

EFFECTS OF ULTRASOUND AND PULSED ELECTRIC FIELD IN EDIBLE INSECT PROTEINS - A REVIEW

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Abstract

The increasing global demand for sustainable protein sources amid environmental concerns related to conventional livestock production has necessitated the exploration of alternative food ingredients, particularly edible insects. This review investigates the effects of Pulsed Electric Field (PEF) technology and ultrasound on the extraction efficiency and functional properties of various insect proteins. The findings indicate that PEF treatment significantly improves protein and fat extraction yields, with higher intensities enhancing antioxidant activity. Conversely, ultrasound enhances the digestibility and solubility of extracted proteins, contributing to improved emulsifying and foaming characteristics essential for food applications. Comparative analysis shows that while both methods enhance protein recovery and functional qualities, PEF demonstrates superior results in optimizing extraction efficiency from insect sources like crickets, whereas ultrasound facilitates the production of high-quality protein isolates. Both technologies represent promising avenues for improving the viability and attractiveness of edible insects as sustainable protein sources, addressing the growing challenges of food security and environmental sustainability.

Keywords: Edible insect; Emerging technology; Protein; Pulsed electric field; Ultrasound

Introduction

In recent years, the negative environmental impact of conventional livestock, including its role in greenhouse gas emissions and the depletion of natural resources, has emerged as a critical global issue (Huis *et al.*, 2013). At the same time, it is predicted that global consumption of meat and dairy products will rise by 173% and 158% between 2010 and 2050 respectively due to population growth (Mazzocchi and Traill, 2016). To fulfill the

increasing need for innovative food components, it is important to investigate and create viable protein sources.

It is necessary to assess these fresh components for their characteristics as raw materials, their intended functionality, nutritional composition, and consumer acceptance (Pereira *et al.*, 2022). The innovative substitutes for sustainable protein sources are insects, microalgae,

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microbial cells, seaweed, duckweed, and rapeseed which are entered or anticipated to enter the human food markets (Van der Spiegel *et al.*, 2013). Plant-based proteins originate from a variety of plant sources, encompassing proteins from legumes, grains, nuts, seeds, and vegetables. These proteins play a crucial role in building and repairing the body's tissues, similar to animal proteins. They are particularly appreciated for their health advantages, as they generally have lower levels of saturated fats and cholesterol compared to proteins from animal sources, making them a beneficial option for the diet (Kyriakopoulou *et al.*, 2019). Nevertheless, there are drawbacks, such as the possibility of lacking certain amino acids, especially lysine and methionine, when depending exclusively on plant-based sources. Additionally, some plant-derived proteins may have anti-nutritional components, such as phytates, which can impede nutrient absorption (Karabulut *et al.*, 2023) and those plant-based proteins might be less easy to digest. Insects are the more efficient source of protein for example species like crickets which contain about 60% protein, along with healthy fats and essential minerals such as calcium and iron than conventional livestock (Wood and Tavan, 2022). All the essential amino acids needed for human nutrition are found in edible insects, making them an excellent source of protein and they have superior health benefits due to their high levels of vitamin B12, iron, zinc, fiber, omega-3 and omega-6 fatty acids, and antioxidants (Gravel and Doyen, 2020; Nowakowski *et al.*, 2022). Moreover, insects like crickets need six times less feed than cattle to produce the same amount of protein, along with emitting fewer greenhouse gases, which make them more environment friendly.

Proteins can have various non-nutritional roles in food, but their primary function is to create and maintain the distinct structure of each food item. The functional characteristics of proteins include their capacity to form and stabilize emulsions, foams, and gels. The emulsifying capability of proteins allows them to stabilize mixtures of oil and water. Additionally, the ability of proteins to gel is vital for achieving textures in products such as meat alternatives, where the protein framework mimics the fibrous structure found in animal products (Foegeding and Davis, 2011).

Insect processing techniques include blanching, which is a heat treatment used to decrease the microbial load of insects and deactivate naturally occurring enzymes, typically performed with hot water. Blanching leads to the denaturation of insect proteins, altering their properties along with the quality of fats (Larouche *et al.*, 2019; Leni *et al.*, 2019). Wet fractionation is the primary technique used to isolate soluble proteins from the

insoluble components that include various insect materials like insoluble proteins and chitin (Zhao *et al.*, 2016). The ability to extract proteins can be improved through further sonication treatment or the use of enzymes. Specifically, sonication-assisted extraction has been previously utilized for extracting proteins from traditional sources like soy, and it has also proven effective in extracting proteins from mealworms, crickets, and silkworms. (Choi *et al.*, 2017). An alternative approach is dry fractionation, which involves milling the raw material followed by fragmentation using air classification or tribo-electrostatic separation. The fractions produced from this method tend to be less pure than those obtained through wet fractionation, but they frequently exhibit high functionality (Schutyser *et al.*, 2015). Several innovative processing techniques have been assessed recently for edible insects to eliminate pathogenic microorganisms as an alternative to traditional blanching and to enhance techno-functional characteristics.

Improving the functionality of proteins through sustainable technologies can be challenging and may require alternative approaches (Pereira *et al.*, 2022). Several approaches have been proposed to raise awareness and acceptance of eating edible insects. Much thought was devoted to the various procedures and treatments meant to improve the nutritional profile and palatability of the insects in order to accomplish this goal (Mannozi *et al.*, 2023). Recently, ohmic heating (OH), pulsed electric field (PEF), high hydrostatic pressure (HHP), and ultrasound techniques were suggested for the above purpose. However, due to a lack of awareness regarding these advancements, sustainable protein sources are still being extracted and functionalized using inefficient processing techniques. The goal of the current study was to determine the most recent methods used on insect proteins in-order to increase understanding in this field and provide insights into how these approaches can improve the use of insects as food ingredients.

