Review

Enhancing Product Value and Energy Efficiency in Seafood By-Product Processing Using Pulsed Electric Fields: A Critical Review

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Enhancing Product Value and Energy Efficiency in Seafood By-Product Processing Using Pulsed Electric Fields: A Critical Review

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Abstract

The global seafood industry generates millions of tons of by-products each year, creating environmental and economic challenges but also presenting a valuable opportunity for resource recovery. These by-products, rich in bioactive compounds such as proteins, omega-3 fatty acids, collagen, chitin, and antioxidants, have traditionally been underutilized due to inefficient and energy-intensive conventional extraction processes. Pulsed electric field (PEF) technology has emerged as a promising, non-thermal, and environmentally friendly method for valorizing seafood by-products by enhancing the permeability of biological membranes through electroporation, thereby facilitating the efficient extraction of high-value compounds. This manuscript critically reviews the scientific principles underpinning PEF, including dielectric breakdown and transmembrane potential generation, and explores its mechanisms for improving mass transfer during extraction and dehydration. Applications of PEF for recovering proteins, lipids, and antioxidants from diverse seafood side streams are comprehensively discussed, with emphasis on its advantages—such as reduced energy consumption, preservation of thermolabile compounds, and improved product quality—compared to conventional methods. Despite demonstrated laboratory-scale successes, industrial adoption of PEF remains limited due to challenges in process optimization, economic feasibility, and regulatory frameworks. This review synthesizes current knowledge and provides guidance for future research to advance the industrial implementation of PEF as a sustainable and efficient tool for seafood by-product valorization.

Keywords: pulsed electric fields; extraction; electroporation; seafood; protein; bioactive compounds

1. Introduction

The global seafood industry processes over 200 million tons of fish and shellfish annually, generating vast quantities of by-products—estimated to be up to 50% of the total catch weight [1,2]. These by-products, including fish heads, skins, bones, viscera, and crustacean shells, are largely discarded or underutilized. This often leads to significant environmental burdens and economic inefficiencies within the sector [3,4]. However, these side streams represent a rich source of high-value bioactive compounds such as collagen, gelatin, peptides, omega-3 polyunsaturated fatty acids (PUFAs), chitin, chitosan, minerals, and antioxidants [4–6]. Recent trends in circular bioeconomy and sustainable food systems have, therefore, emphasized the critical importance of valorizing marine by-products to improve the resource efficiency of the seafood sector [3,7]. Traditional extraction technologies, such as thermal rendering, acid/base hydrolysis, and enzymatic hydrolysis, are frequently energy-intensive. Furthermore, they can result in the degradation of thermolabile compounds, low yields, and considerable environmental impact [8,9]. Consequently, there is a growing demand for innovative and sustainable processing methods capable of improving extraction efficiency while preserving the functional and nutritional integrity of target compounds.

Pulsed electric fields (PEF) is an emerging non-thermal processing technology that involves applying short bursts of high-voltage electric pulses (typically 1–50 kV/cm) to biological tissues or

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fluids. This process induces electroporation, a phenomenon where cell membranes temporarily become permeable due to dielectric breakdown [10–12]. The resulting enhanced permeability improves mass transfer, thereby facilitating the release of intracellular materials like lipids, proteins, and secondary metabolites [13]. PEF is already well-established in various sectors of the food industry, with applications including juice extraction, microbial inactivation, drying pre-treatment, seed treatment, mycotoxin mitigation and starch modification [14–21].

The application of PEF in seafood by-product valorization is garnering increasing attention. Studies have demonstrated that PEF can significantly enhance the extraction of collagen from fish skins [22,23], omega-3-rich oils from fish heads [24], and chitin from crustacean shells [23,25]. Importantly, these improvements are often accompanied by substantial reductions in processing time and energy requirements [20]. Compared to conventional thermal processes, PEF facilitates improved retention of compound bioactivity and functionality due to its minimal thermal footprint [26,27] (Figure 1). Moreover, PEF can be synergistically combined with other green extraction methods, such as enzymatic hydrolysis, ultrasound-assisted extraction (UAE), and supercritical fluid extraction, to further boost process efficiency and selectivity [10,18,28–30].

Despite these promising laboratory-scale results, the industrial-scale adoption of PEF technology in seafood processing remains limited. Challenges such as process optimization, economic feasibility, and regulatory acceptance must be thoroughly addressed to enable widespread commercial implementation [11,20,31,32]. A critical evaluation is presented concerning the scientific principles, technological developments, and application potential of PEF for extracting value-added compounds from seafood side streams. This includes an examination of PEF's influence on cell structure and compound release, a comparison of its performance with conventional methods, an assessment of its energy and cost efficiency, and an exploration of prospects for industrial scaling.

By synthesizing current literature comprehensively, a deeper understanding of PEF's role as a green and sustainable tool for seafood by-product valorization is achieved, and future directions for research, innovation, and commercialization in this domain are concurrently outlined.

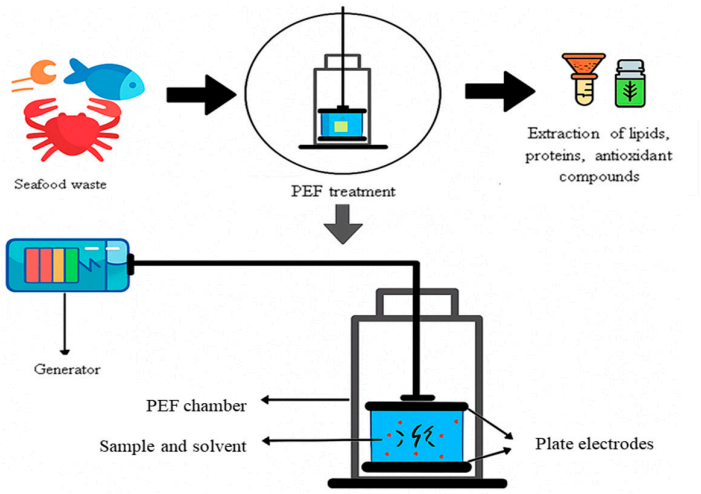

Figure 1. PEF extraction of bioactive materials from seafood waste.

2. Mechanism of Pulsed Electric Fields: Electroporation, Dielectric Breakdown, and Detailed Cellular Effects

At its core, PEF technology operates by initiating electroporation, or electropermeabilization, within biological cell membranes [12,33]. This critical phenomenon involves the application of short,

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high-intensity electrical pulses that induce temporary or permanent structural alterations. Such changes constitute the principal mechanism through which PEF enhances mass transfer, ultimately facilitating the release and extraction of intracellular compounds from diverse biological tissues [34].

