, including proteins, lipids, polysaccharides, polyphenols, micronutrients, and vitamins. Rosello et al. describe the potential use of some novel non-conventional methods, including enzyme-assisted extraction, pulsed electric field, ultrasound, microwaves, and subcritical and supercritical fluid extraction, for the recovery of valuable compounds from mushrooms [107]. PEF technology is one such essential tool that can improve the functionality, extractability, and recovery of nutritionally valuable compounds as well as the bioavailability of micronutrients and components in a diverse variety of foods. To exploit mushroom extracts for various applications in food, pharmaceutical, and nutraceutical industries, they need to be characterized for their bioactive compounds through several extraction processes. The residues from the protein extraction can be further valorized into valuable products based on their potential, such as prebiotics, dietary fiber, and compost. Several research studies on mushrooms have provided a sustainable solution in the valorization of mushroom byproducts to produce valuable functional ingredients with health benefits, especially for the gastrointestinal system [108,109].

3. Protein Extraction Methods

An extraction process is described as the recovery of valuable bioactive compounds from plant cells by means of a mass transport phenomenon, where the intracellular components of the cell are transmitted to the solvent [110–112]. The stages of an extraction process typically include penetration of the solvent into the plant matrix, dissolution of solutes in the solvent, diffusion of the solute out of the matrix, and collection of extracted solutes [113]. Since these extracted solutes are bioactive compounds for which a high degree of purity

is preferred, it is necessary to find efficient extraction techniques that are quick, easy, safe, and reliable, provide better yields, and, most importantly, require a minimal number of purification steps. Existing conventional protein extraction methods, including maceration, percolation, heat reflux extraction, and Soxhlet extraction, are widely adopted due to their simple operational techniques. However, these methods use large volumes of solvents and heat, have a long processing time, and are inefficient [114]. Under these methods, increasing the temperature increases the solubility and diffusion, leading to increased extraction. However, the huge rise in the temperature leads to the extraction of other undesirable impurities, which require additional processing for purification. While we discuss a few examples, alkaline extraction is an inexpensive chemical extraction method in which protein dissolution occurs in an alkaline medium, with the disadvantage of protein denaturation under highly alkaline conditions during the extraction procedure [115]. Also, the increase in temperature and extraction time induces thermal precipitation and denaturation effects that may degrade the quality of proteins [115]. Enzyme-assisted extraction is a biochemical method that relies on the use of enzymatic breaking of the rigid cell wall to boost the protein yield. Though the enzyme usage maintains the optimal pH in order to minimize protein denaturation, the process is an expensive one for industrial applications and the enzymes cannot achieve complete cell breakdown [116].

Since the primary goal is to achieve an enhanced yield in a shorter extraction time without affecting the protein quality to a high degree, the selection of the extraction technique is essential. As discussed earlier, conventional extraction methods have a long extraction time, have a high solvent requirement, cause increased damage to cells, and have low extraction efficiency. Modern extraction methods, including ultrasound-assisted extraction, high-voltage electrical-discharge-assisted extraction, high hydrostatic pressure extraction, and pulsed electric field-assisted extraction, are crucial in preserving a sustainable food chain by the recovery of essential bioactive compounds from plant wastes. These ecofriendly methods have several advantages, such as a minimal temperature increase, less solvent usage, a short extraction time, a quicker process, less damage to the plant matrix, and a higher quality of extracted products [117–120]. This can be made possible by optimizing the extraction parameters throughout the experimental approach. These physical methods of cell degradation provide enhanced protein yields with minimal or no usage of solvents and heat with no environmental impacts. Although research on these techniques is promising, large-scale applications would require a long period of time [121]. In the following section, we discuss the Pulsed Electric Field technique, which works under the phenomenon of electroporation. This method has shown favorable extraction qualities with minimal damage to the plant cell matrix.

4. PEF Technique

4.1. General Features

PEF is a non-thermal technique that uses ultrashort electric field pulses in various applications such as food processing, tissue ablation, electrochemotherapy, and electro-extraction processes [20,122–124]. In food applications, PEF has been used in microbial inactivation studies, the extraction of bio-compounds, assisting with the drying process, and oil extraction [125–128]. While applications in microbial inactivation were initially invented and earned a greater amount of potential in research, recent research has focused on using the method for the extraction of bioactive compounds from the plant cell matrix [129,130]. When compared with thermal methods, the qualities of plant-protein-based foods were minimally affected after the PEF treatment and were maintained throughout the storage period [24]. The PEF method involves subjecting the plant cells to intense high-voltage pulses at an electric field strength range between 0.5 and 50 kV/cm with a pulse duration of milliseconds, microseconds, or nanoseconds in samples positioned between two electrodes to inactivate microorganisms in foods and to improve the rate of mass transfer for extracting intracellular bioactive compounds [131,132]. Basically, the cell membrane is made up of a lipid bilayer with embedded protein channels that play a critical role in separating the

intracellular organelles of the cell from the surrounding environment and modulate the transfer of ions in and out of the cell to maintain the cell balance. The application of high-voltage electric field pulses destabilizes the lipid bilayer by increasing the cell wall permeability, providing adequate contact between the solvent (plain water in this case) and the intracellular compounds. Hence, while the plant cell matrix receives the electric field pulses, the permeability of the cell wall would increase, resulting in the induction of a transmembrane voltage across the cell membrane, the intensity of which depends on the intensity of the electric field, the pulse profile, the nature of cells, and the cell orientation with reference to the direction of the electric field vector [133]. In general, increasing the transmembrane potential increases the electromechanical compression of the cell membrane, leading to electropermeabilization or electroporation [134–136]. As a consequence of electroporation, plant tissues will be softened, enabling them to be well equipped for the subsequent extraction of bio-compounds in a shorter processing time. Thus, electroporation has a wide range of potential applications, from changing the structure and function of plant cells to causing permanent cell death. Figure 2 illustrates the potential uses of PEF in food applications.

