DEPARTMENT OF AGRICULTURAL, FOOD AND
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DEGREE COURSE: FOOD AND SCIENCE TECHNOLOGY

STUDY OF THE EFFECT OF EMERGING FOOD TECHNOLOGIES ON NOVEL FOODS: PULSED ELECTRIC FIELD AND EDIBLE INSECTS

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SUMMARY

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ACRONYM AND ABBREVIATIONS

PEF	Pulsed Electric field.
MR	Moisture ratio.
EHD	Electrohydrodynamic.
TPC	Total polyphenol content
GHG	Greenhouse gases
EU	European Union
FAO	Food and Agriculture Organization of the United Nations

INTRODUCTION AND PURPOSE OF THE THESIS

In recent years, food industry, has undergone significant transformations driven by the need to create innovative and sustainable technologies to develop and implement the existing food and the less known nourishment sources.

The aim of the Emerging food technologies is to improve food quality and safety; among these, the Pulsed electric field (PEF) technology represents one of the most promising innovations. Thanks to the work of the researchers, PEF is already being applied in various fields in the industry and has still room to improve.

At the same time, novel foods, represent an alternative food source that could integrate and diversify the human diet. Edible insects, represent one of the most important subjects of study due to their great potential, given the huge source of macronutrients that can be obtained from the processing of this natural resource (Ojha *et al.*, 2021).

This thesis, reviews, the impact of the PEF technology has on the processing and the nutritional and techno functional proprieties of the edible insects.

Chapter 1

PULSED ELECTRIC FIELDS TECHNOLOGY (PEF)

1.1 **Principles of Pulsed Electric Fields**

Pulsed Electric Field (PEF) is a non-thermal food processing technique that has gained significant attention, in recent years, from food technologists and industry professionals as an innovative and promising technology. PEF operates by applying high-voltage, short-duration electrical pulses to biological materials, including plants, animals, and microorganisms, which are positioned between two electrodes. This method offers a unique approach to food processing, as it enhances the preservation of nutritional and sensory qualities without relying on heat. As a result, it is being increasingly explored for its potential to improve food safety, extend shelf life, and reduce energy consumption in the production of a wide range of food products. The versatility of PEF technology also makes it applicable to various sectors within the food industry, offering potential benefits in everything from juice extraction to microbial inactivation and texture modification (Raso *et al.*, 2016).

Process parameters that define Pulsed Electric Field (PEF) technology include the amplitude of electric pulses (U), electric field strength (E), treatment duration (t), pulse shape, pulse width (τ), number of pulses (n), pulse-specific energy (W), and pulse repetition frequency (f). Among these, electric field strength and treatment time are the two most critical factors that determine the overall intensity and effectiveness of the PEF treatment (Raso *et al.*, 2016). Electric field strength refers to the magnitude of the electrical field within the treatment chamber during the processing of the sample. This parameter is influenced by several factors, including the voltage applied between the electrodes, the geometry of the treatment chamber, and the distribution of the material's dielectric properties between the electrodes. In setups with parallel plate electrodes, commonly used in both batch and continuous PEF treatment systems, the electric field is generally uniform within the space between the electrodes, except for minor edge effects. The electric field strength can be calculated by dividing the voltage (U) applied across the electrodes by the distance between them (L), following the equation: $E=L/U$. This relationship is essential in ensuring accurate control over the PEF process, as adjusting the voltage or the electrode distance can directly impact the intensity of the electric field and, consequently, the effectiveness of the treatment (Raso *et al.*, 2016b).

1.1.1 *Electroporation*

Electroporation is a process induced by applying a Pulsed Electric Field to food material and impacts the cellular membrane. This physical phenomenon is used to increase the permeability of cell membranes, whether in animal or plant cells, through the application of short, high-intensity electric pulses. This method has gained widespread application across various fields, including biotechnology, medicine, and food processing. In biotechnology and medicine, electroporation facilitates the introduction of different molecules into cells and is employed in water treatment and sterilization processes. In food processing, it is used to enhance the efficiency of pressing, extraction, drying, and diffusion operations (Kotnik *et al.*, 2024). Despite its extensive use, electroporation's precise mechanisms remain only partially understood. Several theories have been proposed to explain the process of reversible electroporation and/or the breakdown of the electrical membrane. These theories are based on experimental studies on model systems such as liposomes, planar lipid bilayers, and phospholipid vesicles. Each theory offers unique insights and has its strengths and limitations. However, a key aspect common to all theories is the role of the cell membrane in amplifying the applied electric field. This is due to the significantly lower conductivity of the intact membrane compared to the conductivities of the extracellular medium and the cell cytoplasm. This differential conductivity is crucial as it affects how the electric field interacts with the cell membrane, thereby influencing the electroporation process (Donsì *et al.*, 2010).

Electroporation is a phenomenon with a range of valuable applications in food science, from improving extraction processes to enhancing preservation methods. This technique utilizes short bursts of high-voltage electric pulses to manipulate cell membranes in food products. Depending on the number of pulses and their voltage, electroporation can be classified into two types: reversible and irreversible (Saulis, 2010).

Reversible electroporation involves the application of electric pulses that temporarily increase the permeability of the cell membrane. This controlled enhancement allows substances such as nutrients, flavors, and preservatives to penetrate the cells of food products or to be released from them. One of the key benefits of reversible electroporation is that the cell membrane can recover and return to its normal state once the electric pulses are discontinued, provided that the process is managed carefully. In the food industry, reversible electroporation is particularly useful for optimizing extraction processes. For instance, when applied to fruits and vegetables, this technique can significantly improve the efficiency of juice extraction. By temporarily making the cell membranes more permeable, it facilitates the release of juice and other valuable compounds. Additionally, reversible electroporation can enhance the infusion of flavors or preservatives into food products, leading to better taste and longer shelf life (Genovese *et al*., 2021).

Irreversible electroporation, instead, employs more intense electric pulses that cause permanent damage to the cell membrane, leading to cell death. This form of electroporation is less commonly used in food processing but has specific and valuable applications, particularly in sterilization and decontamination. For example, irreversible electroporation can effectively eliminate microorganisms present in food products without the need for high temperatures or chemical preservatives (Nowosad *et al*., 2021). By using irreversible electroporation for microbial inactivation, the food industry can achieve enhanced safety and preservation of nutritional and sensory qualities without compromising the integrity of the food product (Kotnik *et al*., 2024).

Electroporation offers distinct advantages depending on the type used. Reversible electroporation is ideal for applications requiring temporary changes in membrane permeability, such as improving extraction and flavor infusion processes. Irreversible electroporation, on the other hand, is suitable for applications demanding permanent membrane damage, such as sterilization and microbial control. Understanding these differences allows food scientists and engineers to choose the appropriate electroporation method to achieve specific processing goals, leading to more effective and innovative food production techniques (Demir *et al*., 2023)(fig.1-1).