2.1. The Biophysical Interaction: Cell Membrane as a Capacitor and Dielectric Breakdown

The effectiveness of PEF is intrinsically linked to the unique electrical properties inherent in the cell membrane. Primarily composed of a lipid bilayer, this membrane functions as an electrical insulator, efficiently segregating the conductive aqueous environments found both inside (cytosol) and outside the cell. Consequently, a cell immersed in a conductive medium can be conceptually understood as a micro-capacitor [35].

The system typically includes a pulse generator capable of delivering controlled high-voltage pulses (commonly 10–80 kV/cm), a treatment chamber with well-insulated electrodes, and a flow-through system that ensures uniform exposure of the food matrix. A cooling unit is often integrated to mitigate temperature rise and maintain product quality during processing (Figure 2). Critical design parameters such as pulse width, frequency, energy input, electrode geometry, and treatment time are carefully optimized to achieve targeted microbial inactivation or compound extraction while preserving nutritional and sensory attributes. Electric pulses in a PEF system can be applied in either exponential decay, sinusoidal, monopolar or bipolar modes (Figure 3), depending on the intended application and the electrical characteristics of the target material. In monopolar mode, the polarity of the pulses remains constant, while bipolar mode alternates the polarity, which can reduce electrode polarization and improve treatment uniformity. The pulse waveforms themselves may take various forms, including square waves, exponential decay, logarithmic decay, or oscillatory patterns, each influencing the electroporation effect differently. Square waves, for instance, deliver a constant electric field over the pulse duration, providing consistent membrane permeabilization, whereas exponentially decaying or logarithmically decaying pulses can reduce energy consumption and thermal load on the treated material. Oscillatory waveforms may be useful for enhancing diffusion processes following electroporation. Selection of the pulse type, duration, and frequency is critical to achieving optimal microbial inactivation, extraction efficiency, or structural modification, while maintaining product quality and minimizing adverse thermal effects. Safety features and robust control systems are incorporated to ensure reliable, continuous, and hygienic operation of the PEF treatment line [11,18].

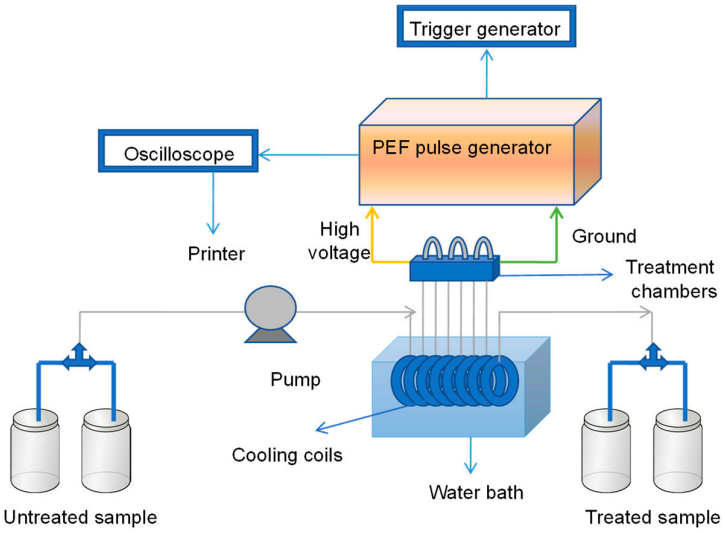

Figure 2. PEF treatment system.

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When an external electric field is applied, the cell, acting as a non-conducting entity within the surrounding conductive medium, induces a localized perturbation of the electric field lines. This phenomenon leads to a significant enhancement of the local electric field intensity across the cell membrane, most notably at its poles—the regions oriented parallel to the applied field [12]. The magnitude of this localized field can considerably exceed the average field applied to the bulk suspension or tissue.

Several studies have demonstrated the feasibility of PEF for different applications in food industry. PEF technology is therefore a valuable tool that can improve functionality, extractability, and recovery of nutritionally valuable compounds as well as the bioavailability of micronutrients and components in a diverse variety of foods. This opens the doors to new PEF applications in the food industry. This review focused on some of the most renowned traditional and emerging PEF applications for improvement of osmotic dehydration, extraction by solvent diffusion, or by pressing, as well as drying and freezing processes [11,34].

Processing of foods by thermal processing with the main objective of shelf life extension and safety may bring some disadvantages on physical or sensory properties as well as compounds classified as functional. Alternatives such as PEF with minimization of undesirable changes for food processing to produce foods with better quality are on demand. The main advantage of PEF technology comes with its mode of application that it is practiced at ambient, above, or below ambient temperature which minimize changes in physical properties of foods. When electric field current is applied to food samples, it is transmitted through the food by the presence of ions; thus, food should have certain amount of ions for PEF processing. In this respect-due to the presence of the hydrogen ions-fruit juices are one of the most suitable food products to be processed by PEF. Different juices have successfully processed by PEF for the safety and shelf life extension, but more studies are needed to prove that PEF is equivalent or superior to heat treatment for fruit processing. Therefore, there is a need to have a comprehensive review for the PEF applications on beverage processing to determine the potential of the technology for beverage production and preservation [11,18].

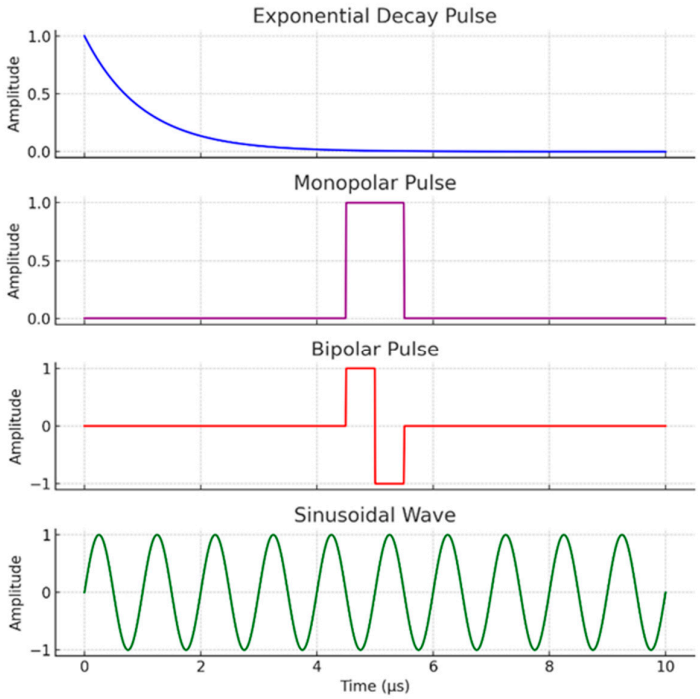

Figure 3. Different types of PEF electric field pulses.