Insect Proteins

Insects are a significant source of protein with a protein content of 30% and 65% (Villaseñor *et al.*, 2022). Early humans most likely consumed insects previously (Van Huis, 2017). According to recent research, insects were a significant source of food in Tanzania 1.8 million years ago (Van Huis, 2003). As latitude rises, there is a typical gradient pattern in the decline of edible insects (Lesnik, 2017) because insects are more common and easier to collect in tropical areas (Van Huis, 2018). The most

commonly consumed insects are the house cricket (*Acheta domesticus*), mealworm (*Tenebrio molitor*), silkworm (*Bombyx mori*), wax worm (*Galleria mellonella*), European honeybee (*Apis mellifera*), and black soldier fly (*Hermetia illucens*) (Ortiz *et al.*, 2016). Insects are a rich source of high-quality protein, often containing 30% to over 80% protein by weight, depending on the species and preparation method. They provide all essential amino acids, making them a complete protein source comparable to meat and fish. This makes insect protein particularly valuable in addressing protein deficiencies among people (Hasnan *et al.*, 2023). Table 1 shows that most insects have a protein content around 60% and this value can range between 7% and 91% (dry weight) (Van Huis, 2016).

Moreover, insect proteins are 76% to 98% easier to digest than animal proteins (beef and egg white, 100%) and plant proteins (lentils and peanuts, 52%). According to (Roncolini *et al.*, 2019), insects are rich in essential minerals and vitamins such as zinc, potassium, iron, sodium, calcium, phosphorus, magnesium, riboflavin, biotin, folic acid, and pantothenic acid (Van Huis, 2013; de Castro *et al.*, 2018). The nutritional composition of different types of insects are discussed in the Table 2. In a healthy adult, proteins constitute 16.5% of the body and play a crucial role in delivering nitrogen, an essential nutrient. Since protein contains all of the

nitrogen, most studies use the Kjeldahl standard technique to quantify the amount of protein using the conversion factor of 6.25, which is widely accepted for foods. (Kim *et al.*, 2019). The exoskeleton of arthropods like insects primarily consists of polysaccharides, nitrogen, and chitin fibers, and the Kjeldahl method is typically employed to determine their crude protein content, which ranges from 8% to 70% of dry mass by measuring total nitrogen in it.

The creation of novel foods which have stable protein-based ingredients demand new and sustainable processing methods, paying much attention to the techno-functional properties of the raw materials, nutritional properties, and consumer acceptance (Zhang *et al.*, 2021). Recently ultrasound (Rahman and Lamsal, 2021), pulsed electric field (Vanga *et al.*, 2021) and ohmic heating (Ferreira-Santos *et al.*, 2021) have been suggested as an emerging green technology to determine the functionality of proteins, which is shown in Table 3.

Non-Thermal Technology

Traditional food preservation methods involves extremely high temperatures, which undoubtedly lowers the amount of contamination or microbiological load on the food but also causes some un-favorable changes in the food, such as the

Table 1. Protein content in edible insects

Type of insect	Insect name	Protein content (%)	References
Cricket	<i>Acheta domesticus</i>	71.7	(Udomsil <i>et al.</i> , 2019)
	<i>Gryllus bimaculatus</i>	58.32	(Ghosh <i>et al.</i> , 2017)
	<i>Teleogryllus emma</i>	55.65	(Ghosh <i>et al.</i> , 2017)
Larvae	<i>Gryllobates sigillatus</i>	56.8	(Hall <i>et al.</i> , 2017)
	<i>Tenebrio molitor</i>	56	(Zhao <i>et al.</i> , 2016)
	<i>Rhynchophorus phoenicis</i>	22.06	(Banjo <i>et al.</i> , 2006)
	<i>Gonimbrasia belina</i>	56.95	(Siulapwa <i>et al.</i> , 2014)
	<i>Hermetia illucens</i>	57.6	(Lu <i>et al.</i> , 2022)
Bee	<i>Apis mellifera</i>	22.1	(Mishyna <i>et al.</i> , 2019)
Dragon fly	<i>Aeschna multicolor</i>	54.24	(Ramos-Elorduy <i>et al.</i> , 1998)

Table 2. Nutritional composition of insects

Scientific Name	Protein	Fat	Fiber	References
<i>Gryllus bimaculatus</i>	58.32	11.88	9.53	(Ghosh <i>et al.</i> , 2017)
<i>Hermetia illucens</i>	34.9	27.93	-	(Vanga <i>et al.</i> , 2022)
<i>Apis mellifera</i>	21	14.5	2	(Banjo <i>et al.</i> , 2006; Ghosh <i>et al.</i> , 2016; Payne <i>et al.</i> , 2016)
<i>Analeptes trifasciata</i>	20.1	NA	3.3	(Banjo <i>et al.</i> , 2006)
<i>Gryllobates sigillatus</i>	70	18.23	3.65	(Zielińska <i>et al.</i> , 2015)
<i>Schidtocerca gregaria</i>	76	12.97	2.53	(Zielińska <i>et al.</i> , 2015)
<i>Musca domestica</i>	19.7	1.9	6.5	(Finke, 2013)
<i>Tenebrio molitor</i>	18.40	16.80	5.40	(Finke, 2015; Nowak <i>et al.</i> , 2016; Payne <i>et al.</i> , 2016)
<i>Acheta domesticus</i>	20.50	6.80	10.00	(Finke, 2002, 2015; Rumpold and Schlüter, 2013)