Beneficial features of the PEF method rely on not using harsh chemicals, heavy solvents, acids, alkalis, or high-frequency mechanical effects on cells. It merely focuses on optimizing ultrashort electric field pulses to enhance the electroporation effect on plant cells through mass transport phenomena for efficient protein extraction [112,126]. When compared with thermal methods, it is a promising method for preserving the quality of proteins throughout the storage period [24]. Additionally, using plain water as a solvent minimizes environmental impacts. The interest in using PEF-assisted extraction has increased significantly since it has developed into an environmentally friendly and green extraction technique [137,138] that can contribute to sustainable development in future food systems. During the PEF bio-extraction procedure, electroporation selectively obtains the valuable bio-compounds from the plant cells by optimizing the intensity of the electric field strength and pulse parameters [139]. Thus, PEF-assisted extraction is advantageous in providing high protein yields when compared with other conventional techniques [130,140]. Moreover, combining other extraction processes with PEF has been reported to increase the protein yield [141,142] when compared with PEF standalone treatments. In addition to the enhanced yield, this method also preserves the quality attributes and nutrition of extracted bio-compounds [143,144]. For instance, PEF enhanced the functional and emulsifying properties of rice bran protein by about 20.29–22.64% and 3.3–12.0%, respectively. Additionally, the foaming capacity and foam stability were increased by 1.8 to 2.9-fold, respectively. Moreover, the in vitro digestibility of proteins was also enhanced [145].

Optimizing process parameters plays a significant role in changing the structure and function of proteins. Some of the structural changes, such as protein unfolding, dissociation, protein denaturation, and reaggregation, highly depend on optimizing the intensity of electroporation, which in turn depends on the electric field strength. When PEF effects on proteins are analyzed, the PEF-induced electric field stress causes the electrostatic interactions to be disrupted inside the polypeptide chain of the protein, leading to the deformation of secondary structures [146,147]. Multi-protein food systems have more complex interactions in an electric field, which can impact the stability of the systems [148]. For instance, with an increase in the electric field strength and number of pulses, the rheological properties of soy milk, such as the viscosity, increase with the change in consistency [149]. Enhancing the surface hydrophobicity of the protein structure enhances the flavor of foods. In this regard, increasing the electric field strength and number of pulses resulted in protein unfolding and the destruction of hydrophobic interactions, leading to structural modifications that increased the surface hydrophobicity in the case of whey protein isolate [150].

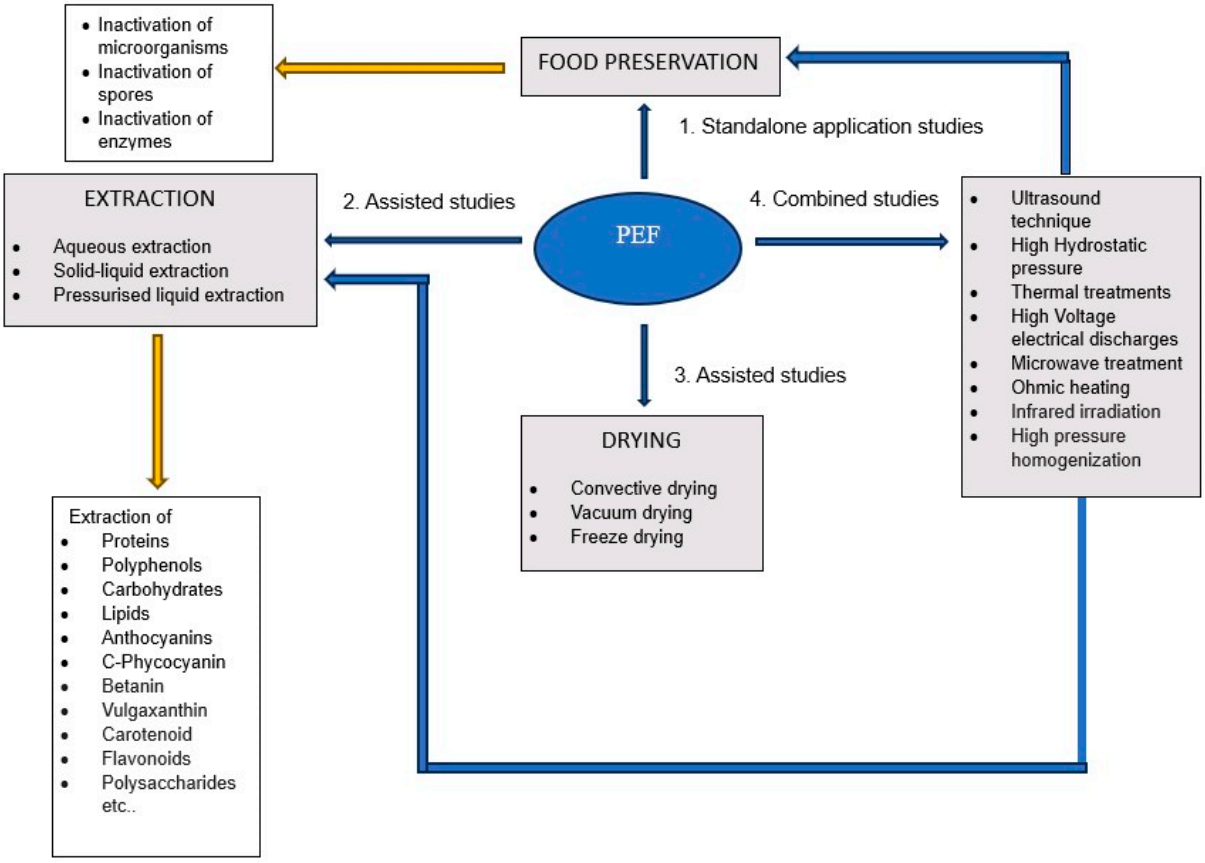

Figure 2. Potential uses of PEF in food applications.