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This enhanced local field drives the swift accumulation of electrical charges on opposing sides of the membrane, resulting in a rapidly developing induced transmembrane potential (TMP). The TMP represents the voltage difference generated across the cell membrane by the external field. Its magnitude peaks at the cell poles, gradually diminishing towards the cell equator, where it is theoretically zero [36–40].

The pivotal event culminating in electroporation is the dielectric breakdown of the cell membrane. As the induced TMP across the membrane intensifies, the electrostatic forces acting upon the lipid bilayer correspondingly increase. Once this induced TMP surpasses a critical threshold (typically ranging from 0.5 to 1.0 V for most biological membranes, though it varies with cell type and membrane composition), the localized electrical stress overwhelms the membrane's inherent structural integrity [41]. This ultimately prompts a swift, localized rearrangement of lipid molecules within the bilayer, leading to the transient formation of nanoscale hydrophilic pores [42–44].

2.2. Detailed Cellular Effects: A Sequential Process of Permeabilization

The intricate interaction between an applied electric field and the cell membrane unfolds as a dynamic, sequential process:

2.2.1. Rapid Membrane Charging and Transmembrane Potential Generation

Immediately following the application of a high-voltage electric pulse, the cell membrane behaves much like an electrical capacitor, undergoing rapid charging. This swift charging process establishes a pronounced induced TMP across the lipid bilayer. It's important to note that the polarity of this induced TMP will oppose itself at the cell's two poles relative to the external field, leading to both depolarization and hyperpolarization in different membrane regions [12].

2.2.2. Electrostatic Stress and Mechanical Compression

The elevated TMP at the cell poles generates substantial electrostatic pressure, also known as electromechanical compression, on the cell membrane. This pressure causes localized thinning of the membrane, leading to its mechanical instability in these highly stressed polar regions [39]. As this localized stress intensifies and ultimately surpasses the membrane's inherent elastic limit and mechanical stability, it triggers a cascade of structural destabilization [45].

2.2.3. Nucleation and Formation of Hydrophilic Pores

Once the critical TMP is reached, it's theorized that infinitesimally small, unstable, hydrophobic defects may spontaneously nucleate within the lipid bilayer. These are regions where water molecules are initially repelled by the lipid tails. Crucially, these unstable structures swiftly transition into more stable, hydrophilic pores (aqueous channels) through a dynamic process involving the influx of water molecules and the reorientation of lipid headgroups to line the pore [46,47]. These newly formed pores establish pathways that bypass the membrane's normally selective permeability.

2.2.4. Enhanced Molecular Transport

The creation of these hydrophilic pores permits the facilitated passage of various substances across the membrane. Initially, smaller ions and water rapidly traverse these channels. Subsequently, larger intracellular molecules—such as proteins, lipids, nucleic acids, and various secondary metabolites—can be released into the surrounding solvent. This process would otherwise be severely restricted by an intact, impermeable membrane or would occur at a significantly slower rate through passive diffusion [20,34].

The degree and duration of electroporation, along with the subsequent efficiency of target compound release, are precisely modulated by several key PEF parameters. These include the applied electric field strength (typically expressed in kV/cm), the duration of individual pulses (microseconds

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to milliseconds), the number of pulses applied, the pulse repetition frequency, and inherent biological factors like the specific cell type, tissue architecture, and membrane composition [11,21].

Crucially, depending on the specific combination of pulse parameters and cellular susceptibility, electroporation can be classified as either reversible or irreversible. In reversible electroporation, the pores reseal over time, allowing the cell to regain its viability and physiological function. Conversely, irreversible electroporation (often achieved with higher field strengths, longer pulse durations, or more pulses) leads to the permanent opening of pores, causing sustained membrane damage, cellular lysis, and ultimately cell death. For the purpose of extracting high-value compounds from seafood by-products, irreversible electroporation is frequently the desired outcome to maximize the recovery of intracellular contents [18,21].

3. How PEF-Induced Electroporation Enhances Extraction

For extraction processes, the goal is to efficiently recover valuable intracellular compounds (e.g., proteins, lipids, pigments, antioxidants) that are otherwise trapped within the cell. PEF facilitates this by making the cell membrane more permeable to these compounds.

3.1. Increased Mass Transfer Rate

The formation of transient or permanent pores in the cell membrane (electroporation) significantly reduces the barrier properties of the membrane. This directly leads to an enhanced mass transfer rate of intracellular components into the surrounding solvent or extraction medium [33,48]. Without PEF, the diffusion of these compounds across an intact, selectively permeable membrane would be slow and inefficient, often requiring harsh conditions (e.g., high temperatures, strong solvents) or extensive mechanical disruption.

3.2. Improved Solvent Penetration

The electroporated membrane allows for better penetration of the extraction solvent (e.g., water, ethanol, mild acid/base solutions) into the cell's interior. This improved access to the intracellular matrix facilitates the solubilization and release of target compounds that might otherwise be poorly accessible [31,34].

3.3. Reduced Processing Time and Energy Consumption

By accelerating mass transfer, PEF pretreatment can drastically reduce the overall extraction time needed to achieve a desired yield [21,49]. This, in turn, translates to lower energy consumption compared to conventional methods that rely on prolonged heating or mechanical grinding.

3.4. Preservation of Thermolabile Compounds

A major advantage of PEF is its non-thermal nature. Since the electroporation process itself causes minimal bulk temperature increase, heat-sensitive bioactive compounds (such as certain vitamins, enzymes, polyunsaturated fatty acids, and delicate peptides) are largely preserved from thermal degradation that occurs in conventional hot extraction methods [22,23,33]. This helps maintain the functional quality and nutritional value of the extracts.

3.5. Higher Yields and Purity

The more efficient release of intracellular components due to electroporation often results in higher extraction yields [50]. Moreover, by avoiding harsh thermal or chemical treatments that can lead to degradation or undesirable side reactions, PEF can contribute to extracts with higher purity and better functional characteristics [51,52].

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3.6. Reduced Solvent Use and Environmental Impact

The enhanced permeability of cells often means that less solvent or milder solvents can be used to achieve effective extraction. This reduces the chemical footprint of the process, making it more environmentally friendly [50,53].