Table 3. Shows functional properties of protein

Insect	Method	Protein functionality	References
<i>Schistocerca gregaria</i> (grass hopper), <i>Apis mellifera</i> (honey bee)	Ultrasound	FC, FS, EC, and ES	Mishyna <i>et al.</i> , 2019
<i>Acheta domesticus</i> (house crickets)	Pulsed electric field	WHC, OHC, EC and FC	Psarianos <i>et al.</i> , 2022
<i>Hermetia illucens</i> (black soldier fly)	Ohmic heating	Emulsifying property	Queiroz <i>et al.</i> , 2021
	Ultrasound	Gelling property	Kumar <i>et al.</i> , 2022

loss of temperature-sensitive nutritional components, changes in the texture of the food due to heat, and modifications to the food's organoleptic characteristics (Hernández-Hernández *et al.*, 2019) additionally food bio-actives such as bio-availability, stability, functionality also affected (Meléndez-Martínez *et al.*, 2015). The modern consumer demands for a safe and hygienic without sacrificing its nutritional value or flavor. High quality, and minimally processed meals, non-thermal technology in food processing is a good substitute for conventional thermal processing (Barbhuiya *et al.*, 2021). Cold plasma, pulsed electric field, high hydrostatic pressure, ultrasound, and ozone technology are the promising emerging non thermal technologies. Numerous studies have shown how that the functionality of food proteins are impacted when food is processed using emerging technologies (Farjami *et al.*, 2021). Hydrodynamic properties such as texturization, gelation, and thickening; physicochemical features and properties such as shape, size, charge distribution, secondary, tertiary, and quaternary structures; molecular flexibility; and, lastly, topographical and physicochemical properties such as dispersibility, emulsification, foaming, wettability, solubility, and fat and flavor binding were used to assess the quality and functionality of proteins (Phillips and Williams, 2011) and also proteins can undergo conformational changes, such as the unfolding (partial or full unfolding) and displacement of hydrophilic and hydrophobic groups (Higuera-Barraza *et al.*, 2017).

The comparative analysis of various processing techniques with their mechanism, its effect on protein structure, techno-functional properties, pros and cons of the emerging technologies are summarized in the Table 4. In this paper, two novel emerging technologies namely ultrasound and pulsed electric field were briefly overviewed. PEF is highly effective in enhancing extraction yields of proteins from edible insects (Taha *et al.*, 2022). This non-thermal technology applies short bursts of high voltage to destabilize cell membranes, facilitating the release of intracellular components, including proteins,

without substantial thermal degradation (Psarianos *et al.*, 2022).

Ultrasound complements this by providing mechanical agitation that further aids in disrupting cellular structures, allowing for deeper penetration of solvents and enhancing the solubilization of proteins (Psarianos *et al.*, 2022). One of the significant advantages of PEF and Ultrasound is their ability to maintain the structural integrity and functional properties of proteins during extraction. Unlike thermal methods, which can denature proteins and alter their functional characteristics, both techniques operate at lower temperatures, thereby preserving bioactive compounds that could be lost during conventional heating (Pan *et al.*, 2022).

Ultrasound Processing Technology

Ultrasonic waves are defined as sound waves with a frequency greater than the threshold of the human ear, which is approximately 20 kHz (Chan and Perlas, 2011). It can be categorized into two categories based on the intensities: high and low-intensity ultrasound (Laugier and Haïat, 2011). High-intensity ultrasound disrupts and affects the mechanical, chemical, or biological properties of food at frequencies ranging from 20 to 500 kHz and intensities greater than 1 W/cm² (Chavan *et al.*, 2022). Low-intensity ultrasound uses power levels (usually less than 1 W/cm²) that are so low that the ultrasonic wave is destructive (McClements, 1995), meaning it doesn't alter the physical or chemical characteristics of material it passes through. Hydrodynamic shear forces and an increase in temperature at the location of bubble collapse are caused by the cavitation phenomenon (Zheng *et al.*, 2022) and have an impact on the physicochemical characteristics of proteins. Protein application in food systems can be improved by ultrasonic treatment, which has been regarded as a green technology due to its shorter processing times and low solvent requirements (O'donnell *et al.*, 2010). When ultrasonic waves are applied to a liquid, they usually produce microbubbles that quickly collapse