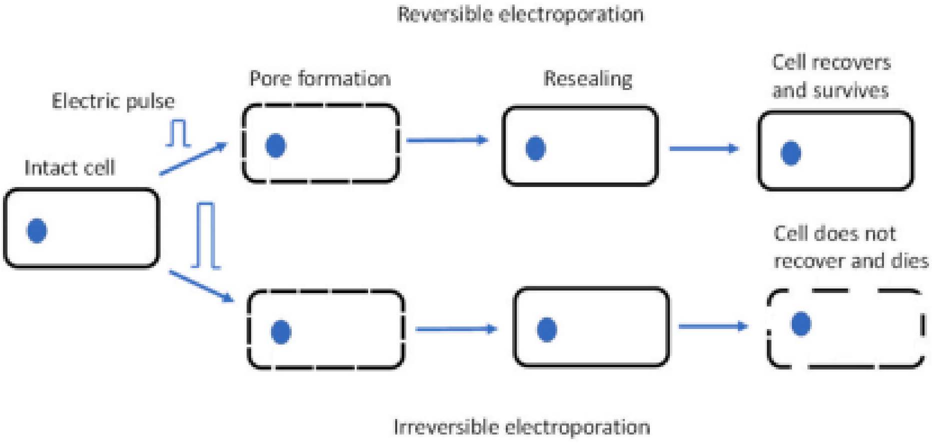


Figure 1-1example of reversible and irreversible electroporation of a living cell (Demir et al., 2023).

1.1.2 *Pulse shape*

In Pulsed Electric Field treatments, pulse shapes are typically either exponential or square-wave and can be unipolar or bipolar. To ensure precise control over the treatment intensity, it's essential to continuously monitor the voltage and current waveforms of the electric pulses delivered to the treatment chamber. This monitoring should be done using high-voltage and high-current probes placed as close as possible to the treatment chamber. This is particularly important because the voltage output from the pulse power supply often differs from the voltage measured within the treatment chamber, especially in configurations with low intrinsic electrical resistance. Accurate measurement is therefore crucial to achieving the desired treatment effectiveness (Raso *et al.*, 2016).

Electric field strength and total specific energy input are often recommended as key parameters for comparing data obtained from different conditions and equipment in PEF treatments (Demir *et al.*, 2023). Among these, total specific energy input is particularly valuable and should be prioritized over treatment time, especially when dealing with exponentially decaying pulses. This preference is because the treatment time alone does not account for the variations in energy delivered to the sample (Raso *et al.*, 2016). Furthermore, specifying the total energy input provides an estimate of the energy consumption associated with the PEF process, expressed in kilojoules per kilogram (kJ/kg). This measure not only facilitates comparisons between different experimental setups but also helps in assessing the efficiency and cost-effectiveness of the PEF treatment. By considering total specific energy input, researchers and engineers can gain a more comprehensive understanding of the energy dynamics involved in the process and make more informed decisions about optimizing PEF conditions for various applications (Saulis, 2010)(fig.1-2).

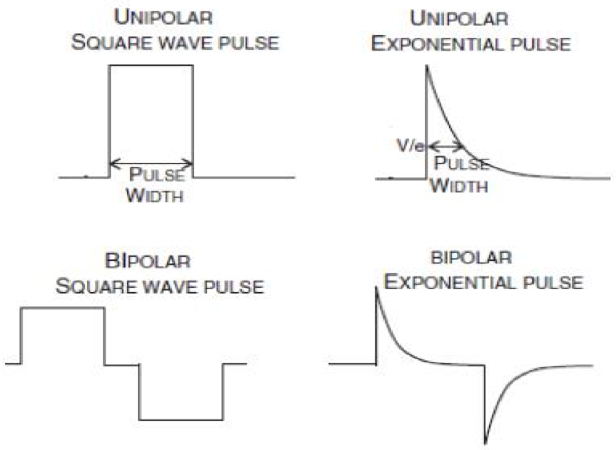


Figure 1-2 figure representing the pulse shapes (Coban & Fidan, n.d.) .

1.1.3 *Treatment chamber*

Treatment chambers are specifically engineered to function under high electric field intensities while maintaining strict control over the temperature during the Pulsed Electric Field (PEF) process. These chambers are designed to ensure that the desired electric field strength is achieved without causing significant temperature fluctuations that could affect the integrity of the product or the effectiveness of the treatment. Managing temperature is critical, as excessive heat could compromise the non-thermal nature of PEF processing and lead to unwanted thermal effects, such as product degradation or nutrient loss (Alkhafaji & Farid, 2007). Each treatment chamber is equipped with a pair of stainless-steel mesh electrodes. These electrodes serve as critical components in the PEF system, ensuring the effective delivery of electric pulses across the food or biological material being processed. Stainless steel is chosen due to its durability, conductivity, and resistance to corrosion, making it ideal for maintaining consistent performance under the high-intensity conditions of PEF treatment (Alkhafaji & Farid, 2007). Various types of treatment chambers have been tested, yielding different results. Cylindrical chambers, for instance, have shown potential for higher throughput and the ability to be integrated into automated production lines, making them suitable for industrial-scale applications. However, their primary drawback is the lack of a uniform electric field, which can lead to inconsistent treatment of the product.

On the other hand, square or rectangular treatment chambers offer a more uniform electric field, ensuring consistent exposure of the material to the electric pulses. This uniformity is advantageous for achieving more reliable and controlled results in the processing of food products. Despite this benefit, these chambers are less suitable for large-scale industrial use, as they are more difficult to automate, limiting their application in production environments where high efficiency and continuous processing are required (Jeyamkondan *et al*., 1999).

1.2 **Application of PEF in the food industry**

In the food industry, the demand for innovations is increasingly important to improve both the shelf life of products and the efficiency of processing. This requires leveraging a combination of well-established technologies alongside cutting-edge, emerging techniques. By integrating these advancements, companies can not only extend the freshness and safety of their products but also streamline production processes, reduce waste, and meet growing consumer expectations for quality and sustainability. The continuous evolution of food technology is essential to addressing these challenges while ensuring that the industry remains competitive in an ever-changing market (Casti *et al.*, n.d.).

PEF technology is one such innovation and has been applied across various processes in the food industry. Its versatility has made it a valuable tool for improving efficiency, enhancing product quality, and optimizing a wide range of food processing operations (Gazda & Glibowski, 2024). The application of PEF has been extensively documented in scientific literature. For instance, a search on the Scopus database using the keywords "pulsed electric field" and "oil extraction" yields over 50 relevant articles. This highlights the significant attention the technology has received in research related to various food processing applications (Ferraz & Silva, 2025). Pulsed electric field technology presents unique opportunities to modify the structure and properties of milk and dairy products. Using low-energy pulses, it provides a mild, non-thermal treatment that enhances the safety of milk and its derivatives without subjecting them to aggressive heat. This approach helps preserve the nutritional and sensory qualities of dairy products while improving their microbial safety (Gazda & Glibowski, 2024). It is important to highlight that this treatment has minimal impact on key compounds of technological importance, such as proteins and essential nutrients, particularly vitamins. This ensures that the quality and nutritional value of the food product are largely preserved during processing (Gazda & Glibowski, 2024).