4. How PEF Effects on Cell Membrane Aid Drying and Dehydration

Drying and dehydration processes aim to remove water from biological materials to extend shelf life and reduce weight/volume. Water is primarily located within cells and in the extracellular space. PEF's impact on cell membranes directly facilitates water removal:

Increased Cell Permeability to Water: The electroporation effect, by creating pores in the cell membranes, significantly increases the permeability of cells to water [54,55]. This means water can more easily move from the intracellular space, where it is tightly held, to the extracellular space and then to the surface of the material, from where it can evaporate during drying.

Enhanced Water Diffusivity: By compromising the cell membrane's barrier function, PEF pretreatment effectively increases the effective water diffusivity within the tissue. This means water molecules can migrate faster through the porous cellular structure towards the product surface for evaporation. This internal mass transfer is often the rate-limiting step in drying [56,57].

Reduced Drying Time: The improved water mobility directly translates to a significant reduction in drying time. For example, studies have shown PEF can reduce the drying time for carrots by up to 45%. Shorter drying times are advantageous for energy savings and increased throughput in industrial operations [58,59].

Lower Energy Consumption: Shorter drying times inherently lead to reduced energy consumption in energy-intensive drying processes like hot-air drying or freeze-drying. By replacing or reducing the need for prolonged thermal input, PEF contributes to more energy-efficient and sustainable dehydration [60,61].

Improved Product Quality

Preservation of Thermolabile Components: Similar to extraction, the non-thermal nature of PEF means that heat-sensitive nutrients, pigments (e.g., carotenoids), and volatile aroma compounds are better preserved compared to conventional high-temperature drying methods. This results in dried products with better nutritional value, color, and flavor [33].

Reduced Shrinkage and Enhanced Rehydration: By creating controlled pores rather than causing complete structural collapse, PEF can help maintain the cellular integrity and porosity of the material. This often leads to less structural shrinkage during drying and improved rehydration capacity of the dried product, as water can more easily re-enter the porous structure during rehydration [48,62].

Improved Texture: In some cases, PEF pretreatment can also lead to desirable textural changes, such as softening of plant tissues, which can be beneficial for subsequent cutting or further processing [63,64].

Facilitating Combination Technologies: PEF can be effectively combined with other drying methods, such as osmotic dehydration or freeze-drying, to enhance their efficiency. For instance, PEF can increase mass transfer during osmotic dehydration, accelerating water removal into a hypertonic solution. For freeze-drying, PEF can improve water removal by sublimation and reduce drying time [57,58,65]

In essence, by strategically weakening the cellular barrier through electroporation, PEF makes cells more "leaky" to water and target compounds, fundamentally altering the kinetics of mass transfer processes in both extraction and dehydration, leading to more efficient, higher-quality, and often more sustainable outcomes [45,59].

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5. Fish By-Products: From Waste to High-Value Resources

Global population growth and the associated demand for food, particularly high-quality protein, have brought significant attention to marine food sources over the last two decades. In developed nations, fish and seafood are primary dietary components. In 2017, combined wild catch and aquaculture fish production was approximately 175 million tons, with projections indicating an increase to 194 million tons by 2026. This figure excludes the capture of other marine organisms, such as mollusks and crustaceans [66,67].

According to FAO statistics, over 70% of all fish caught undergoes processing methods like canning, smoking, filleting, curing, and salting [68]. These processes generate substantial quantities of solid waste and by-products, often constituting 30% to 70% of a fish's total weight, depending on the species. Annually, more than 28 million tons of by-products, including heads, viscera, blood, and skin, are produced. Harnessing fish proteins from these by-products presents a significant opportunity to enhance their value and improve the efficiency of the fishing industry, while also mitigating environmental and sustainability concerns. Although fish and seafood processing by-products (e.g., skin, fins, viscera, backbone, and head) have potential uses in animal feed, human food, and other industries, they are frequently discarded into the environment, leading to organic pollution [69,70]. Given the environmental harm caused by such waste disposal, current research is focused on developing strategies to extract valuable compounds from these by-products for use in the food and pharmaceutical sectors, owing to their antimicrobial, antioxidant, and anti-inflammatory properties. While significant amounts of compounds like proteins, bioactive peptides, essential fatty acids, and pigments have been isolated from fish and seafood by-products, there remains a need for environmentally friendly and cost-effective extraction techniques [71–73].

Fish by-products typically contain 8% to 35% crude protein, and extracting these proteins can help alleviate environmental concerns associated with their disposal [74]. However, producing high-quality fish protein necessitates the development of appropriate extraction methods and the use of raw materials with high protein quality and content [74–76]. Due to fish protein's susceptibility to degradation from factors such as oxidation, denaturation, and excessive heat during traditional extraction methods, emerging extraction technologies have gained considerable attention in recent years. Furthermore, environmental considerations have spurred researchers to explore and optimize greener extraction approaches. Conventional chemical extraction methods are resource-intensive, contributing to ecological issues, while heat-based methods are often time-consuming and can compromise beneficial compounds. Consequently, novel techniques such as ultrasound (US), microwave assisted extraction (MAE), supercritical fluid extraction (SFE), PEF, and pressurized-liquid extraction (PLE) are being investigated for biomolecule recovery from various sources. These contemporary methods are generally more environmentally conscious and have demonstrated effectiveness in protein recovery [76–79].

PEF extraction from seafood by-products is influenced by multiple operational parameters. Key factors include the material-to-liquid ratio, electric field strength, pulse number, and pH conditions, all of which must be optimized for maximum recovery of target compounds [80]. For example, He et al. [23] investigated the extraction of chondroitin sulfate from fish bones using PEF and found that the extraction yield was significantly affected by all tested parameters. Under optimized conditions—material-to-liquid ratio of 1:15 g/mL, electric field intensity of 16.88 kV/cm, pulse number of 9, and NaOH concentration of 3.24%—a maximum yield of 6.92 g/L was obtained. Additionally, a short PEF treatment (5 minutes, 9 pulses) produced a substantially higher yield of chondroitin sulfate compared to conventional methods: 2.02 times greater than enzymatic extraction (4 hours), 1.84 times more than alkaline extraction (2 hours), and 1.42 times higher than ultrasonic extraction (23 minutes). Similarly, [81] optimized the PEF-assisted protein extraction from the viscera of fresh abalone (Haliotis discus hannai Ino). Key variables included electric field intensity (5–20 kV/cm), material-to-solvent ratio (3:1–10:1 W/V), and treatment duration (100–800 μs). The optimal yield of 39.99% was achieved at 20 kV/cm, 600 μs, and a 4:1 solvent ratio—outperforming enzymatic extraction (35.14%). Furthermore,

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the protein hydrolysates obtained via PEF demonstrated superior functional properties, including higher solubility (91.54%), greater degree of hydrolysis, and improved emulsifying capacity.