Table 4. Comparative analysis of different non-thermal technologies on proteins

Processing Technique	Mechanism	Effects on Protein Structure	Effects on Techno-Functional Properties	Pros.	Cons.
Pulsed Electric Field (PEF)	Polarization of protein molecules and release of free radicals can induce changes in protein structures and functionalities (Nunes and Tavares, 2019)	Increases soluble aggregates, promotes larger protein particle size (Zhang <i>et al.</i> , 2023)	Enhances viscosity and improves emulsification and foaming abilities (Zhang <i>et al.</i> , 2023).	- Improves functional properties of proteins (Xiang <i>et al.</i> , 2011) - Reduces heat-induced damage (Malik <i>et al.</i> , 2024)	- High initial cost - May not be suitable for all protein types (Malik <i>et al.</i> , 2024)
Ultrasound	Acoustic cavitation (the formation and collapse of air bubbles) induces chemical reactions and physical effects, which influence the structure and techno-functional properties of proteins (Hu <i>et al.</i> , 2022)	Alters protein aggregation and can denature proteins (Ommat Mohammadi <i>et al.</i> , 2023)	Improves solubility and emulsion stabilization, enhances textural properties (Ommat Mohammadi <i>et al.</i> , 2023)	- Increases protein solubility - Improves emulsification and foaming properties (Flores-Jiménez <i>et al.</i> , 2022)	- Effects can be highly variable - Scaling up challenges in implementation (Flores-Jiménez <i>et al.</i> , 2022)
High Pressure Processing (HPP)	Protein unfolding can occur due to the penetration of water into the protein matrix (Liu <i>et al.</i> , 2019)	Modifies protein solubility and water/oil binding capacities (Ommat Mohammadi <i>et al.</i> , 2023)	Improves gelling properties and maintains nutritional value (Ommat Mohammadi <i>et al.</i> , 2023)	- Maintains nutritional value - Extends shelf life - Effective at inactivating pathogens (Abera, 2019)	- Expensive equipment - Limited applicability to certain foods (Abera, 2019)
Cold Plasma	At the molecular level, exposed proteins interact with electromagnetic energy. Then, heat is generated from the electromagnetic energy through the motion of molecules during treatment proteins (Hu <i>et al.</i> , 2022)	Changes secondary structure from α -helices and random coils to β -sheets (Ommat Mohammadi <i>et al.</i> , 2023)	Alters interfacial properties, enhancing emulsification and surfactant properties (Ommat Mohammadi <i>et al.</i> , 2023)	- Effective microbial inactivation - Enhances protein properties (Pan <i>et al.</i> , 2022)	- Limited penetration depth - Requires optimization for specific proteins (Pan <i>et al.</i> , 2022)
Microwave	Several high-energy radicals, such as nitric oxide, atomic oxygen, superoxide, and hydroxyl radicals to break the covalent bonds and promote several chemical reactions (Ji <i>et al.</i> , 2018)	Changes in the secondary structure. Protein aggregation (Cao <i>et al.</i> , 2019)	Gelling properties improved (Solaesa <i>et al.</i> , 2021)	- Fast processing - Retains nutrients (Chandrasekaran <i>et al.</i> , 2013)	- Risk of uneven heating - Requires specialized packaging (Chandrasekaran <i>et al.</i> , 2013)

with a subsequent wave propagation that causes a high shearing effect, also known as cavitation (Rahman and Lamsal, 2021). The process can break non-covalent bonds, which unfold proteins without significantly altering amino acid composition. Figure 1 illustrates the process involved in ultrasound processing and its effects on insect proteins.

Comparing the technique to conventional methods, there are number of advantages: higher efficiency, treatment uniformity, affordability,

quality, and functionality of processed material (Gharibzahedi and Smith, 2020).

Applications of Ultrasound processing technology:

Ultrasound processing not only enhances extraction efficiency but also positively impacts the functional properties of insect proteins. This includes improvements in solubility, emulsification, and foaming capabilities, which are essential for various food applications (Ding *et al.*, 2022).

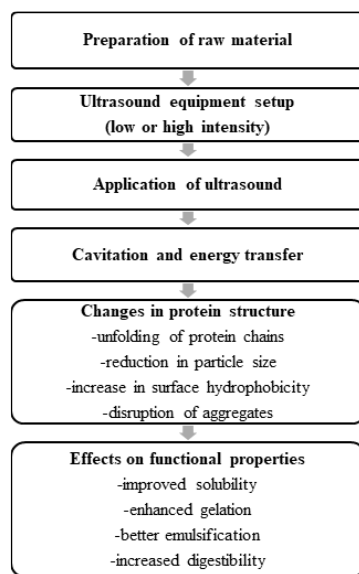


Figure 1. Shows the process involved in ultrasound processing and its effect on insect proteins

The use of ultrasound can improve the nutritional profile of insect proteins by increasing the availability of essential amino acids and bioactive compounds. The technology has been reported to alter the molecular characteristics of proteins, thereby enhancing their digestibility and accessibility in food applications (Liang *et al.*, 2024).

Studies have shown that ultrasound can significantly improve the emulsifying properties of cricket protein, enhancing its application in salad dressings and sauces (Pan *et al.*, 2022). Ultrasound has been shown to enhance the water-holding capacity (WHC) and gel strength of soy protein isolate when induced by calcium sulfate. This enhancement is crucial for the production of meat analogs and other texturally demanding food products. The ultrasound-assisted gelation process results in denser gel networks, which contribute to improved mouthfeel in finished products (Taha *et al.*, 2024). Further investigation into the applications of PEF technology in optimizing insect protein properties for industrial food-processing contexts is recommended.