Applications of this technology have also been observed in the production of blood orange juice (Fabroni *et al.*, 2024). After extracting blood orange juice and reducing the pulp through refining processes, a stabilization phase is essential to prevent chemical, physical, and microbiological changes. This step ensures the juice maintains its quality and safety during storage, in this case, the study refers to a pre-treatment using PEF to preserve the micronutrients in the product. This process is still under investigation, with ongoing research aimed at optimizing various PEF parameters to achieve the best possible results (Fabroni *et al.*, 2024). One application that has become increasingly prevalent is the use of Pulsed Electric Field technology in the processing of potatoes within the food industry (Baltacıoğlu *et al.*, 2023). This technology has been explored extensively for various potato products, including the production of potato chips. Potato chips are a popular fried snack, but their preparation involves the Maillard reaction that, during the process of frying, may cause the formation of acrylamide. Acrylamide is a compound that has garnered significant attention due to its potentially harmful effects on human health (Santiago-Mora *et al.*, 2024).

Researchers have also demonstrated that the application of PEF combined with ultrasounds has beneficial effects beyond just enhancing safety and quality in food processing.

Specifically, the combination of the treatments has been shown to positively influence moisture content and cutting mechanics. For instance, potatoes subjected to PEF pre-treatment exhibit improved characteristics in terms of slicing. The pre-treatment with PEF results in a finer and more precise cut, which is advantageous for achieving consistent product quality and optimizing processing efficiency. This enhanced cutting precision is attributed to the structural changes induced in the potato cells by the PEF treatment, which affects how the potatoes are sliced and processed. Thus, PEF not only contributes to reducing harmful compounds but also improves various aspects of food texture and processing performance (Ostermeier *et al*., 2021) (fig.1-3).

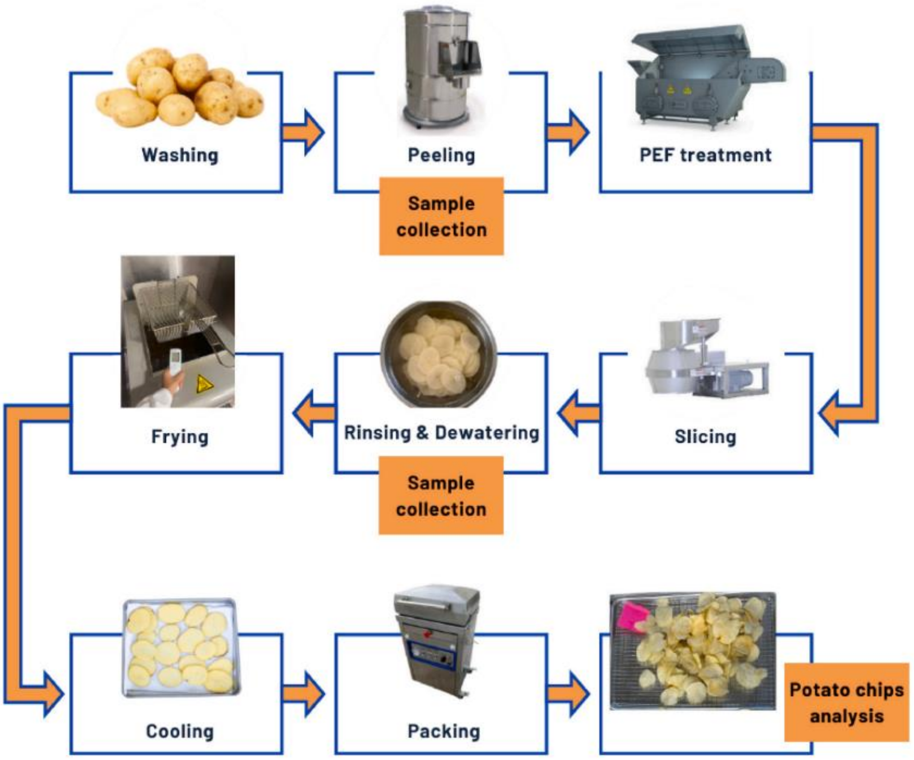


Figure 1-3 Scheme of the process of industrial potato chips with PEF pre-treatment (Santiago-Mora et al., 2024)

1.3 **Advantage of the application of PEF as a pre-treatment**

The main advantages of PEF-assisted processing over conventional methods include:

- Improved extraction yields
- Enhanced mass transfer efficiency
- Reduced processing time
- Lower intensity of conventional extraction parameters

- Decreased degradation of heat-sensitive compounds
- Lower energy costs
- Reduced environmental impact

The growing interest in utilizing PEF as a pre-treatment comes from its ability to address several critical requirements in the food industry. These include the need for enhanced efficiency in processing, improved quality and safety of food products, and the ability to meet stringent regulatory standards. By implementing PEF technology, the industry can better manage factors such as extraction yields, energy consumption, and the preservation of sensitive compounds, ultimately leading to more sustainable and effective food processing solutions. The discussion has extensively explored the critical issues of reducing processing times and increasing extraction yields in the food industry. Addressing these aspects is essential for improving overall efficiency and productivity in food processing. By focusing on strategies to minimize processing durations and optimize extraction outcomes, the industry aims to achieve more effective and cost-efficient methods. These improvements not only enhance the quality and quantity of extracted products but also contribute to better resource management and reduced operational costs, aligning with the broader goals of sustainability and innovation in food production (Parniakov *et al*., 2022).

The application of PEF in food industry has led researchers to study the effects of the electric pulsed field on edible insects.

Chapter 2
EDIBLE INSECTS

2.1 **Overwiew on edible insects**

Edible insects have been a component of human diets since prehistoric times, reflecting their longstanding role in our nutritional history. Despite this ancient tradition, it is only within the last century that significant interest has emerged in studying and promoting edible insects. This recent surge in attention is driven by factors such as sustainability, food security, and the exploration of alternative protein sources, which highlight the potential benefits of incorporating insects into modern diets (Olivadese & Dindo, 2023).

The renewed interest in edible insects can be attributed to the dramatic rise in the global population, which has significantly increased the demand for sustainable and alternative sources of nutrition. As the world's population continues to grow, traditional food resources are being stretched to their limits, making it essential to explore new and efficient ways to meet the nutritional needs of a burgeoning population. Edible insects offer a promising solution due to their high protein content, low environmental impact, and efficient feed-to-protein conversion rates. This renewed focus on insects as a viable food source reflects a broader effort to address the challenges of food security and sustainability in the face of escalating demands on conventional agricultural systems (Puteri *et al*., 2024).

Currently, there is a growing interest in a diverse range of edible insects, and this thesis specifically examines three species: Tenebrio molitor, Hermetia illucens, and Acheta domesticus. These edible insect species were selected because the available literature discusses only those species.