4.2. PEF Setup

The working principle of PEF relies on the application of high-voltage pulses to samples placed between two electrodes, where one electrode will be charged and the other will be at the ground potential. A PEF prototype comprises a high-voltage pulse generator unit, a treatment chamber with two electrodes, a peristaltic pump for circulating samples, an oscilloscope for displaying pulses, and a control unit [22]. The untreated sample will be transferred to the PEF treatment chamber before it is subjected to the required high-voltage pulse. Samples will be tested in batches in the case of static treatment chambers or will be pumped into the chamber for continuous testing purposes. The pulse-forming network (not shown) plays a significant role in delivering the required pulse waveshape to the testing medium placed inside the PEF chamber. The pulses can be delivered in the form of square, exponential, or oscillatory waveshapes. The treatment chamber and the electrodes must be carefully designed since they govern the electric field distribution inside the chamber. Figure 3 represents a simplified sketch of a Pulsed Electric Field setup.

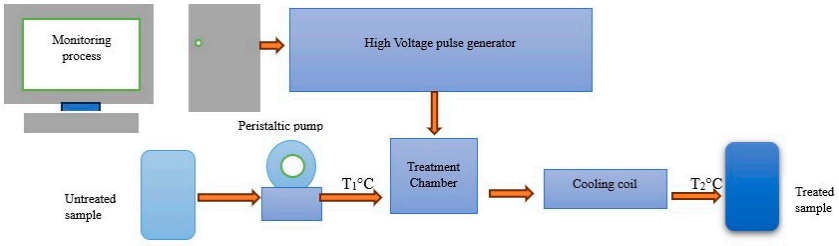

Figure 3. Pulsed Electric Field setup.

There are three basic electrode configurations, including parallel plate, coaxial, and collinear electrodes [151], the shapes of which can be optimized further to suit either

batch or continuous modes of operation [152–155]. Among the designs, the parallel plate configuration was the earliest-invented design of high importance, with a simple construction that delivers a uniform electric field inside the treatment gap [156]. While applying the electric field pulses to the testing medium, the electrodes act as a mediating factor between the high-voltage pulse generator and the treatment gap. Though the treatment chamber and electrode designs play the primary role in providing the optimum electric field distribution, process parameters such as electric field intensity, pulse waveshape, pulse duration, and pulse frequency should be adjusted to produce beneficial impacts on the treated samples [157,158]. The impact of the treatment also depends on the properties of the testing medium, such as the electrical conductivity, pH, concentration, and permittivity [111,159]. Hence, to obtain the best treatment outcomes, the researcher has to optimize the combination of all the aforementioned parameters.

High-voltage pulse generators deliver the desired electric pulses repetitively to treatment chambers through a pulse-forming network (PFN) and high-voltage switches. These generators provide the required waveshape, polarity, and amplitude of the pulses based on the construction of the PFN. While square and exponential pulses were frequently used in earlier studies [160,161], the square waveshape has been shown to be more efficient in treating the samples due to the longer retainment of the peak voltage during the treatment [161]. Exposing the cells to oscillatory decay pulses were found to be less effective due to the shorter cell exposure time. To minimize undesirable electrochemical reactions and associated thermal effects, bipolar pulses are generally preferred over monopolar pulses [160,162,163] due to the alternate stress effects on the cell membrane.

During the PEF method's application, the sample will be treated either in batch or in continuous mode inside the treatment chamber [164]. Insulating spacers will be positioned between two electrode edges to facilitate the application of high-voltage pulses only in the treatment gap [112]. In earlier studies, batch testing modes were preferred for small-scale research and continuous modes were used for industrial testing purposes. Nowadays, lab-scale studies use both modes of operation. Batch operation involves testing the samples in batches using a standard treatment chamber. In this mode, there will be no continuous flow of the testing medium and the samples will be subjected to the static application of an electric field in the treatment gap for a specific treatment time. This mode of operation is slow to process due to the manpower involved in placing the testing medium inside the treatment chamber, transferring the medium, and collecting it after the treatment. Using a batch treatment chamber, the extraction of essential bio-compounds, including proteins, has been observed in several plant cells and waste plant tissues [4,165]. The sample to be treated will be placed either in small cuvettes or in a customized chamber, depending on the testing volume requirements. The product will be mixed with mild solvents or tap water in a prescribed ratio before it is subjected to the required high-voltage pulses [143].

During continuous operation, the peristaltic pump circulates the untreated samples and delivers them to the treatment chamber for the application of high-voltage pulses. When compared with batch systems, continuous systems are attractive due to their increased capacity to treat foods continuously in a shorter treatment time. Due to their high efficiency [166], continuous systems are preferred for large-scale industrial testing purposes not only in PEF-assisted extraction but also in other areas such as freezing, drying, cutting, and cold pasteurization [111,167]. In continuous mode, the electrodes and the chamber design will be constructed to facilitate the continuous flow of the testing medium inside the chamber at a constant flow rate per second/minute/hour depending on the volume of the testing sample. Tamborrino et al. used a collinear tubular electrode configuration for a continuous PEF-assisted olive oil extraction process, which resulted in an increase in the process efficiency and quantity [168]. One minor drawback of continuous chambers lies in the difficulty of having the product flow uniformly experience the electric field and subsequent electroporation effects. This has been observed in both microbial inactivation and bio-compound extraction studies [168,169]. The parallel plate and coaxial electrodes were generally used for both batch and continuous treatment processes [154,155,164,170–172]

whereas collinear designs were allocated for the continuous treatment process in the majority of studies [168,173]. Despite fringing effects [174], the parallel plate design provides a more homogeneous electric field in the treatment area, whereas the coaxial shape ensures a uniform flow of the sample that is suitable for industrial-scale applications. Though all three electrode shapes are used for continuous PEF operations [175], the collinear design facilitates the easy flow of product through the chamber. The efficiency of both the batch and continuous testing modes is dependent on several factors, such as the chamber design, electrode design, electric field strength, number of pulses, pulse width, pulse waveshape, and properties of the testing medium such as the electrical conductivity, pH, permittivity, and concentration of the sample.