Effects of Ultrasound in Insect Proteins:

During ultrasonic treatment, the Black Soldier Fly Larvae (BSFL) protein extract was assessed and determined to be 35.67% and 62.24%, respectively. Till 15 minutes of ultrasonic treatment, the particle size dramatically increased to 245.3 ± 1.0 nm alone and decreased to 197.1 ± 1.1 nm after 30 min of ultrasound treatment. The results obtained for the increase in particle size of BSFL protein are

comparable to the dispersions of black bean protein isolate as reported by (Jiang *et al.*, 2014). As the power of the ultrasonic treatment is increased, however, the particles become smaller, leading to a broader particle size distribution. This phenomenon occurs because the unstable aggregates are violently broken down into smaller, soluble protein aggregates due to the intense forces of cavitation generated during the ultrasonic treatment. All the dispersions have ζ -potentials between -20 and -30 Mv, which indicates that they are slightly stable (Cano-Sarmiento *et al.*, 2018). The protein dispersion's absorbance significantly increased to 0.554 ± 0.005 after 15 minutes of ultrasonic therapy exposure. This can be explained by the fact that the turbidity increased as a result of the aggregate formation and the increase in particle size. After 15 minutes of ultrasonic treatment, the surface hydrophobicity increased significantly to 617.9 ± 17.59 . This finding is consistent with the results observed in the dispersions created from soybean protein isolate (Zhao *et al.*, 2021), which showed an increase to around 200 and 225 after 5 and 15 minutes respectively, in contrast to the untreated sample, which was approximately 95. After 30 minutes of treatment, the gel was found to have the maximum water retention capacity, measuring $96.02 \pm 0.28\%$, which is similar to the results obtained by (Zhang *et al.*, 2023) regarding the protein from *Tenebrio molitor* larvae, which showed that as ultrasound treatment duration increased, the water retention capacity initially rose before declining, reaching its peak at the 30-minute mark ($P < 0.05$). The physicochemical and functional properties of the BSFL protein were altered by ultrasound treatment. These changes are strongly linked to molecular alterations, primarily an increase in the exposure of hydrophobic groups and particle size variation (Kumar *et al.*, 2022). The exceptionally high in vitro digestibility value of 99.79% attained with ultrasound-assisted extraction of BSFL was ascribed to higher solubility and looser surface morphology (Xu *et al.*, 2023).

The ultrasonic extraction can yield high quantities of protein from Bombay locusts. Protein recovery and solid yield are important metrics for assessing the efficiency of this extraction process (Karnjanapratum and Benjakul, 2020). The ultrasonic conditions had a substantial effect on the extract's functional and antioxidative capabilities. Ultrasound Assisted Process (UAP) was found to considerably boost the yield of BL solids in all evaluated conditions ($p \leq 0.05$). Additionally, the use of amplitude enhanced BL's protein recovery (12.03-15.57%), UAP's considerably ($p \leq 0.05$) improved foaming.

Functional Properties of Insect Proteins Using Ultrasound Technology

Ultrasound processing technology significantly enhances the functional properties of insect proteins, improving their solubility, emulsification, gelation, and overall nutritional quality. This technology leverages ultrasonic waves to modify protein structures and interactions, resulting in improved performance in various food applications.

Solubility

Protein solubility refers to the proportion of proteins that are in a soluble state under specific conditions (Haque *et al.*, 2016). In general, the solubility of insect proteins is pH-dependent with the lowest solubility at the isoelectric point which lies, for instance, in the region of pH 4 for mealworms and black soldier flies (Bußler *et al.*, 2016). Notable variations in the solubility of proteins were identified for Bombay locust when extracted using ultrasound-assisted processing (UAP) at different amplitudes (40%, 50%, and 60%) across various pH levels. The peak protein solubility for Bombay locusts occurs at certain pH values, with levels reaching nearly 99% at pH 2. However, this solubility diminishes at elevated pH levels, particularly dropping to roughly 56% at pH 4, which is approaching the protein's isoelectric point, resulting in increased precipitation (Kingwascharapong *et al.*, 2021). The use of sweep frequency ultrasound in the preparation of proteins and hydrolysates from black soldier flies indicated a substantial enhancement in thiol value and re-constitutability across the pH range of 2-10 (Mintah *et al.*, 2020). Such advancements in solubility are crucial for the incorporation of insect proteins into food products, ensuring they can effectively enhance the nutritional profile of formulations (Queiroz *et al.*, 2023).

Emulsifying properties

The effects of ultrasonic pretreatment on the emulsifying properties of *Clanis Bilineata* Tingtuaica Mell protein found that applying ultrasound significantly reduces protein particle size, increases surface hydrophobicity, and strengthens the protein network, which enhances emulsion stability. Particularly at an optimal power level of 400 W, the emulsifying capacity improved from 4.16 to 27.62 m²/g, demonstrating the efficacy of ultrasound in promoting better emulsification. The resulting emulsions exhibited greater stability under stress conditions, suggesting that ultrasound could enhance the functional applications of this insect protein in the food industry (Wang *et al.*, 2021). This enhancement occurs due to the

reduction of protein particle size and the alteration of surface characteristics during treatment, which increases the proteins' ability to stabilize oil-water interfaces. Improved emulsification is critical for the formulation of stable emulsions in products such as sauces and dressings, which often require high emulsifying capacity (Queiroz *et al.*, 2023).

Gelling properties

The ultrasound treatment affects the gelling properties of proteins from the black soldier fly (*Hermetia illucens*). It enhances the gelling ability of these proteins by promoting molecular interactions and structural transformations. This process facilitates the denaturation and unfolding of protein molecules, leading to improved gel formation. Ultrasound treatment also assists in reducing particle size, which increases the surface area for gelation and enhances the protein's ability to stabilize emulsions. This property is particularly beneficial for developing meat alternatives and other texturized food items where specific mouthfeel and consistency are desired (Mishyna *et al.*, 2021; Xu *et al.*, 2023).