6. Protein Recovery and Functionality by Pulsed Electric Fields

In recent decades, PEF technology has gained considerable interest as a non-thermal method for food processing and preservation [22]. Firstly, PEF can effectively destroy living cells, including microorganisms, by facilitating the outflow of their contents and the influx of surrounding materials, leading to cell demise. Secondly, the increased permeability of plant and animal cells through PEF allows for more efficient and rapid extraction of intracellular components. This latter effect is valuable as a pre-processing step for recovering important cellular substances such as enzymes, sugars, and secondary metabolites [82–84].

PEF has demonstrated its utility in enhancing both the rate and yield of protein extraction, while also preserving the quality of the extracted proteins. Numerous recent studies have explored the use of PEF as a pre-treatment to improve protein recovery from a variety of plant sources, including soybean [85], beer waste [86], rapeseed stems and leaves [87], sesame cake [88], and macroalgae [89]. Additionally, PEF's application has been investigated for extracting proteins from animal-derived sources such as eggs [90], milk, chicken meat waste [91], beef [92], and seafood [22,23]. Compared to conventional methods, PEF significantly boosted protein extraction yields, ranging from 3.34% to an impressive 105%. These findings underscore PEF's potential as an effective method for protein extraction [22].

A study investigating the combined impact of PEF and enzymatic extraction on protein recovery from abalone waste (Haliotis discus hannai Ino) evaluated various processing parameters, including treatment duration, electric field intensity, and the solvent-to-material ratio. The optimal conditions—a solvent-to-material ratio of 4:1, an electric field strength of 20 kV/cm, and a treatment time of 600 μs—resulted in the highest protein extraction efficiency. Additionally, the combined treatment significantly enhanced the emulsifying properties of the extracted protein compared to enzymatic extraction alone. However, the application of PEF was associated with a reduction in the foaming capacity and viscosity of the recovered proteins [81].

Different conditions of PEF intensity strength, treatment time and the ratio of material to solvent of PEF-assisted enzymatic extraction of the abalone viscera protein (AVP) were studied in the article. Optimal PEF extraction conditions were achieved (PEF intensity strength of 20 kV/cm, treatment time of 600 μs and the ratio of material to solvent of 4:1). Detailed comparison of various properties (degree of hydrolysis, nitrogen yield, hydrolysis rate, function properties) of two AVPs was investigated. Application of PEF-assisted enzymatic extraction resulted in fully hydrolyzed, high AVP product yield and good emulsifying properties of AVP when compared to purely enzymatic extraction; however, the foaming properties and viscosity were reduced. In particular, the increase of solubility (91.54%) and the reduction of viscosity of AVP had a good effect on the improvement of solubility of spices, providing a theoretical basis for the development of abalone condiments. The AVP obtained by pulsed electric field-assisted enzymatic extraction had good hydrolysis (high solubility) and good properties (low foaming properties, viscosity and high emulsify properties [81].

PEF was also used to extract protein from mussel. Results show that PEF extraction speed is much faster, and the extraction yield of protein is higher compared with traditional methods. Variation of PEF parameters and the extraction yield of protein were determined by single-factor experiments. The processing conditions were optimized by response surface methodology. The maximum extraction yield of protein of 77.08% was achieved under the following conditions: electric field intensity of 20 kV/cm, pulse number of 8 and enzymolysis time of 2 h. PEF can be widely used to extract protein with high speed and low pollution [93].

Taurine is an amino acid, and product rich in taurine is widely used in the field of functional foods and medicines. There are many wild mussel resources which is inexpensive in the Songhua River Basin of Jilin Province. The wild mussel contains abundant taurine, but its processing utilization rate is low. In this paper, taurine was extracted by using a pulsed electric field (PEF)

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assisted enzymatic from mussel meat, and the single-factor test and response surface methodology were used to optimize the extraction process by using a central composite design (CCD). When a PEF assisted enzymatic was applied to the mussel meat, the cells ruptured, and substances such as taurine flowed out. A three-dimensional graphical surface and a two-dimensional contour map were constructed based on the RSM test results, which could intuitively reflect the influence of various factors and their interaction on the extraction of taurine. We could see that the results were consistent with the initial single-factor experiment. The optimal extraction conditions were the electric field strength of 25 kV/cm, the number of pulses of 10, and the enzymolysis time of 2.95 hr. The maximum taurine yield was achieved of 13.77 mg/g. Compared with enzymatic method; the taurine yield was significantly increased by 28.21% with the PEF-assisted enzymatic extraction process. Taurine could be effectively separated and purified by cation exchange and crystallization and recrystallization. Novelty impact statement PEF-assisted enzymatic method was used to extract taurine from mussel meat, and the taurine yield was 13.77 mg/g under the best conditions. The single-factor test and response surface methodology were used to optimize the extraction process by using a central composite design. Taurine could be effectively separated and purified by cation exchange chromatography and crystallization and recrystallization to obtain better purity and yield [93].

Application of 20 kV/cm electric field strength, 40–3000 Hz pulse frequency, of 2 μs pulse duration, with eight pulses, and a 2-hour enzyme reaction time, provided approximately 77% protein extraction from mussels. This performance was notably faster than traditional techniques involving NaCl, alkaline solutions, or enzymatic procedures. Furthermore, this method considerably reduced the processing time and labor required for protein extraction, establishing it as a more efficient and cost-effective option [93].

The influence of combining enzymatic and PEF extraction on taurine quality was investigated, and optimal processing parameters—an electric field intensity of 25 kV/cm, 10 pulses, and an enzymolysis duration of 2.95 hours—were identified. These conditions yielded the highest taurine concentration (13.77 mg/g), representing a 28.21% increase compared to the control. The authors concluded that the combined enzymatic–PEF extraction approach offers a promising strategy for producing high-quality taurine with enhanced yield and improved functional properties [94].