4.3. Mechanism of Extraction

PEF assists in the efficient extraction of intracellular proteins by means of a mass transport phenomenon through electroporation. Basically, the cell plasma membrane is considered to be a barrier, which protects the inside of the cell from dynamic environmental conditions. It is a semipermeable membrane that regulates the passage of nutrients and the ionic flow in and out of the cell, thereby creating an osmotic balance between the cell and its surroundings. This membrane acts as an insulator made up of lipid molecules consisting of polar (i.e., hydrophilic) and non-polar (i.e., hydrophobic) sections that together form a bilayer structure. When this membrane is subjected to high electric stress, the permeability and conductance of these membranes increase, leading to cell disruption that results in electropermeabilization or electroporation [176]. Electroporation is currently gaining recognition due to its cost-effective, time-saving, and chemical-free application in several areas, such as food processing, electrochemotherapy, tissue ablation, and bio-compound extraction protocols. Fundamentally, it is the process of inducing pore formation in the biological cell membrane, the radius of which depends on the intensity of the electric field strength [177]. The capacity of electroporation to cause the mass transport phenomenon in the plant matrix depends on the electric field strength, pulse number, pulse frequency, and total treatment duration [178]. The rationale behind producing electrical pores is to extract valuable bio-compounds from the cell wall matrix. Due to the increase in the electric field, transmembrane potential (tmp) will be induced across the cell membrane that enables the membrane to experience electromechanical stress leading to the creation of pores. The pores will continue to increase depending on the supercritical electric field Ec [177]. Hence, the electroporation mechanism involves a significant number of stages, including application of ultrashort electric field pulses, lipid bilayer charging, local structural rearrangements within the cell, perforation of the cell membrane, and increased ionic and molecular transport through the pores [134]. The structural rearrangements indicate the permeation of the lipid bilayer leading to the formation of hydrophilic pores for an increase in the transmembrane potential value [134]. After being exposed to an electric field, cells cannot regain their original semipermeable properties completely [178].

When the electric field E is increased such that E > Ec, structural changes, cell rupture, and an increase in the cell membrane permeability will take place, leading to pore formation. Based on the increase in the electric field E, a larger number of pores will be formed, resulting in the leakage of intracellular biomolecules, including proteins, lipids, and carbohydrates. After the pulse's application and the subsequent release of intracellular components, the resealing of pores will occur, and the cell will return back to its original state in the case of reversible electroporation (RE). The life span of pores ranges from milliseconds to minutes after the pulse's application [176]. When the increase in the electric field E is very high such that E >> Ec, which progresses beyond the radius of the pores, permanent cell damage will occur, leading to irreversible electroporation (IRE) [179,180]. Therefore, the electroporation parameters should be optimized based on the treatment requirements and cell types. Figure 4 illustrates the electroporation stages in a plant cell.

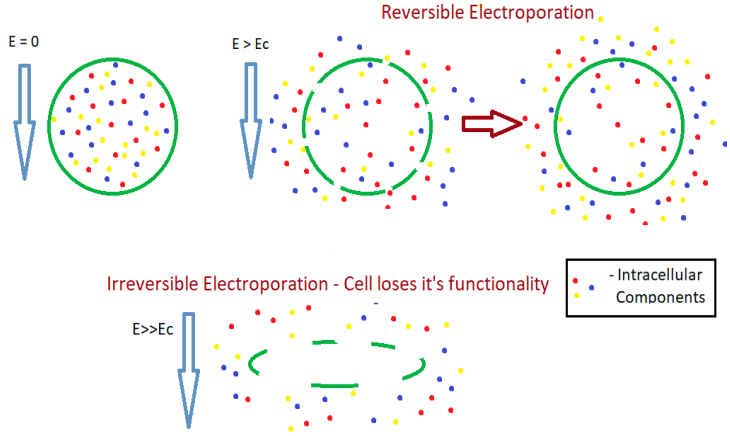

Figure 4. Electroporation stages in a plant cell.

5. PEF-Assisted Extraction of Proteins

In PEF-assisted extraction, the intensity of the electric field can significantly influence electroporation to soften the plant tissues and facilitate a better protein yield from the cell matrix. Hence, PEF-induced permeability changes in the cell membrane depend on the electric field intensity, extraction time, pH, specific energy, temperature, and properties of the cell matrix, especially the conductivity [181]. These electroporation effects can be observed on the cell surface using scanning electron microscopy (SEM), which can help the researcher to make analogous studies with other treatment effects on the cells [182]. Figure 5 illustrates the PEF-assisted protein extraction process.

Over the past few years, studies on PEF-assisted protein extraction have escalated exponentially due to the potential of this treatment to provide better extraction yields with quality retainment. This technique to extract proteins through the mass transport mechanism works by the diffusion of the solvent into the intracellular medium and the dissolution of the solute out of the cell matrix. Due to its beneficial advantages, several studies have incorporated PEF into protein extraction research [4,114,119,129,159,183–185]. Moreover, thermolabile compounds are well preserved using PEF when compared with existing thermal methods [18,112]. Table 3 presents several remarkable studies on PEF-assisted protein extraction from waste plant parts and mushrooms. These studies represent the protein yield through process parameter optimization.