Foaming properties

The ultrasound treatment significantly improves the foaming ability of grasshopper and honeybee insect proteins by promoting protein denaturation and increasing the exposure of hydrophobic amino acids, which enhances air incorporation during foaming. Additionally, ultrasound reduces protein aggregate size, leading to more stable foams due to increased surface area and better interfacial film formation (Mishyna *et al.*, 2019). Table 5 provides a summary of the effects of ultrasound on various insect species, highlighting the diverse findings across different parameters.

Challenges and limitations of ultrasound in insect proteins

One significant challenge of ultrasound technology is the potential for protein structure degradation during the extraction process. High-intensity ultrasound can lead to the formation of free radicals and thermal effects, altering protein structures and potentially affecting their functional properties. This raises concerns about the quality of the extracted proteins, as undesirable modifications could affect their nutritional value and functional applications (Liang *et al.*, 2024). The extraction efficiency of ultrasound-assisted processes can differ significantly among various insect species, which may require different ultrasound configurations for optimal results. This variability necessitates comprehensive studies that explore the specifics of ultrasound application for each insect

Table 5. Effects of ultrasound treatment on insect species

Insect	Parameter	Findings	References
Black Soldier Fly Larvae (BSFL)	Protein Extraction Yield	35.67% and 62.24% protein extraction efficiencies.	Kumar <i>et al.</i> , 2022
	Particle Size	Increased to 245.3 ± 1.0 nm after 15 minutes of ultrasonic treatment.	Jiang <i>et al.</i> , 2014
	ζ -Potential	Ranged between -20 and -30 mV, indicating moderate stability.	Cano-Sarmiento <i>et al.</i> , 2018
	Surface Hydrophobicity	Increased to 617.9 ± 17.59 after 15 minutes.	Zhao <i>et al.</i> , 2021
	Water Retention Capacity	Maximum capacity measured at $96.02 \pm 0.28\%$.	Amiri <i>et al.</i> , 2018
	In Vitro Digestibility	Achieved a high digestibility value of 99.79%.	Xu <i>et al.</i> , 2023
Bombay Locusts	Protein Recovery	Ranged from 12.03% to 15.57% with ultrasonic extraction.	Karnjanapratum and Benjakul, 2020
	Yield of Solids	Considerably boosted in all evaluated conditions ($p \leq 0.05$).	Karnjanapratum and Benjakul, 2020
	Foaming Capacity	Improved to 140.00-150.00% at 40% amplitude but decreased at 60%.	Kingwascharapong <i>et al.</i> , 2021
	Emulsion Activity Index	No negative impact under all test conditions.	Kingwascharapong <i>et al.</i> , 2021
Clanis Bilineata Tingtauica Mell (CBTM)	Emulsion Stability	Maintained stable interfacial layer even at 500 mM NaCl.	Wang <i>et al.</i> , 2021
	Emulsification Results	Best results observed at a power intensity of 400 W.	Wang <i>et al.</i> , 2021

type to ensure reliable extraction outcomes. Current research emphasizes the need for species-specific protocols that consider these differences (Pan *et al.*, 2022). Limited research results in a lack of consensus regarding optimal extraction methods, which can hinder its broader acceptance and application in commercial settings. More in-depth studies are essential to understand the full potential and limitations of ultrasound technology in this field.

Pulsed Electric Field Technology

The primary drawback of PEF technology is the potential for bubbles to form during treatment, which could lead to inconsistent treatment and operational issues. Furthermore, commercial PEF units are not generally accessible in many parts of the world. However, PEF is currently gaining popularity due to its eco-friendly methodology and numerous applications in the biotechnology and food industries. For example: It can be used for inactivation of microbes (Wan *et al.*, 2009) enhancing the kinetics of drying and extraction (Shorstkii *et al.*, 2022) and enhancing the functional characteristics of proteins (Han *et al.*, 2018). More protein recovery was obtained when pulsed electric field extraction was used at lower temperatures, longer pulse durations, and higher field intensities (Kumar *et al.*, 2021). Modifications in the structure of proteins can affect their functional characteristics, such as solubility, apparent viscosity, emulsifying, foaming, gelling, and

immunoreactivity, which are crucial in the processing of food (Zhang *et al.*, 2019). Figure 2 illustrates the process of pulsed electric field (PEF) technology and its effects on insect proteins. PEF has numerous benefits over alternative methods (Gómez *et al.*, 2019), such as quick processing times and a waste-free procedure. Reduced energy use; ecologically friendly method; enhanced nutrient, flavour, and colour retention; and appropriateness for processing foods that are heat-sensitive.

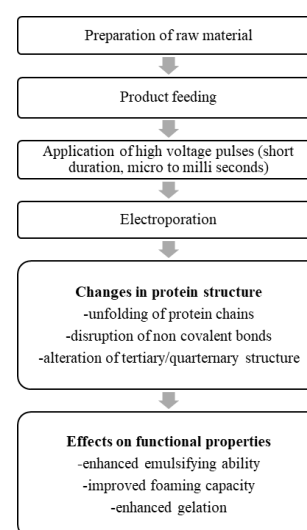


Figure 2. Shows the process involved in pulsed electric field processing and its effects on insect proteins

Applications of Pulsed electric field technology

Pulsed Electric Field (PEF) technology has diverse applications in the processing of edible insects. PEF also improves oil extraction from insect material. The application of electric fields enhances lipid release from insect cells, which can be particularly beneficial for sourcing oils from fatty insect species like the Black Soldier Fly (Alles *et al.*, 2020). PEF-treated proteins often exhibit improved emulsifying and foaming capabilities, which are crucial for various food applications. This enhancement allows for the effective use of insect-derived proteins in products such as sauces, dressings, and nutritional supplements, thereby expanding the market potential for insect protein products (Psarianos *et al.*, 2022).