A recent study applied PEF technology to sea bass (*Dicentrarchus labrax*) side streams—specifically heads, skin, viscera, and muscle—using optimized treatment conditions tailored to each tissue type. Following PEF pre-treatment, extractions were performed using either 100% water or 50% ethanol. The results showed that both the solvent type and PEF treatment significantly influenced protein recovery in the liquid extracts, although a notable amount of protein remained in the solid residues. ICP-MS analysis revealed enhanced mineral recovery in head and muscle extracts following PEF treatment, while lower levels of heavy metals were detected in the liquid extracts, all within safe consumption limits [84].

Another study compared PEF and accelerated solvent extraction (ASE) methods for protein recovery from rainbow trout (*Oncorhynchus mykiss*) waste (head, skin, and viscera). ASE conditions involved a temperature of 45–55 °C for 15 minutes, a pH of 5.2–6.8, and a pressure of 103.4 bar. PEF extraction conditions included an intensity of 1–3 kV/cm, 123–300 kJ/kg, and a duration of 15–24 hours. Both ASE and PEF methods increased protein extraction efficiency from rainbow trout by 80%, with the highest yields observed from the head, skin, and viscera, relative to a control. For Dover sole samples, PEF significantly improved protein extraction from the skin and head compared to the control, although no significant increase was noted for rainbow trout in this specific comparison. Interestingly, the results also suggested that PEF facilitates the extraction of smaller protein molecules from fish skin but not the dissolution of larger protein molecules from sole skin [95].

The exact mechanism behind PEF-induced changes in protein distribution remains unclear. However, related research indicates that at low PEF intensities, protein molecules exhibit polarization. As the electric field intensity increases, their hydrophobic amino acids progressively become more exposed to the solvent. At moderately high electric field intensities, the final folded protein might transform into a complex held by weak covalent and non-covalent bonds. Studies have also revealed

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that PEF can modify the secondary structures of proteins, leading to a shift towards more β-sheets and away from α-helices. The alterations in protein bond distribution due to PEF are primarily linked to two aspects: One is that PEF accelerates protein breakdown by disrupting fish by-product cell walls; another hypothesis suggests that PEF makes the protein's hydrophobic amino acids more accessible, promoting further protein aggregation. These extraction procedures ultimately result in changes to the protein's molecular weight [81,94,96].

Protein extracts are frequently utilized in various industries for their antibacterial and anti-inflammatory properties. In line with this, the effects of proteins extracted by ASE and PEF methods on bacterial growth and anti-inflammatory activity was investigated. Their findings demonstrated that extracts from ASE-treated rainbow trout skin and PEF-treated sole viscera could function as natural antibacterial agents, effectively inhibiting the growth of pathogenic bacteria like *Staphylococcus aureus* and *Salmonella*. Moreover, the results indicated that both PEF and ASE extracts possess antimicrobial and probiotic characteristics, making them potentially useful for protecting food from spoilage and pathogenic bacteria, and for promoting the growth of beneficial probiotic bacteria (e.g., *Lactobacillus casei* growth with PEF and *Bifidobacterium lactis* growth with ASE). These insights suggest that fish extracts hold promise for applications in food preservation, probiotic supplements, and pharmaceutical development [95].

Pulse duration has been identified as a critical parameter for effective protein extraction from microalgae with intact cell walls [97,98]. A single pulse lasting 2×10^3 μs was found to be sufficient for efficient extraction, whereas shorter microsecond-range pulses failed to induce protein release, even when the total exposure time extended over several milliseconds. The efficiency of protein extraction improved with the number of applied pulses, with one cycle of 15 bipolar pulses resulting in a significant yield [83].

Protein release from microalgae occurred gradually. A substantial portion of the proteins was released within the first 30 minutes following PEF treatment, while more complete extraction was achieved after allowing the cells to incubate overnight at ambient temperature. Interestingly, despite the observed protein leakage, ultrastructural analysis did not reveal any visible damage to the cells, leaving the exact mechanisms of protein transport unclear. Beyond pulse characteristics, other factors such as the composition and concentration of the lysing buffer, the pH of the medium, and the temperature during PEF processing have also been shown to influence protein extraction efficiency from microalgal biomass [99,100].

Microalgae are recognized as a promising source of a wide array of functional compounds, including proteins, lipids, and pigments [101]. PEF has proven particularly effective in facilitating the release of ionic solutes, carbohydrates, and low-molecular-weight, water-soluble proteins [83,97,98]. However, the presence of a rigid and complex cell wall in many microalgae species presents a significant barrier to the recovery of larger intracellular compounds, such as high-molecular-weight proteins. Enhanced extraction of large water-soluble proteins has been reported in microalgal strains with weakened or absent cell walls [102,103]. The efficiency of protein recovery also depends heavily on process parameters, such as pH and extraction sequence. For example, the synergistic effect of PEF pre-treatment followed by alkaline extraction at various pH levels (8.5, 11, and 12) demonstrated that the highest protein yield, approximately 10%, was achieved at pH 12, highlighting the importance of optimizing extraction conditions when using PEF for microalgae biomass valorization [99,103].

7. Antioxidant Recovery Using Pulsed Electric Field Technology

Antioxidants are crucial bioactive compounds that mitigate oxidative stress by neutralizing free radicals, thereby playing a vital role in food preservation, human health, and disease prevention. In the context of sustainable food systems, the recovery of natural antioxidants from food industry by-products has become a focal area of research [74,105]. Fish processing residues, such as gills, heads, and bones, are often discarded despite being rich sources of proteins, peptides, and antioxidant compounds. Leveraging innovative, green extraction methods to valorize these low-value by-products can offer both economic and environmental benefits [49,106]. In this regard, a recent study

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explored the potential of PEF-assisted water extraction for enhancing antioxidant recovery from *Sparus aurata* (sea bream) and *Dicentrarchus labrax* (sea bass) residues. The results demonstrated that PEF treatment significantly improved the antioxidant capacity of water-based extracts compared to conventional water or methanol extraction. Antioxidant activity, evaluated through DPPH, ABTS, and FRAP assays, was markedly higher in sea bream residues than in sea bass, with DPPH values showing substantial increases in extracts from heads, bones, and gills—by 35.8%, 68.6%, and 33.8%, respectively, for sea bream, and 60.7%, 71.8%, and 22.1% for sea bass. Although methanolic extracts exhibited higher ORAC values, the overall antioxidant performance was superior in PEF-assisted aqueous extracts [49].