These insects are increasingly being processed in the food industry to create various products, including "insect flour." This flour is valued for its exceptional nutritional profile, which includes high levels of protein, essential amino acids, vitamins, and minerals. By incorporating these insects into food products, the industry aims to offer sustainable and nutrient-rich alternatives to traditional protein sources. This exploration of edible insects highlights their potential to contribute significantly to nutritional needs while also addressing environmental and resource challenges (Li *et al.*, 2024).

Proteins and lipids are two critical factors that have attracted considerable attention from researchers, primarily because of the exceptionally high protein content in these insects compared to traditional food sources. This elevated protein percentage makes them a particularly valuable alternative for addressing nutritional needs. The substantial presence of proteins and lipids in insects offer potential benefits for developing new food products and enhancing dietary quality, providing a compelling reason for ongoing research and exploration in the field (Sharma *et al.*, 2024).

Lipids have garnered considerable interest from researchers for two key reasons. Firstly, the substantial quantity of lipids present in these insects provides a valuable source of nutrients. Secondly, and perhaps more importantly, the quality of these lipids is particularly noteworthy, as they contain all the essential fatty acids necessary for a balanced human diet. This includes essential fatty acids that the human body cannot synthesize on its own and must be obtained through dietary sources. The presence of these vital nutrients in a highly accessible form underscores the potential of edible insects to contribute significantly to nutritional health and dietary supplementation (Bogusz, Bryś, *et al.*, 2023).

Edible insects have become increasingly popular because they can be reproduced with relative ease, making them a sustainable and efficient food source. Additionally, their versatility allows them to be incorporated into a wide variety food product, from traditional recipes to innovative food alternatives. Their adaptability in both culinary and industrial contexts makes them a valuable resource for addressing global food challenges and promoting more sustainable consumption habits (Parniakov *et al.*, 2022).

Figure 2-1 examples of the adult stage of Tenebrio molitor, Hermetia illucens, Acheta domesticus

2.2 **Nutritional benefits of edible insects**

In recent years, there has been a growing interest in the rediscovery of the beneficial effects that edible insects can have when included in both human and animal nutrition. This emerging area of study highlights the potential of insects as a sustainable and nutrient-rich food source, offering promising alternatives to traditional nutrient sources. While the research is still ongoing, scientists are investigating the full extent of the health improvements these insects can bring, particularly in terms of their nutritional value and functional properties (Sharma *et al.*, 2024).

Among the bio-functional compounds found in edible insects such as *Hermetia illucens* (black soldier fly), *Tenebrio molitor* (mealworm), and *Acheta domesticus* (house cricket), there are key substances like chitin, which has been linked to gut health, oleic acid, a beneficial fatty acid, high-quality proteins, and bioactive peptides known for their potential antioxidant, antimicrobial, and immune-modulating properties. These compounds are being studied for their wide range of potential applications in promoting health, both in humans and animals, as researchers continue to explore the full spectrum of benefits that edible insects might offer (Tanga & Ekesi, 2024).

The fortification of foods with insect-based ingredients has been shown to enhance levels of protein, fats, fiber, provitamin A, riboflavin, vitamin C, and minerals such as iron, zinc, magnesium, and calcium. It also increases the presence of saturated fats, caproic acid, arachidic acid, lauric acid, myristic acid, as well as monounsaturated and polyunsaturated fatty acids, including essential amino acids. This fortification improves the overall energy content, texture, and proportion of unsaturated fats, particularly those rich in omega-6 and omega-9. Studies have also revealed that foods enriched with insect proteins exhibit significantly higher digestibility compared to those using alternative protein sources (Tanga & Ekesi, 2024).

The composition of the lipid fraction plays a crucial role in facilitating the absorption of essential fatty acids, a fact documented in scientific literature. These essential fatty acids are vital for numerous biological processes, including cell membrane integrity, inflammation regulation, and overall metabolic health. The ability of lipid-rich food products, particularly those derived from insect sources, to enhance the bioavailability of these important nutrients has been increasingly recognized (Bogusz, Bryś, *et al.*, 2023).

Edible insects offer a rich supply of essential minerals and vitamins, with especially high concentrations of phosphorus and B-complex vitamins, which are vital for energy metabolism and overall health. These nutrients make insects a valuable addition to the diet. In contrast, their carbohydrate content is remarkably low, particularly in simple sugars, making them a suitable option for those looking to reduce carbohydrate intake. However, it's important to note that certain vitamins and minerals, such as vitamins A, D, and E, along with calcium and iodine, are present in smaller amounts, meaning additional sources may be necessary to meet daily nutritional needs. Overall, their impressive vitamin and mineral profile, combined with low carbohydrate levels, makes them a highly nutritious and functional food option (Mohd Zaini *et al*., 2023).

Ongoing research indicates that edible insects may offer a range of health benefits, including anticancer, anti-inflammatory, and antioxidant properties. Such compounds could play a significant role in preventing or mitigating the development of certain cancers, reducing inflammation, and neutralizing harmful free radicals in the body. As studies continue, the evidence supporting these health-promoting properties of edible insects is expected to grow, further highlighting their potential value in functional foods and medical applications (Tanga & Ekesi, 2024).

2.3 **Environmental impact and sustainability**

The continuous expansion of the global population is creating significant challenges for the food industry, as the demand for food products becomes increasingly intense. This growing need for essential nutrients in human diets necessitates the exploration of alternative food sources. In this context, edible insects emerge as a highly promising solution. They provide a rich source of proteins and lipids, which are crucial for a balanced diet, and their production involves relatively low costs. Moreover, the environmental footprint of insect farming is notably smaller compared to conventional agricultural practices, which often require substantial resources such as water, land, and feed. Adopting insect-based food sources, could potentially reduce the strain on natural resources and mitigate environmental impacts, making this approach a sustainable and efficient strategy for addressing future food security challenges (Olivadese & Dindo, 2023).

Insects are highly efficient at converting plant-based proteins into their body proteins. For instance, crickets need less than 2 kg of feed to achieve 1 kg of body weight gain. Additionally, crickets have an estimated edible and digestible portion of up to 80%. This high efficiency in feed conversion means that insect farming has a significantly lower environmental impact, requiring fewer resources and producing less waste compared to traditional livestock farming. This makes insects a more sustainable option for meeting food demands while minimizing environmental strain (Lange & Nakamura, 2021).

Livestock farming plays a significant role in greenhouse gas emissions, accounting for approximately 18% of the total global emissions of gases such as carbon dioxide, methane, nitrous oxide, and ammonia. This impact is substantial, considering the critical role these gases play in climate change. Beyond greenhouse gas emissions, industrial animal agriculture also places considerable demands on global water resources. It is responsible for consuming about 8% of the world's freshwater, with an additional 7% of this water being used specifically for producing animal feed. Additionally, the disposal of waste generated by the food production sector contributes to around 6% of total greenhouse gas emissions. These figures, derived from data as of 2019, highlight the significant environmental footprint of traditional livestock farming, underscoring the need for more sustainable practices in the food industry (Lumanlan *et al*., 2022).