It has been shown to at a temperature range of 25-45 °C has been investigated. A moderate electric field treatment at 20 V was found to reduce the interfacial tension at the sunflower protein solution/water interface (Subaşı *et al.*, 2021). The application of PEF 20V/cm during the holding phase and 80 V/cm during the heating phase, at a frequency of 20KHz for 5 to 7 minutes, results in alterations to the secondary structure of β -lactoglobulin (Rodrigues *et al.*, 2019) which is an whey protein isolate. While insect protein is unique in its source, it shares similarities in terms of amino acid profile and nutritional value with other protein sources like pea protein, soy protein, fish protein, etc. In pea protein isolate, treatments with PEF of 5, 10, and 20 V/cm at frequencies of 50 Hz and 20 kHz were examined. Specifically, a moderate electric field treatment at 50 Hz and 20 V/cm resulted in increased surface hydrophobicity and enhanced gelling properties of pea protein (Chen *et al.*, 2022). The effects of PEF on the functional properties of soybean protein isolates significantly enhances solubility, with increases observed from 70% to 90% at an electric field strength of 20 kV/cm. The gelling properties improve as well, with gel strength rising by approximately 40% compared to untreated samples. Additionally, emulsifying activity increases by about 30%, while foaming capacity can reach up to 120% higher than that of untreated proteins (Li *et al.*, 2007).

Effects of Pulsed Electric Field in Insect Proteins

PEF was applied to house crickets (*Acheta domesticus*) under various conditions between (4.9-49.1 kJ/kg). The PEF treatment at 4.90 kJ/kg increased the protein (>18%) and fat (>40%) extraction yields; on the other hand, the treatment at 24.53 kJ/kg increased the cricket flour's oil binding and emulsifying capacity as well as its antioxidant activity by 28.10, 64.88, and 58.20%, respectively.

The PEF treatment had no effect on the ability to bind water or foam it (Psarianos *et al.*, 2022).

The PEF-treated *Hermetia illucens* larvae, sample at 3 kV/cm (PEF1) had a lower crude protein content $59.3 \pm 2.65a$ (g/100g) than the control sample at 2 kV/cm (PEF 2) which is $63.5 \pm 1.66a$ (g/100g). The two PEF-treated samples (PEF1 and PEF2) appear to have had lower crude protein contents as a result of the stronger electric field. This could be explained by the possibility that the combination of increased PEF intensity and mechanical expression at high temperatures could alter the structure of the proteins (e.g., denaturing them), which would decrease their extractability (Alles *et al.*, 2020). Pulsed electric field (PEF) treatment (40 kJ/kg) significantly increased the antioxidant activity of black soldier fly larvae dried using either convective drying (CD) or infrared-convective drying (IR-CD). Fat extraction was influenced by PEF intensity ($\eta^2 = 0.992$, $p < 0.001$) and drying method ($\eta^2 = 0.724$, $p < 0.001$) and it was noted that extraction yield increased as the intensity of the PEF treatment increased. Protein content was higher in CD-dried samples but could be increased with low PEF intensities. Higher intensities decreased protein content (Bogusz *et al.*, 2023).

PEF has been used in a variety of fields of study, such as cell disintegration, extraction, drying, and microbial inactivation. (Shorstkii *et al.*, 2022). Drying is one of the most important steps in processing of edible insects biomass for their potential utilisation in food applications. Drying typically denatures proteins, increasing their water-binding capacity (Ye *et al.*, 2024). This is due to the reduced solubility of denatured proteins, which allows them to bind more water. Pulsed electric field (PEF) treatment significantly improves the drying rate of edible insects, particularly at high moisture contents. For example, after 20 minutes of drying, *Hermetia illucens* larvae treated with 20 kJ/kg PEF had a moisture ratio (MR) of 0.72, compared to 0.86 for untreated larvae. While PEF treatment was less effective at low moisture ratios or with other insect species, it did not affect the water-binding properties of dried insect biomass. In fact, PEF-treated samples often exhibited the same or even higher hygroscopic properties as untreated samples (Bogusz *et al.*, 2022).

Both Ultrasound and Pulsed Electric Field (PEF) technologies serve as effective non-thermal methods for enhancing protein extraction from insects, yet they operate through distinct mechanisms and yield unique benefits. Ultrasound employs cavitation to disrupt cellular structures, resulting in a high extraction yield of proteins and

lipids from various insect species, such as black soldier fly larvae, and significantly improving the quality of the extracted protein by enhancing its digestibility and solubility (Xu *et al.*, 2023). In contrast, PEF utilizes high-voltage electric pulses to increase the permeability of insect cell membranes, leading to enhanced extraction efficiency while improving the functional properties of proteins, including emulsification and foaming capabilities. While Ultrasound extraction has shown remarkable results in producing high-quality protein powders and isolates. Unlike PEF, UAP considered to be a gentler extraction method, which helps preserve the structural integrity of proteins. This may lead to better nutritional profiles and functionality in the final protein products (Kozlu *et al.*, 2024). PEF has been highlighted for its superior results in increasing both protein and fat yields from insect sources like crickets. Both PEF and UAP have been shown to significantly improve protein extraction yields compared to traditional methods, offering sustainable and efficient alternatives for processing insect proteins. Together, both technologies demonstrate considerable potential for optimizing insect protein production, addressing the growing demand for sustainable protein sources in human and animal nutrition. The summary of the effects of pulsed electric field technology on insect species are given in Table 6.