PEF was applied to various *D. labrax* processing by-products—specifically heads, skin, viscera, and muscle—using tissue-specific parameters: 1.0 kV/cm and 220.5 kJ/kg for heads; 3.0 kV/cm and 299.4 kJ/kg for skin; and 3.0 kV/cm and 123.7 kJ/kg for both viscera and muscle. This treatment was followed by extraction using either pure water or a 50% ethanol solution. The results showed that PEF significantly enhanced the total antioxidant capacity (TAC) of extracts from viscera, indicating a more efficient release of antioxidant compounds from this tissue. Both solvents proved effective in recovering valuable bioactive compounds from both the liquid extracts and solid residues. Overall, the findings highlight PEF as a sustainable and efficient technique for improving the recovery and valorization of nutritional and functional components from sea bass side streams [84].

Shrimp processing side streams represent an important natural reservoir of antioxidant carotenoids, with astaxanthin (ASX) being one of the most valuable due to its potent biological activity and its widespread use in aquaculture feed formulations [107,108]. While conventional extraction methods rely heavily on organic solvents, there is a growing interest in eco-friendly and innovative technologies such as PEF treatment to improve both yield and sustainability. In a recent study, PEF was applied using two parallel plate electrodes set 10 cm apart, with an electric field strength of 3 kV/cm, a specific energy input of 100 kJ/kg, and 74 pulses. The effects of PEF and ASE—both separately and in combination—were evaluated for their efficiency in extracting astaxanthin from shrimp by-products using two different solvent systems. The astaxanthin concentration in the resulting extracts was quantified using spectrophotometric and high-performance liquid chromatography (HPLC) analyses, with HPLC also used to characterize the carotenoid profile.

Results showed that both PEF and ASE significantly enhanced astaxanthin recovery compared to conventional solvent extraction, regardless of the solvent used. The highest yield, 585.90 μg/g, was obtained from *Aristeus antennatus* when dimethyl sulfoxide (DMSO) was used as the solvent in a sequential PEF-ASE process. Additionally, the antioxidant activity of the extracts improved, although the degree of enhancement varied depending on the solvent applied. HPLC profiling revealed a complex composition, including free (all-E) astaxanthin, four Z-isomers of unesterified astaxanthin, and a mixture of unresolved esterified forms. These outcomes confirm the potential of combining PEF and ASE as effective methods for maximizing the recovery of high-value carotenoids like astaxanthin from shrimp side streams, supporting the development of sustainable and value-added strategies in seafood processing [109].

8. Lipid Extraction Using Pulsed Electric Field Technology

Omega-3 fatty acids and carotenoids are among the most extensively studied bioactive compounds due to their substantial health-promoting properties. Omega-3 polyunsaturated fatty acids (PUFAs), particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have been associated with numerous physiological benefits, including cardiovascular protection, anti-inflammatory effects, cognitive support, and a reduced risk of chronic diseases such as diabetes, cancer, and neurodegenerative disorders. Similarly, carotenoids like β-carotene, astaxanthin, lutein, and zeaxanthin function as powerful antioxidants, protecting cells from oxidative stress and contributing to immune regulation, skin health, and eye function.

Seafood, especially marine organisms, serves as a rich and natural source of these essential bioactive compounds. In particular, underutilized seafood by-products such as heads, shells, and

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viscera contain significant quantities of high-value lipids and pigments. Valorizing these side streams not only supports sustainable processing practices but also enhances the economic value of seafood resources [110]. For instance, lipids extracted from the cephalothorax of Pacific white shrimp (*Litopenaeus vannamei*) have been shown to contain considerable amounts of omega-3 fatty acids, especially EPA and DHA, as well as carotenoids including β-carotene and astaxanthin [111]. Astaxanthin, a prominent carotenoid found in crustaceans, is recognized for its potent antioxidant activity—reported to surpass that of canthaxanthin, zeaxanthin, and lutein [112]. Due to its established antioxidative and anti-inflammatory properties, astaxanthin is commercially available in supplement form, particularly in capsule formulations [113].

Recent advances in extraction technologies have further enhanced the recovery efficiency of these compounds. In one study, shrimp cephalothorax was subjected to PEF pre-treatment at varying electric field strengths (4, 8, 12, and 16 kV/cm) and pulse numbers (120, 160, 200, and 240) to improve lipid extraction. Following PEF treatment, lipids were extracted using ultrasound-assisted extraction (UAE) at 80% amplitude for 25 minutes in continuous mode. The combination of PEF and UAE significantly improved lipid yield, reaching up to 30.34 g per 100 g of dry solids. Moreover, PEF pre-treatment contributed to reduced lipid oxidation, as evidenced by lower peroxide values (PV) and thiobarbituric acid reactive substances (TBARS). Lipids extracted under these conditions also showed increased concentrations of polyunsaturated fatty acids and carotenoids—including astaxanthin, astaxanthin mono- and diesters, canthaxanthin, and β-carotene—demonstrating the effectiveness of PEF-UAE synergy in enhancing both yield and quality of bioactive compounds from crustacean by-products [110].

PEF has been recognized as a highly effective technique for extracting intracellular compounds from microalgae. Application of extended-duration pulses (approximately 103 μs) was shown to permeabilize the plasma membrane and induce modifications in the cell wall structure. This treatment resulted in the gradual release of soluble cytoplasmic proteins while minimizing the formation of cellular debris, which often complicates downstream purification steps. By carefully tuning the PEF parameters, it is possible to preserve the integrity of internal organelles such as vacuoles, thereby reducing the release of proteolytic enzymes that could degrade target proteins [114].

A flow-based process designed to handle industrial-scale microalgal suspensions demonstrated the feasibility of this approach [80]. During passage through the pulsing chambers, each algal cell received an optimized number of electric pulses to maximize extraction efficiency. However, a key technical challenge was heat accumulation due to the Joule effect, necessitating the use of multiple pulsing chambers to maintain thermal control. Experimental findings confirmed that effective extraction of soluble cytoplasmic proteins required the application of long-duration square-wave pulses, rather than the summation of short ones [80,83]. One limitation of using long pulses is the occurrence of electrochemical reactions at the electrode surfaces. This issue was mitigated by employing pulse trains with alternating polarity and incorporating brief pauses (less than ~104 μs) between pulses [114].

Before PEF treatment to enhance lipid extraction from *Auxenochlorella protothecoides* microalgae water-soluble compounds were first removed from the PEF-treated biomass, after which 70% ethanol was employed to extract lipids from the residual material [115]. Similarly, it was demonstrated that PEF pretreatment significantly improved both crude lipid and fatty acid methyl ester yields from Scenedesmus species [116]. Additional research involving various microalgae has confirmed that PEF facilitates the enhanced recovery of a wide range of intracellular compounds, including proteins, carbohydrates, polyphenols, and carotenoids [89,117–121]. Collectively, these findings highlight PEF as a promising strategy—either as a standalone treatment or in combination with other techniques—for the efficient extraction of valuable bioactive compounds from microalgal biomass.