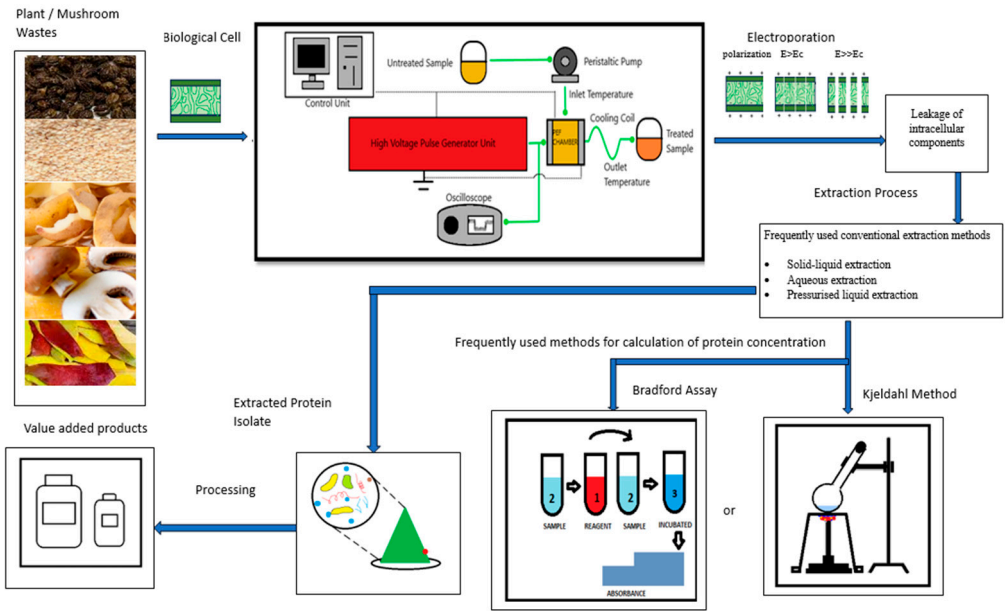

Figure 5. PEF-assisted protein extraction process.

Table 3. PEF-assisted protein extraction studies from waste plant parts and mushrooms.

Protein Source	Process Parameters	Outcome	Essential Findings	Reference
Agaricus Bisporus	38.4 kV/cm, Pulse width of 2 µS at both 400 Hz and 800 Hz frequencies Inlet Temperature: 20 °C	Protein extraction of 40.8%	* Significant increase in protein yield when combined with mild heating * Protein concentration increased with an increase in the electric field, temperature, and treatment time	[119]
Agaricus Bisporus	2.5 kV/cm, Specific energy of 50 kJ/kg, and 6 h of extraction time	Maximum protein recovery of 140.72 +/− 15.14 mg BSA/g DM*	* PEF increased the recovery of proteins	[143]
Papaya peels	13.3 kV/cm, 400 pulses, 40 kV applied voltage, treatment time of 2720 secs.	Protein concentration of 20 mg/L	* Two stages of PEF + supplementary aqueous extraction resulted in good recovery of bioactive compounds including proteins	[165]
Rapeseed stems and leaves	20 kV/cm 200 pulses, 40 kV applied voltage, pulse duration of 10 µs	Protein extraction of up to 80%	* PEF treatment increased the yield of proteins. * Protein contents changed during plant development	[4]
Rapeseed green biomass (stems)	8 kV/cm, Pulse duration of 10 µS, pressure of 10 bar, maximum applied voltage of 40 kV, frequency of 0.5 Hz, 200 pulses	0.14 g BSA/100 g DM*	* Protein content increased by about 2 times compared with the untreated sample and 50 pulse applications	[186]
Sesame cake	13.3 kV/cm Pulse duration of 10 µs Frequency of 0.5 Hz (2 s between pulses or discharges) 0–700 pulses Temperature of 40 °C Variable treatment time between 1 and 7 ms Energy inputs between 0 and 291 kJ/kg	Between 374 and 2001 mg BSA/100 g DM*.	* Protein contents increased with energy inputs until 83 kJ/kg was reached. * PEF improved the protein extraction yield.	[183]
Tomato wastes	5 kV/cm Treatment time of 1.5 ms 200 pulses	145.1 mg/100 g	* Protein concentration increased with increasing the electric field strength and time	[158]
Rice bran	2.3 kV/cm Treatment time of 25 min 250 pulses per minute	20.71–22.8%	* Improved the extraction yield of rice bran protein * Improved oil's holding and emulsifying properties * Increased foaming ability and stability * Enhanced in vitro digestibility	[145]

Table 3. *Cont.*

Protein Source	Process Parameters	Outcome	Essential Findings	Reference
Brewers spent grains (BSG) Light Dark	2.8 kV/cm 3000 pulses 20 µs pulse-width	20.31 ± 0.01 (%DWe*) 23.78 ± 0.16 (%DWe*)	* Increased yield of proteins from dark BSG compared with light BSG * Light BSG exhibited higher antimicrobial activity than untreated ones	[187]
Nettle leaves	3 kV/cm Specific energy of 10–24 kJ/kg Temperature between 70 and 78°C Extraction time of 5 min	>60%	PEF specific energy input, extraction temperature, and particle size had high impacts on solid liquid extraction kinetics	[188]

DM*, Dry Matter; DWe*, Dry weight extract.

The integration of PEF into the protein extraction process in mushrooms and waste plant parts is evident from earlier studies [4,119,143,165]. In addition to protein extraction, PEF–US combination techniques increased the drying rates, decreased the drying time, provided better mass transfer and rehydration capacities, changed the structure of the cell tissue, and showed high antioxidant activity in shiitake mushrooms [189]. In 2014, Boussetta et al. studied the impact of pulsed electric field-assisted extraction of polyphenols from waste flaxseed hulls. Their findings highlighted a research gap in protein extraction from flaxseed hulls [190].

PEF has also been proven to be effective in bio-compound extraction from seeds. Sesame seeds are extensively used in cooking to prepare tasty dishes and act as a flavoring agent in many recipes. These seeds are highly nutritious, are a good source of vitamins, healthy fats, protein, minerals, fiber, and antioxidants, and contain other beneficial characteristics for health. These seeds contain about 20% protein [191]. After oil extraction, the remaining byproducts of sesame seeds are still high in nutritional value. Sarkis et al. carried out extraction studies on sesame press cake for the protein and polyphenol content. 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 [183]. The PEF method's application increased the electrical conductivity and cell permeability of the residual seed cake, causing minimal cell damage. This enabled an easier extraction in a shorter time [183].