A comprehensive study was conducted to compare the environmental impacts of producing edible insects versus pork. The aim was to evaluate the ecological footprint of both production methods while achieving an equivalent amount of protein. The results were interesting: it was found that producing the same quantity of protein from edible insects incurs only about 9% of the environmental impact compared to pork production. This significant difference underscores the potential of insect farming as a more sustainable alternative, offering a way to meet protein needs while substantially reducing environmental strain. This research highlights the efficiency of insect-based protein sources in minimizing resource use and lowering greenhouse gas emissions, making them a compelling option for more eco-friendly food production (Vinci *et al*., 2022)(fig.2-2).

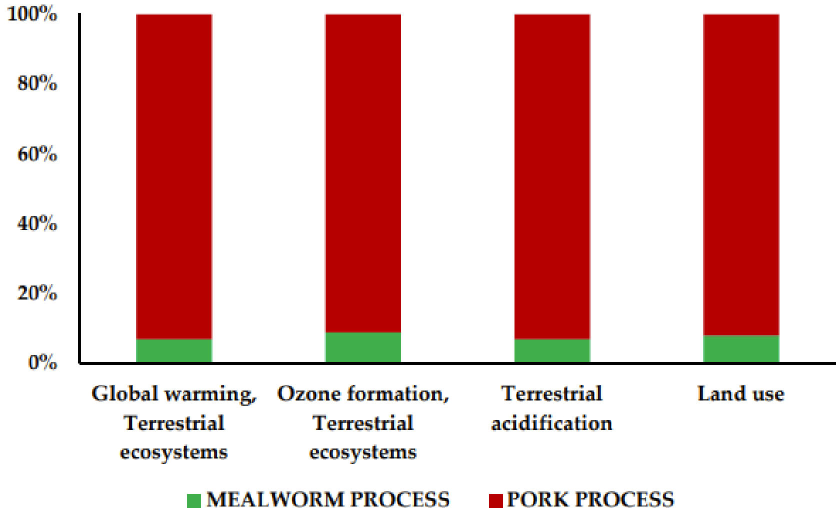


Figure 2-2 Comparison of the environmental impact of Tenebrio molitor and pork industry (Vinci et al., 2022).

Chapter 3
PEF TECHNOLOGY APPLIED TO EDIBLE INSECTS

Processing insects is crucial for ensuring that the resulting raw materials, ingredients, and products meet safety and quality standards for large-scale food and feed applications. The processing techniques used will vary depending on the initial insect material and the intended final product. These techniques often involve a range of operations that are already established in the food and feed industries (Ojha *et al.*, 2021). Processing insect biomass is becoming increasingly essential to ensure safety and minimize potential biological and chemical risks. To achieve this, a variety of techniques are utilized, combining established methods from the food and feed industries. These include thermal treatments such as blanching, boiling, and drying, alongside mechanical processes like grinding and milling. Additionally, fractionation methods such as extraction, purification, and centrifugation are employed. This integrated approach helps in effectively managing and processing insect biomass for safe and high-quality applications (Parniakov *et al.*, 2022).

One of the emerging technologies that has garnered significant interest in the field of edible insect production is the Pulsed Electric Field, which utilizes high-intensity electric fields delivered in brief, rapid pulses. This method applies an electric field of high strength for very short intervals, effectively creating a series of pulsed electrical discharges. These pulses penetrate the cell membranes of microorganisms or plant cells, disrupting their structures and enhancing the efficiency of subsequent processing steps. This technique is particularly useful in various applications, including food and beverage processing, as it can improve extraction, preservation, and overall product quality (Psarianos *et al.*, 2022).

3.1 **Purpose of PEF application on edible insects**

PEF has numerous potential applications in the field of food production, offering innovative approaches to various processing stages. Some of the applications of PEF are:

- Drying
- Enhancing extraction yield
- Microbiological inactivation

3.1.1 *Drying.*

For edible insects, the focus has predominantly been on applying PEF to enhance drying processes. Researchers are exploring how this technology can improve the efficiency and effectiveness of drying insect biomass, potentially leading to better preservation and quality of the final product. By integrating PEF into drying techniques, the aim is to optimize the processing of edible insects, making them more suitable for large-scale food applications (Parniakov *et al.*, 2022). It is also significant that the drying process for edible insects is one of the most versatile methods in the agrifood industry. In addition to decreasing moisture, drying lowers the water activity in the product, which helps to minimize microbial growth and extends the shelf life. This process is particularly beneficial for storing insects at room temperature, as it ensures stability throughout the storage period. It is estimated that when cricket and black soldier fly larvae powders are dried, they can be stored at 25°C for up to 7 months (Parniakov *et al.*, 2022).

3.1.2 *Enhancing extraction yield.*

Using Pulsed Electric Field (PEF) as a pretreatment offers significant advantages for both nutrient extraction and helps in the process of analysing the nutrient profile of edible insects.

This technology improves the effectiveness of extracting valuable nutrients from insect biomass and offers a more detailed understanding of their chemical composition.

Researchers are actively exploring how PEF can be combined with traditional extraction methods to optimize results and improve processing outcomes.

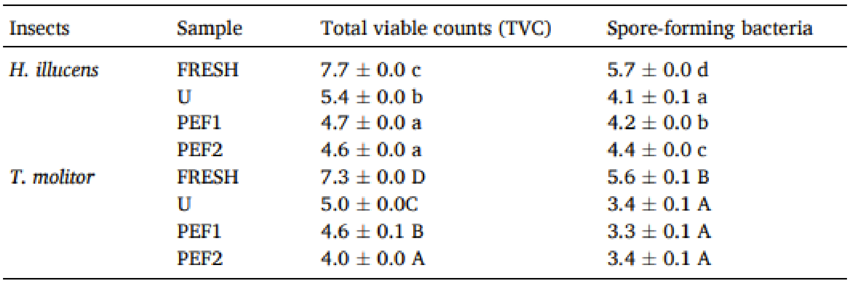
Furthermore, PEF has also been shown to positively impact techno-functional properties of dried edible insects. It improves factors such as rehydration rate, which is crucial for the usability of insect powders in various food products, and hygroscopic properties, which affect the moisture absorption and stability of the dried insects. These improvements contribute to better quality and performance of insect-based ingredients in food and feed applications (Bogusz, Pobiega, *et al.*, 2023).

3.1.3 *Microbiological inactivation.*

One of the emerging areas of interest in PEF technology is its potential for microbiological applications. Researchers are increasingly focusing on how PEF can be used to enhance food safety by targeting pathogen or alterative microorganisms. Ongoing studies are specifically looking at the use of PEF for microbial inactivation through a process known as irreversible electroporation. This process involves the application of short, high-intensity electric pulses that disrupt the cell membranes of both active bacterial cells and more resistant spores, leading to their inactivation.