Functional Properties of Insect Proteins Using Pulsed Electric Field Technology

Emulsifying properties

The effects of PEF on the emulsifying properties of cricket (*Acheta domesticus*) flour find that PEF treatment enhances the emulsifying capacity of cricket protein by promoting structural changes that expose hydrophobic sites, facilitating better interaction with oil and water phases. Specifically, applying PEF at optimal energies significantly increases protein extraction yields while simultaneously improving emulsification. The treated cricket flour showed enhanced stability of emulsions due to smaller droplet sizes and stronger interfacial films, indicating that PEF is an effective technique for optimizing the functional properties of insect proteins for food applications (Psarianos *et al.*, 2022).

Gelling properties

Pulsed electric field (PEF) treatment has been shown to significantly enhance the gelling properties of edible insect proteins, particularly those derived from *Tenebrio molitor* (mealworms) and *Gryllus campestris* (crickets). For instance, PEF increases gel strength and reduces gelation time, with notable improvements shown at specific electric field strengths. In studies, the gelation temperature of *Tenebrio molitor* proteins ranged between 51 and 63 °C, with optimal performance observed at higher temperatures. Additionally, final

Table 6. Effects of pulsed electric field treatment on insect species

Insect Species	PEF Conditions	Key Findings	References
House Crickets (<i>Acheta domesticus</i>)	4.9 - 49.1 kJ/kg	- At 4.90 kJ/kg: Protein and fat extraction yields increased by >18% and >40%, respectively. - At 24.53 kJ/kg: Increased oil binding (28.10%), emulsifying capacity (64.88%), and antioxidant activity (58.20%). - No effect on water binding or foaming ability.	Psarianos <i>et al.</i> , 2022
Black Soldier Fly Larvae (<i>Hermetia illucens</i>)	3 kV/cm (PEF1) vs. 2 kV/cm (PEF2)	- PEF1 (3 kV/cm) showed lower crude protein content (59.3 ± 2.65 g/100g) than PEF2 (2 kV/cm) with 63.5 ± 1.66 g/100g.	Alles <i>et al.</i> , 2020
Black Soldier Fly Larvae (<i>Hermetia illucens</i>)	40 kJ/kg	- increase in antioxidant activity with both convective and infrared-convective drying methods. - Fat extraction yield increasing with higher PEF intensity. - Protein content was higher in CD-dried samples but could decrease with high PEF intensities	Bogusz <i>et al.</i> , 2023
General Application of PEF in insects	Various conditions	- PEF is used for cell disintegration, extraction, drying, and microbial inactivation. - For <i>Hermetia illucens</i> , 20 kJ/kg PEF reduced moisture ratio (MR) from 0.86 to 0.72 after 20 minutes of drying.	Shorstkii <i>et al.</i> , 2022; Bogusz <i>et al.</i> , 2022

gel strength values can vary significantly, emphasizing the importance of optimizing PEF parameters to achieve desirable gelling characteristics in edible insect proteins (Taha *et al.*, 2022).

Oil-binding properties

The oil-binding capacity of cricket (*Acheta domesticus*) flour reveals that PEF treatment significantly enhances the oil-binding capacity of cricket protein, increasing it from 4.27 g/g to 6.02 g/g, an improvement of approximately 41.8%. This enhancement is attributed to PEF's ability to promote structural changes in the protein, exposing more hydrophobic sites that facilitate better interaction with oils. (Psarianos *et al.*, 2022).

Challenges and limitations of pulsed electric field in insect proteins

One of the primary challenges in utilizing PEF for insect protein extraction involves the optimization of process parameters, such as electric field strength, pulse duration, and temperature. Finding the ideal combination to maximize extraction yield while minimizing protein degradation is complex. Research indicates that the protein extraction research on PEF process parameter optimization should be broadened to promote the applications in a large-scale manner (Ramaswamy *et al.*, 2024). PEF can potentially lead to structural changes in proteins, which may affect their functionality. Studies have shown that PEF treatments at high strengths could change the structure of proteins, causing denaturation and loss of functional properties such as solubility and emulsification (Taha *et al.*, 2022).

Conclusions

The review emphasizes the significant potential of non-thermal technologies, specifically Ultrasound Processing and Pulsed Electric Field (PEF) technologies, in enhancing the extraction and functional properties of insect proteins. Both ultrasound and PEF are proven to increase protein and lipid extraction yields from various insect species, such as Black Soldier Fly larvae and crickets. This is crucial for maximizing the utilization of insect biomass in food applications. Unlike traditional thermal methods, these non-thermal technologies preserve the structural integrity of proteins, enhancing their digestibility, solubility, emulsification, and foaming capabilities, which are vital for various food formulations.

The improvements in technical functionalities of insect proteins position them as attractive

ingredients for developing high-quality, sustainable food products, including protein powders, nutritional supplements, sauces, and dressings. As consumers increasingly demand safe, nutritious, and minimally processed food options, the application of these technologies presents a promising solution. They align with consumer preferences for high-quality proteins that maintain flavor, nutritional value, and safety.

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