Papaya seeds are rich in isothiocyanates, phenolic compounds, and proteins. Parniakov et al. observed an increased degree of disintegration in papaya seeds at 13.3 kV/cm and 2000 pulses when coupled with supplementary aqueous extraction (SAE). The authors recovered a significant amount of protein after three hours of PEF-assisted extraction. Upon the PEF method's application, the degree of extraction of ionic components facilitated the release of embedded proteins from the cell wall matrix during SAE [159]. Therefore, PEF and supplementary aqueous extraction together produced a higher protein yield, opening the door for industrial processing [159]. Yu et al. studied the PEF-assisted extraction of proteins from rapeseed stems and leaves, which primarily depended on the electric field strength and growth phase of the plant. The specific parameters that caused tissue damage were observed at 800 V/cm and a protein extraction yield of up to 80% was achieved at 20 kV/cm [4]. Kamboj et al. observed an increase in the protein yield of up to 10.58% on perilla seed meal at processing parameters of a 4 kV/cm electric field and a 120 µS pulse width [192]. Though PEF-assisted protein extraction studies have been performed on mushrooms and plant wastes, these edible products are currently in the amateur stage of study and require further exploration to improve the productivity economically.

6. Optimization of the PEF-Assisted Extraction Process

The optimization of PEF-assisted extraction requires the execution of systematic procedures to enhance the recovery of proteins from the cell matrices. The primary factors that influence the PEF performance in the extraction process include the treatment chamber design and the electrode shape, which govern the electric field distribution inside the chamber. These factors must be carefully designed in order to provide the optimal treatment to the testing samples. Other process parameters that need to be adjusted include the electric field strength, number of pulses, pulse width, pulse waveshape, specific energy input, and temperature of the untreated sample, as these factors optimize the electroporation effects on the cells to be treated [193], facilitating the mass transport phenomenon to assist with extraction. If these process parameters are increased, the ability of electroporation to puncture the plant cells would increase, enhancing the overall extraction process. Also, a decrease in impedance would be noted on the cell tissues upon increasing the electric field strength [194]. However, increasing the process parameters by many folds beyond the extractable threshold of the cell matrix and a prolonged extraction time would cause a significant reduction in protein extraction levels. On the other hand, the electroporation effects also depend on the characteristics of the cell, such as the cell wall rigidity, cell arrangement, structure, water content, and usage of solvents. Hence, the process parameter optimization may be variable depending on each cell matrix.

Electric field strength is one of the most significant parameters to be optimized in order to obtain an enhanced protein extraction rate. While PEF technology relies on the application of an electric field in a lower range from 0.1 to 0.3 kV/cm and in a higher range from 20 kV/cm to 80 kV/cm [131,195], reducing the pulse duration with a higher electric field would aid in providing a higher extraction yield in a short processing time [126]. In dairy and plant proteins, the intensity of the electric field can modify the structure and techno-functional characteristics of proteins. In addition, a higher electric field strength can yield better emulsifying properties of proteins and protein–polysaccharide complexes [196]. Xue and Farid analyzed the impact of PEF-assisted protein extraction on white button mushrooms (*Agaricus Bisporus*) and obtained 49% $\pm$ 0.09 protein at a 38.4 kV/cm field intensity for 272 µs of treatment time and a treatment temperature of 85 °C. In addition, reducing the pulse width to 136 µs while keeping constant the electric field value retained the pink-to-reddish color similar to fresh extracts [119]. Although a high electric field intensifies the electroporation effect, care must be taken not to increase the electric field exponentially as it may synergistically increase the product temperature, resulting in a loss of nutrients in the extracted outcome. So far, more research findings have been reported on the fact that the electric field strength induces higher protein yields in microalgal cells [124,188,197] than in plant resources and mushrooms. The aim of this article is to open up new avenues for more PEF-assisted protein extraction research on waste plant tissues and mushrooms.

The PEF treatment time can be calculated by multiplying the pulse duration by the number of pulses. Modulating either the pulse duration or the pulse number would modify the treatment time. The extraction time and temperature are the essential parameters to be optimized during the extraction process. An exponential increase in either of these parameters would degrade the quality of the testing medium. For instance, Boussetta and Segovia reported an increase in the yield of valuable compounds in grape seeds and borage leaves at a lower temperature and shorter extraction time than the control samples [137,198]. Hence, these two factors must be regulated appropriately to preserve nutritional and sensory aspects in the samples.

The efficiency of PEF technology also depends on the conductivity of the sample [20]. In microbial inactivation studies, high-conductivity foods created a peak electric field across the treatment chamber, which intervened into the electric field's application during the treatment, resulting in a low-efficiency outcome. Hence, it is always preferred to choose low-conductivity foods to obtain optimum results in electroporating microorganisms. In plant-based bio-extraction studies, samples with high conductivity increased the ionic

strength of the medium, which hastened the cell membrane charging, resulting in enhanced protein extraction levels. Hence, according to Buchmann, a highly conductive extracellular medium would enable the diffusion of proteins through the permeable membrane, resulting in an enhanced protein yield [124].

The application of ultrashort pulses (microseconds or nanoseconds) is considered to be one of the major advantages of this treatment that renders it non-thermal and consumes less energy when compared with thermal methods. This feature would assist in lowering the processing cost, though the initial cost of the equipment is high [199]. Hence, a suitable combination of optimized pulse parameters would provide effective extraction results. However, the process parameters must be selected based on the plant tissues in order to achieve high protein yields. Generally, the application of high-voltage pulses may not have an impact on intracellular organelles due to the presence of the rigid cell wall. However, if the pulse duration is made to be ultrashort, i.e., nanoseconds, sub-nanoseconds, or picoseconds, the electric shots will directly charge intracellular organelles in the cytoplasm without charging the outer cell membrane [200]. Hence, the cell wall characteristics play a significant role in the extractability of proteins [201]. However, when a long pulse duration is applied, it may lead to an undesirable increase in the temperature, resulting in adverse electrode reactions and thereby degrading the quality of the testing medium [202]. Hence, the application of a large number of ultrashort pulses would help in minimizing the temperature increase and associated detrimental effects [203].