The advantage of this technique is its ability to penetrate and neutralize microbial cells without the need for chemical additives or excessive heat, making it a more natural and energy-efficient method of ensuring food safety. The initial results from these studies are highly encouraging, as they demonstrate that PEF can significantly reduce microbial loads while maintaining the quality of the food product. These promising findings, as detailed in recent publications, indicate that PEF could become an important tool in microbial control for both food and feed production systems, contributing to safer and longer-lasting products (Bogusz *et al*., 2022)(fig.3-1).

Example of microbiological inactivation on *Hermetia illucens* and *Tenebrio molitor* after an infrared-drying with and without PEF pre-treatment



Insects	Sample	Total viable counts (TVC)	Spore-forming bacteria
H. illucens	FRESH	7.7 ± 0.0 c	5.7 ± 0.0 d
	U	5.4 ± 0.0 b	4.1 ± 0.1 a
	PEF1	4.7 ± 0.0 a	4.2 ± 0.0 b
	PEF2	4.6 ± 0.0 a	4.4 ± 0.0 c
T. molitor	FRESH	7.3 ± 0.0 D	5.6 ± 0.1 B
	U	5.0 ± 0.0C	3.4 ± 0.1 A
	PEF1	4.6 ± 0.1 B	3.3 ± 0.1 A
	PEF2	4.0 ± 0.0 A	3.4 ± 0.1 A

Table 3-1 the data in log CFU/g for untreated (U), treated with PEF (PEF1) (PEF2) with 5kj/kg and 20kj/kg of energy consumption (Bogusz et al., 2022).

Chapter 4

PEF, EDIBLE INSECTS AND DRYING TECHNOLOGIES

The purpose of the chapter is to observe the improvements/enhancements of food technologies by applying PEF in combination with them.

4.1 **Comparison of PEF combined with drying methods**

Scientific studies have emphasized the critical impact of thermal processing on edible insects intended for human consumption, resulting in study cases that analyse the effects of non-thermal technologies on edible insects. Often the studies lead to the application of PEF combined to drying processes because these pretreatments have been investigated to enhance the final quality of the edible insects, extend their shelf life, and reduce overall energy consumption (Bogusz *et al*., 2024). The studies have found high interest in a large number of insects, especially three of them: *Acheta domesticus* (Zafar *et al*., 2024)*, Tenebrio molitor* and *Hermetia illucens* (Ojha *et al*., 2021) due the high protein and lipid content that can be extracted from those. Edible insects are frequently used in form of insect flour (Parniakov *et al*., 2022) with the objective to reduce moisture, water activity, microbial activity and providing stability during the storage time. The already known drying processes result more efficient in the task of lowering the moisture content of the edible insects than the new experimental ones, like the electrohydrodynamic (EHD) drying (Psarianos *et al*., 2024).

Based on trials with Acheta domesticus, is noticed that the convective drying process is faster than the EHD drying, which also suggests a lower energy consumption (Psarianos *et al*., 2024). On the other side, the processing of PEF and EHD drying has a higher quality of the nutrients, due the temperature of the process that remains at 25° C for all the EHD drying time (Psarianos *et al*., 2024). The main finding is that these technologies could be applied to a chain process that reduces the drying time and improves the quality, however, increases the total energy consumption. Nevertheless, if the process has a high energy consumption, therefore a high cost, can be comparable to the process to the PEF and freeze-drying combination on *Acheta domesticus.* Freeze drying is a method of drying based on the sublimation process, that removes water from frozen material under reduced pressure and low temperature. The lyophilization process is well known for its costs (Bogusz, Pobiega, *et al.*, 2023). To combine PEF with freeze- drying is needed to apply a low energy consumption PEF pre-treatment with short width. The high temperature caused by a high number of pulses, can cause overheating making the freeze-drying a non-optimal choice. The 4.9kj/kg energy consumption therefore is the best option quality wise enhancing the functional properties of house cricket flour as a food material (Psarianos *et al.*, 2022). Applying the low energy PEF to the freeze-drying treatment, beyond keeping a high level of nutrient quality, enhances the treatment itself reducing the drying time for 45 min (Bogusz, Pobiega, *et al.*, 2023) as shown in the study case regarding the *Tenebrio molitor* experiment. On the other hand, the study cases did not always produce the desired results, as shown on the same experiment (Bogusz, Pobiega, *et al.*, 2023) that was applied to *Hermetia illucens* larvae resulting in the irreversible electroporation that caused leakage into the membrane raising the water activity to 0.405 ± 0.001 form the 0.165 ± 0.008 that is shown in the control sample that was not pre-treated with PEF with the same drying time of about 5160 min and same dry matter content of 83.2 % (Bogusz, Pobiega, *et al.*, 2023). For *Tenebrio molitor* the studies have found that, not only the freeze-drying has the potential as a better drying treatment but also with others drying techniques have positive results such as the convective and infrared-convective drying. In the study case (Bogusz *et al.*, 2024) the first thing to notice is the temperature of the process that was 90°C and 40°C for convective and infrared-convective drying. Both the processes, despite the temperature gap, have given similar results in terms of dried mass but with the infrared-convective processed one has a lower fat yield caused by the migration of the fat globules on the surface of the *Tenebrio molitor* larvae sample. This phenomenon caused by the shrinkage of the sample requires further investigation with scanning electron microscopy (SEM), laser confocal scanning microscopy (LCSM) and nuclear magnetic resonance (NMR) to explain the kinetics of the process (Bogusz *et al.*, 2024). From the same researchers, is stated that PEF pre-treatment on *Tenebrio molitor* and *Hermetia illucens* may reduce the infrared drying time thanks to the reversible electroporation caused by a low energy consumption (5 kJ/kg - 20 kJ/kg) with these parameters: pulse width of 7 µs with 40 µs of duration and a 2Hz frequency (Bogusz *et al.*, 2022). The tries on *Tenebrio molitor* larvae have shown a reduction in drying time of 8-10% reaching a minimum of 58 min to a moisture content on 0.14 with an energy consumption of 20kj/kg of PEF treatment. In the *Hermetia illucens* tries instead researchers state that the PEF pre-treatment of 5kj/kg resulted in no significant changes of the drying time. The researchers state also that the drying time reduction is not only dependant by the PEF pre-treatment and may depend on the structure of the insect because the infrared drying is based on the infrared ray absorption of the food material, the heating of the external part of the insect, and the following water evaporation, could cause a flaw commonly called case hardening (Bogusz *et al.*, 2022). The more intense treatment with an energy consumption of 20 kj/kg reduced the drying time by 6.5%, peaking at 86 min instead of 92 min of the control sample (Bogusz *et al.*, 2022). The researchers state that the convective and infrared-convective techniques combined, may affect the chemical composition of various nutrients that can be found in *Hermetia illucens* such as fats, polyphenols and antioxidants (Bogusz, Bryś, *et al.*, 2023). The researchers also state that with oven-convective drying assisted with microwave drying on *Hermetia illucens* enhances the protein digestibility (Ojha *et al.*, 2021); the PEF pre-treatment of the black soldier fly could assist the polymerization of the proteins. Both drying technologies (convective and infrared-convective) result in a lower moisture content than the control sample not treated with PEF, reaching 16% less moisture content in 100g of dried mass with an energy consumption of 5kj/kg of PEF pre-treatment. The higher energy consumption though responded with higher significantly moisture content ($p<0.005$); this could lead to the hypothesis that the PEF treatment modified the structure of the nutrients enhancing the resistance to water evaporation (Bogusz, Bryś, *et al.*, 2023). *Hermetia illucens* has seen yet another treatment based on PEF and freeze-drying that negatively had significant results, according to the researchers the PEF pre-treatment lengthened the freeze-drying process by 41.9% compared to the control sample, reaching a maximum of 7320 min with an energy consumption of 5kj/kg with these parameters pulse width of 7 µs with 40 µs of duration and a 2Hz frequency. It is assumed that this phenomenon may be caused by an irreversible electroporation that led to a water leakage in the insect. Furthermore, the drying curve shows that the insect may present some water highly bounded to the proteins *(Bogusz, Pobiega, et al., 2023)*. Based on the papers, PEF and freeze-drying result the most efficient combination of technologies regarding the drying process of *Acheta domesticus, Tenebrio molitor and Hermetia illucens* but with negative aspect of the high energy consumption that impacts the ability to produce insect-based food at a lower cost compared to traditional sources like meat. Considering this, other technologies may be sufficient to fulfil the needs of high-quality nutrients at a sustainable cost. Certainly, the PEF pre-treatment needs further research to improve the effectiveness of it due the small amount of research done in this specific scenario that involves edible insects, PEF combined to other technologies (table 4-1).