In *A. protothecoides* suspensions concentrated to 100 g dry weight per liter, rectangular pulses of 1 μs at 34 kV/cm enabled the immediate release of approximately 15% of the biomass into the extracellular medium. This water-soluble fraction included salts, sugars, amino acids, and soluble

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proteins. Intracellular lipid droplets, typically larger than 1 μm, remained within the cells due to their inability to traverse the permeabilized membranes and cell walls [122].

Following separation of the soluble fraction, lipids were extracted from the residual biomass using ethanol. This approach yielded a three- to fourfold increase in lipid recovery compared to untreated samples, achieving over 80% extraction of intracellular lipids on average [115]. The PEF treatment was conducted without prior washing, at a conductivity of 1 mS/cm, corresponding to that of the cultivation medium at harvest. The energy input required was 150 kJ per liter of suspension or 1.5 MJ per kilogram of dry biomass. Notably, increasing biomass concentration did not compromise extraction efficiency [122]; at 200 g/L, energy demand dropped to 0.75 MJ/kg, significantly lower than that of conventional methods. Moreover, PEF treatment did not generate cell debris, simplifying subsequent separation steps.

This energy-efficient fractionation approach enables the simultaneous recovery of both lipid-rich residues and high-value water-soluble products, contributing to the economic viability of microalgal biorefineries. By offsetting the energy costs of cultivation, PEF may also support the development of processing pathways with a favorable net energy balance for bioenergy applications [123].

Green microalgae (*Ankistrodesmus falcatus*) were subjected to PEF treatment using exponentially decaying high-voltage pulses characterized by a decay time of 360 nanoseconds. These pulses generated a uniform electric field with a peak intensity of 45 kV/cm within a treatment chamber composed of parallel stainless-steel electrodes. The effectiveness of the PEF treatment was evaluated through both lipid extraction efficiency and microscopic analysis of cells stained with propidium iodide. At a specific energy input of 26 MJ per kilogram of dry biomass, approximately 90% of the algal cells were lysed, resulting in a 130% increase in lipid yield compared to untreated controls [124]

In a separate investigation, *A. protothecoides* was treated using 1 μs square-wave pulses at an electric field strength of 35 kV/cm, also applied between parallel stainless-steel electrodes. This study utilized a maximum specific energy input of 2 MJ/kg (dry weight). The outcome was assessed by comparing lipid recovery from treated versus untreated samples. The results demonstrated that PEF application at this energy level led to a fivefold (500%) increase in lipid extraction efficiency [125].

9. Comparison of PEF with Alternative Extraction Technologies

A range of innovative technologies—such as US, MAE, enzymatic-assisted extraction (EAE), high-voltage electrical discharge (HVED), and PEF—have been extensively explored to enhance mass transfer and improve extraction efficiency. Among these, US technology utilizes ultrasonic waves to induce mechanical disruption via acoustic cavitation, where the rapid formation and collapse of microbubbles cause turbulence and shear forces that disrupt cell walls, thereby increasing the yield of intracellular compounds [126,127]. MAE operates by delivering electromagnetic radiation within the 300 MHz to 300 GHz frequency range. The interaction of microwaves with intracellular moisture results in rapid evaporation and subsequent internal pressure buildup, leading to cell wall rupture and the release of cellular contents [128,129]. In EAE, specific enzymes such as cellulase, pectinase, and hemicellulase enzymatically degrade the structural components of the cell wall, enhancing membrane permeability and facilitating the diffusion of target molecules [130]. HVED involves the application of high-energy electrical discharges through electrodes submerged in a liquid medium. These discharges create localized plasma, hot spots, and shock waves that mechanically disrupt cell structures, enhancing extraction efficacy [131].

Extraction yield is a critical economic metric for evaluating the industrial scalability of these pretreatment techniques. PEF, in particular, has demonstrated superior extraction yields compared to US, MAE, and EAE under equivalent energy input conditions [132–134]. For instance, it was reported that PEF outperformed both US and MAE in extracting ginsenosides from ginseng, offering higher yields with lower processing costs [135]. Similarly, PEF application at relatively low electric field strengths (0.2–1 kV/cm) significantly enhanced astaxanthin extraction from microalgae cultures, outperforming conventional mechanical (bead beating), ultrasound, and thermal methods [136]. In the case of turmeric (*Curcuma longa*), which is rich in bioactive curcuminoids [137–139]. EAE yielded

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the highest curcuminoid concentration (83.6 mg/g). However, PEF achieved a comparable yield (80.9 mg/g) with reduced energy consumption and shorter processing time [140].

Nonetheless, other investigations highlight the effectiveness of PEF in specific matrices. For example, PEF facilitated higher anthocyanin and total phenolic content (TPC) extraction from blueberry pomace compared to US and HVED [132]. These contrasting results suggest that extraction efficiency is highly dependent on the cellular architecture of the biological matrix. Overall, comparative assessments of these advanced pretreatment methods emphasize that extraction outcomes—such as yield, processing time, and energy efficiency—are highly product-specific. For example, ultrasound demonstrated the highest yields in microalgal matrices, whereas PEF showed greater efficacy in fruit-based extractions. Therefore, selecting an appropriate pretreatment method should be based on the specific characteristics of the target material and the quality attributes desired in the final product [31].

10. Conclusions

PEF technology represents a transformative, sustainable solution for enhancing the valorization of seafood processing by-products. By inducing electroporation, PEF significantly improves the permeability of cellular membranes, enabling higher yields and better preservation of valuable compounds such as proteins, omega-3 fatty acids, and antioxidants, while minimizing energy consumption and environmental impact. Evidence from recent studies highlights its potential to outperform traditional extraction methods in efficiency, selectivity, and product quality. Nevertheless, scaling up PEF for industrial applications faces critical challenges related to equipment design, process standardization, economic viability, and regulatory approval. To fully realize the benefits of PEF in seafood by-product valorization, future research should focus on optimizing process parameters, integrating PEF with other green extraction techniques, and developing comprehensive life-cycle assessments to support commercial adoption. Harnessing PEF's potential will not only advance circular bioeconomy objectives but also contribute to a more sustainable, resilient, and resource-efficient seafood sector.

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