The use of solvent at a suitable concentration has been highly beneficial in achieving the mass transport phenomenon in extraction studies [204]. By using PEF, the amount of energy and organic solvent needed to extract the desired compounds would be minimized [137,201]. Water is recognized as a green solvent liquid. However, the low solubility of valuable compounds in water makes it less preferable [205,206]. While the majority of extraction methods use ethanol, methanol, aqueous NaOH, KOH, $NaHCO_3$, or NaCl as the solvent in a high concentration, PEF-assisted extraction has used different solvent types, such as water alone and a mixture of low-concentration organic solvents with water, for research. For instance, Min Wang [207] reported a higher protein yield from *Chlorella* using water alone as a solvent. This study gave the best extraction efficiency at an extraction time of 180 min at 3 kV/cm, 44 pulses, and 99 kJ/kg energy. Barba et al. studied PEF-assisted extraction of valuable bio-compounds from stevia leaves using water alone as a solvent, which facilitated an increase in the extraction of soluble matter (~33%), an increase in the activity of phenolic compounds (80%), chlorogenic acid (93%), caffeic acids (55%), ferulic acid (90%), and protocatechuic acids (45%), and increased the conductivity by 25% [111,208]. Parniakov et al. [209] compared one-stage and two-stage extraction procedures using the microalgae *Nannochloropsis* spp. In two-stage procedures, the combination of less-concentrated organic solvents and water obtained high non-degraded protein levels at 20 kV/cm for 400 pulses with a one-minute pause after each application of 200 pulses for better temperature control to 20 °C. The one-stage procedure resulted in the undesirable degradation of proteins due to the high concentration of solvents used. Serena Carpentieri et al. studied the PEF-assisted extractability of aroma and bioactive compounds from rehydrated plant materials. Here, green solvents such as a water–ethanol mixture and propylene glycol were used in solid–liquid extraction after the PEF application. PEF improved the solvent's penetration into the plant cell's cytoplasm and the diffusion rate of solubilized compounds during solid–liquid extraction, thereby enhancing the overall extractability rate [210].

7. Industrial Processing

In the 1960s, several possible applications were identified using electroporation in food and bioprocessing areas [211]. A PEF commercial installation for fruit juice processing was first achieved in 2006 in the USA followed by a line of installations in Europe in 2009, which was extended to applications for the pretreatment of vegetables in 2010 [211]. A PEF system has recently been installed in several countries, including Italy (Amica Chips), Canada

(Potatopro), and New Zealand (McCain Foods), from machine manufacturers including Pulsemaster from The Netherlands and Elea Technology GmbH from Germany, allowing these countries to manufacture their own food products. For industrial applications, sustainable production is essential to feasible applicability in PEF-assisted protein extraction research, which requires further insights to become a commercially mature technique. Substantial factors to be considered for this analysis include reliability, economic evaluation, environmental impact, social impact, and relevant contribution to society. Therefore, it is essential to analyze all these factors for the successful integration of PEF into the protein extraction process in order to evaluate its efficiency. Based on earlier studies, PEF has been proven to be highly energy efficient in terms of applying ultrashort pulses when compared with other thermal processing methods. Due to the low energy consumption, the temperature of the PEF-treated sample would be lower when compared with conventional thermal techniques [212]. Hence, the overall processing cost would be lower, even though the initial cost of the machine is high [199].

Currently, there is high demand for green extraction processes that replace the non-ecofriendly conventional chemical and heavy solvent usage techniques. The objective of green techniques is to minimize the interference with solvent usage and enhance the qualitative and quantitative characteristics of the target extractable compounds. The PEF technology started with testing small quantities in batches using simple treatment chamber designs and electrodes at laboratory scales and has grown into a sustainable and commercially established technique in microbial inactivation studies. Later, many other divisions of application emerged, including solid and liquid food processing, spore inactivation, enzyme inactivation, dehydration, freezing, and medical science areas [213]. Moreover, the increase in demand led to an increase in the flexibility of PEF systems appropriate for each testing sample [112,214,215]. Over the past few years, PEF has been found to be effective in bio-compound extraction studies, but they are still in a preliminary stage [126]. Hence, further research is required in extraction studies to streamline the energy efficiency and safety processes suitable for industrial applications.

Due to the growing consumer demand for nutritional, safe, and high-quality food products, PEF technology has upscaled systems for industrial production. Section 4 discusses the PEF layout in detail. There are many significant parameters involved in the successful operation of a PEF system at the commercial scale. An effective treatment depends on optimizing these process parameters based on the volume of the sample treated at a larger scale. Arshad et al. have discussed the interdependency of these process parameters and the difficulty of modulating these parameters in larger-scale operations [216].

PEF systems deliver high-voltage pulses with high functional capabilities that meet large-scale industrial standards [217]. Stefan Toepfl installed high-power semiconductors that facilitated the evolution of industrial-scale PEF systems with a high degree of competence [218]. Research on the scaling-up of the PEF extraction process can be understood from several studies [219,220]. Toepfl's analysis on upscaling from the lab scale to the industrial scale was achieved through an appropriate transfer of processing conditions suitable for larger scales. In this regard, the major processing parameters, such as the electric field strength, specific energy input, and temperature, were optimized, where a specific energy input of 5–10 kJ/kg was required for the permeabilization of plant tissue [38].