Main findings of PEF-combined technologies applied on insects

Insect	stage	Method/ technology	PEF values	PEF energy consumption	Main findings	references
Acheta domesticus	NS	PEF, Oven drying	500 p 25 µs 4.4 kV/cm 41.6 A 20 Hz	40.21 kJ/kg	Shorten oven drying process	(Psarianos *et al.*, 2024)
Acheta domesticus	NS	PEF, EHD (electrohydro-dynamic) drying	500 p 25 µs 4.4 kV/cm 41.6 A 20 Hz	40.21 kJ/kg	Not efficient process	(Psarianos *et al.*, 2024)
Acheta domesticus	adult	PEF, Freeze-drying	100/1000p 15 µs 1.5 kV/cm NS A 20 Hz	4.9 kJ/kg 24.53 kJ/kg 49.10 kJ/kg	Enhanced tecno-functional properties	(Psarianos *et al.*, 2022)
Tenebrio molitor	larvae	PEF, Convective drying	NS p 7 µs 1 kV/cm NS A 2 Hz	5 kJ/kg 20 kJ/kg 40 kJ/kg	Enhanced extraction yield (worst at 5Kj/kg)	(Bogusz *et al.*, 2024)
Tenebrio molitor	larvae	PEF, Infrared-convective drying	NS p 7 µs 1 kV/cm NS A 2 Hz	5 kJ/kg 20 kJ/kg 40 kJ/kg	Enhanced extraction yields only at 5kj/kg	(Bogusz *et al.*, 2024)
Tenebrio molitor	larvae	PEF, Freeze drying	NS p 7 µs 1 kV/cm NS A 2 Hz	5 kJ/kg 20 kJ/kg	Shorten drying time (45min) at 20kj/kg	(Bogusz, Pobiega, *et al.*, 2023)
Tenebrio molitor	larvae	PEF, Infrared drying	NS p 7 µs 1 kV/cm NS A 2 Hz	5 kJ/kg 20 kJ/kg	Shorten drying time by 10% with enhanced	(Bogusz *et al.*, 2022)

					rehydration	
Hermetia illucens	larvae	PEF, Infrared drying	NS p 7 µs 1 kV/cm NS A 2 Hz	5 kJ/kg 20 kJ/kg	Shorten drying time to 86 min (control sample 92 min) with enhanced rehydration	(Bogusz *et al.*, 2022)
Hermetia illucens	larvae	PEF, Freeze drying	NS p 7 µs 1 kV/cm NS A 2 Hz	5 kJ/kg 20 kJ/kg	Irreversible electroporation caused water leakage into the insect lengthening drying time (2000min 5kj/kg)	(Bogusz, Pobiega, *et al.*, 2023)
Hermetia illucens	larvae	PEF, Infrared-convective drying	NS p 7 µs 1 kV/cm NS A 2 Hz	5 kJ/kg 20 kJ/kg 40 kJ/kg	Lower moisture content at 5kj/kg	(Bogusz, Bryś, *et al.*, 2023)
Hermetia illucens	larvae	PEF, Convective drying	NS p 7 µs 1 kV/cm NS 2 Hz	5 kJ/kg 20 kJ/kg 40 kJ/kg	Lower moisture content at 5kj/kg with higher quality of thermolabile nutrients	(Bogusz, Bryś, *et al.*, 2023)

Table 4-1 technologies applied, values of the PEF pre-treatment, energy consumption for the treatment. p (pulses), NS (not stated)