8. Existing Challenges for PEF Systems

At present, the occurrence of electrochemical reactions is a significant concern for pulsed electric field treatment in the food industry [221,222]. The main consequences of electrochemical reactions include corrosive effects, electrode fouling, migration of metallic particles from electrodes, chemical changes, and water electrolysis in the food matrix [222,223]. So far, research has been performed to minimize the electrolysis by (i) using bipolar square wave pulses [162,224], (ii) reducing the pulse duration [220,225], (iii) maintaining the minimum peak voltage [225], and (iv) coating the electrode [155]. Moreover, the availability of commercially established PEF units is limited worldwide, which requires further research

to overcome the existing limitations [199]. Johan Morren et al. studied the electrolysis that occurs during PEF treatment, the effect of which could be minimized in the treated food by using small pulses [221]. If the electrodes are corroded, the PEF-treated samples would be contaminated with metal particles [221].

Another drawback is the occurrence of gas bubbles during the PEF treatment that affect the uniformity of the electric field's application in the treatment area, resulting in operational issues [196]. Moreover, the majority of food samples are homogeneous in nature. The PEF's application in heterogeneous foods leads to dielectric ruptures due to air and gas bubbles in the food product that occur due to the electrical discharge between the two electrodes inside the treatment chamber. This would create sparks, thereby increasing the probability of explosions [226].

The major disadvantage of a pulsed electric field is the extensive initial cost of the machine, which requires significant financial investment [30,227,228]. However, since the operational cost is very low [216,228], many industries and research institutes propose to buy the equipment, thereby evaluating PEF as a long-term profitable food processing business that has the potential to provide high research integrity.

Moreover, during the PEF's application, varying the process parameters would cause electric-field-induced temperature changes in the food samples based on the conductivity value of the food. High-conductivity foods will have a high ionic concentration that would produce a peak electric field across the treatment chamber, thereby inducing heat energy. Hence, the temperature should be monitored and controlled to minimize the degradation of the thermolabile compounds in the plant cells [113]. For this purpose, mild treatments or circulating cooling water would aid in minimizing the associated temperature increase in the system, which would also help to preserve the quality attributes of the tested food [167,229,230].

The extraction of proteins from the plant matrix involves process parameter optimization, which poses a greater challenge to protein extraction research teams. Varying degrees of optimization would be a requirement to suit each type of plant matrix to obtain enhanced protein levels. In addition, promoting this research area on a larger scale requires established research reports pertaining to specific doses of the PEF to each protein source. Hence, further research is still required on molecular perception to find out the changes that occur in the protein levels from the plant matrix for each processing condition. In addition to protein yield, other factors such as the initial cost of equipment, maintenance and operating costs, sustainability, and the feasibility of PEF application under varying environmental conditions should be analyzed to upscale this technology to industrial processing. However, studies involving all of the above factors are still limited and we need deeper insights for further progress.

9. Conclusions and Future Directions

Protein is a mandatory requirement for the body's everyday functioning. The protein requirement varies based on age and conditions in the human body. When it comes to plant-based dietary proteins, the extraction of proteins from plant wastes using green techniques favors both the economy and the environment, promoting sustainable production. From a global perspective, valorizing raw plant wastes and rejected mushroom parts would help to reduce the amount of fruit, vegetable and mushroom wastage and provide value-added products. In this regard, the PEF-assisted protein extraction method has the potential to exponentially grow in the future to meet the ever-escalating protein demand since this technique facilitates a high protein yield in a non-thermal manner that preserves the bioactive and associated thermolabile compounds. However, further studies are required in order to optimize process parameters and scale up the technology for industrial applications. PEF is considered to be a green technique, due to which its utilization will increase in the following years. As a limited number of studies are available on PEF-assisted protein extraction, emerging unknown aspects need to be examined in the future. In particular, the impact of different electric field strength values must be studied to find out the optimum

value for enhanced protein yield at a controlled temperature, since temperature is one of the most important parameters to monitor. Other process parameters should also be optimized with the electric field until we obtain clear conclusions, which should be evaluated multiple times to obtain valid and established reports. These studies would facilitate the technique for industrial applications.

Recently, combinations of techniques with PEF have been found to be effective in obtaining enhanced protein levels when compared with PEF standalone treatments [231,232]. However, the additional method should not use severe processing conditions to degrade the proteins, which may in turn require further processing steps including purification. Zhou et al. studied protein extraction with a combination of PEF and pressurized liquid extraction (PLE) treatments compared with a PEF pretreatment with conventional extraction methods. The enhanced extraction was due to the initial cell breakdown effect caused by PEF followed by the easier diffusion of the solvent into the cell during PLE to achieve a high level of protein recovery from *Spirulina* [233]. Carullo et al. showed improved recovery of bioactive compounds including proteins from a combination of high-shear homogenization (HSH) and PEF treatments on *A. platensis* suspensions due to synergistic effects. The disaggregating effect of *A. platensis trichome* clusters due to the HSH pretreatment enhanced the cell membrane permeabilization, followed by the PEF treatment, thus intensifying the extractability of valuable compounds [142]. The experiment was maintained at a feasible temperature in order to achieve non-thermal effects in the samples. Therefore, the integration of two techniques would aid in enhancing the overall protein levels in the samples. However, further studies should be conducted in order to select an appropriate green technology to combine with PEF to obtain high protein yields, also facilitating the combination for larger scales. Moreover, the cost effectiveness and sustainability should also be considered in order to promote combined techniques to industrial levels.

The challenges and future developments that should be addressed are as follows:

- The protein extraction research on PEF process parameter optimization should be broadened to promote the applications in a large-scale manner.
- Deeper insights should be gained into PEF-assisted protein extraction in non-conforming botanical parts such as rejected stems, leaves, roots, flowers, fruits, and other green waste resources from industrial processing.
- Research on combining other green techniques with PEF to obtain the optimum protein yield should be extended.
- The research on electrolysis is underdeveloped in extraction studies and can be further improved to minimize the effects in the final byproducts.
- Advanced simulation studies should be conducted to find out the protein's structural and functional changes due to electric stress, which would improve our understanding of the interaction between the protein molecule and electric field and consequently be beneficial for future experimental research.

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