Chapter 5
EFFECTS OF PEF ON THE QUALITY OF EDIBLE INSECTS

5.1 **Nutritional composition of the insects post-treatment**

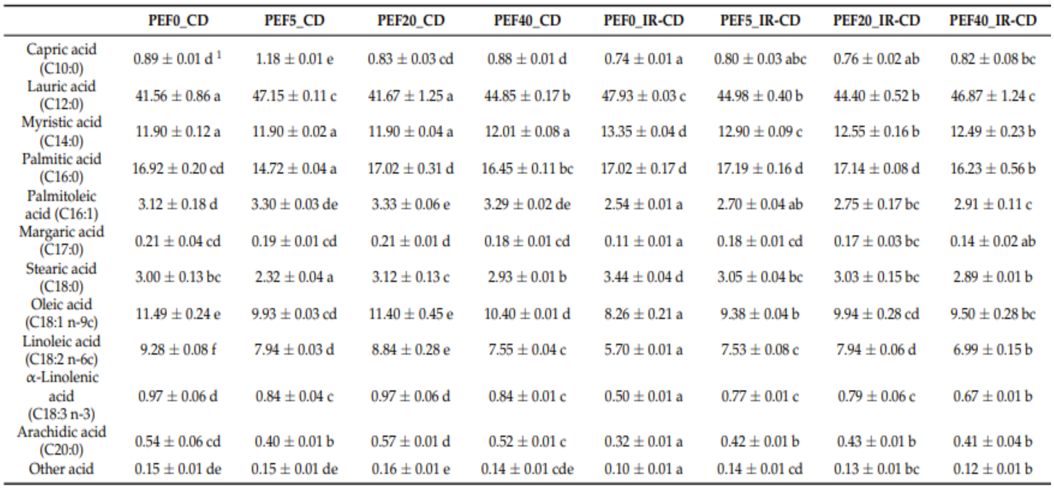
5.1.1 *Proteins*

On top of technical findings made by researchers, the aim of the studies was to value the post-treatment composition of the nutrients, the yield and the tecno-functional proprieties. Equally important is to find cost-efficient treatments that also can guarantee the safety of the product (Mannozzi *et al.*, 2023). The protein content in insects can vary significantly, ranging from 30% to 60%, and is influenced by a wide range of factors. These factors include the stage of development of the insect, the specific type and quality of feed provided, the species of insect, the conditions under which it is reared, and finally, the various processing techniques and methods that are applied after harvesting. All these elements collectively play a crucial role in determining the final protein percentage and quality found in the insects (Queiroz *et al.*, 2023). Most of the studies affirm that a pre-treatment with PEF, enhances the yield of the nutrients, and as shown in the research, PEF could be a possible solution for the future of the processing of insect flour. The combination of PEF pre-treatment and convective drying has shown several times that increase the yield of the protein extraction via Kjeldahl method on *Tenebrio molitor* and *Hermetia illucens* (Bogusz *et al.*, 2024), (Bogusz, Bryś, *et al.*, 2023). This higher yield, however, is only achievable for now with a PEF pre-treatment with an energy consumption of 5kj/kg. The higher energy consumption tries with these treatments, have shown a decrease in protein yield. This notable reduction in protein content could potentially be linked to various structural changes within the insect tissues, including the breakdown of proteins through proteolysis and a decrease in protein extractability. These effects may be attributed to the electroporation phenomenon, which causes disruptions in the cell membranes, as well as the exposure to elevated temperatures during the drying process. The combination of these factors likely contributes to the observed decline in protein availability and overall nutritional quality (Bogusz *et al.*, 2024). These significant variations in yield, specifically an increase observed at energy levels of 5 kJ/kg and a noticeable decrease at higher energy levels of 20-40 kJ/kg, have been consistently mentioned in the studies cited.

Such patterns were particularly evident during the protein extraction process carried out using the Kjeldahl method. Notably, these trends were observed when working with *Hermetia illucens*, also known as the black soldier fly, a species of growing interest in the fields of sustainable agriculture and biotechnology due to its potential as a high-protein resource. The energy efficiency and accuracy of the Kjeldahl method in quantifying nitrogen content further highlight the relevance of these findings (Bogusz, Bryś, *et al.*, 2023). The infrared-convective drying has an overall extraction lower than the convective drying at every PEF energy consumption but has an advantageous aspect: the lower temperature of the process. During the study of the *Tenebrio molitor* larvae, it was further observed that there was a significant increase in the concentration of free amino acids when energy consumption levels reached 20 kJ/kg and 40 kJ/kg. This finding highlights a notable correlation between higher energy input and the enhancement of free amino acid content, suggesting that these specific energy thresholds may play a critical role in influencing the biochemical composition. Such results offer valuable insights into optimizing processes where the concentration of free amino acids is a key factor (Bogusz *et al.*, 2024). The same phenomenon is presented in both infrared-convective and convective drying. Another study also claims that pretreatment with Pulsed Electric Fields (PEF), in combination with freeze-drying, enhances protein extraction yield in both *Tenebrio molitor* and *Hermetia illucens* (Bogusz, Pobiega, *et al.*, 2023). This indicates that the synergistic application of PEF alongside freeze-drying is particularly effective in improving the efficiency of protein recovery from these species. The findings highlight the potential of combining pretreatment methods to optimize the yield of the proteins. Yet in this research is to notice a reduction in protein yield at higher energy consumption PEF pre-treatment on *Tenebrio molitor*, the researchers state that this phenomenon is called “overtreatment”. Overtreatment in food refers to excessive or overly aggressive processing techniques applied to food during preparation, preservation, or production. This can involve physical, chemical, or thermal treatments that exceed what is necessary or optimal, potentially leading to undesirable effects on the food's quality, texture, nutritional value, or flavor. However, the results from studies on *Acheta domesticus* present findings that differ from those observed in previous research. While previous studies indicated a lower yield at higher PEF energy consumption, this research shows a huge increase of 39.55% in yield extraction at the 60 min mark, using cold extraction with NaOH 0.5M (Psarianos *et al.*, 2022).

5.1.2 *Lipids*

Fat plays a crucial role as an ingredient in a wide array of products, underscoring the significance of its properties and quality. The characteristics of fat are influenced by the composition of its fatty acids and the specific chemical structure of these acids within the triacylglycerol molecule. Understanding these aspects is essential because they directly affect the functionality and performance of fat in various applications, from food products to industrial uses. For this reason, numerous studies on edible insects have focused on analysing the composition of fats after treatment processes. These investigations aim to understand how various treatments affect the fat content and quality in edible insects, which is critical for evaluating their suitability and nutritional value in different applications. By examining the changes in fat composition, researchers can gain insights into how processing methods impact the properties of insect fats and their potential uses in food products and other industries. It is widely recognized that the fat content in edible insects is rich in essential fatty acids. These fats are not only abundant but also play a vital role in the nutritional profile of edible insects, contributing significantly to their health benefits. The presence of these essential fatty acids is crucial for various physiological functions and can enhance the overall value of edible insects as a dietary component for the human diet. As discussed in scientific literature, the fat content in edible insects can range from 20% to 30% of their dry mass. Among the lipids found in these insects are sterols, waxes, monoglycerides, diglycerides, triglycerides, and phospholipids. This composition of fats can vary, but typically includes a diverse array of lipid types (Zafar *et al.*, 2024). In *Tenebrio molitor*, for example, the studies affirm after PEF pre-treatment combined with convective drying has enhanced the yield from 22.09 ± 0.07 g /100 g dried mass (0kj/kg) to 24.7 g /100 g dried mass (20kj/kg) (Bogusz *et al.*, 2024), in the same research the higher yield also resulted with PEF and infrared-convective drying but only with an energy consumption of 5kj/kg due the phenomenon of the migration of the fat globules explained in the previous paragraph (Bogusz *et al.*, 2024). Both combinations of technologies were assisted by the Soxhlet, Behrotest ET2 Control Unit extraction. The infrared- convective drying was applied along the PEF pre-treatment with *Hermetia illucens* with results that differ from the tries on *Tenebrio molitor*. For the research, the extraction method was based on the method described by the PN-EN ISO 12966:2017 (Bogusz, Bryś, *et al.*, 2023), with the result given with the ANOVA variation system. The system gives a 0.992 Partial η^2 (eta squared) and the not significant value was $p<0.001$. The study stated that infrared-convective drying combined with PEF did not increase the yield but instead lowered it. The same method of analysis was applied on *Hermetia illucens* with convective drying assisted by PEF resulting in a higher yield. The study, however, was focused specifically on investigating the composition of the lipid fraction of the edible insect, and it produced some intriguing findings. While the overall lipid yield was lower than results from similar studies, the analysis revealed that the quantity of free fatty acids present was significantly higher. This contrast in results suggests that, despite the lower lipid yield, the insect may offer a richer source of free fatty acids, which could have important implications for its nutritional value and potential applications in food (Bogusz, Bryś, *et al.*, 2023) (table 5-1). The researchers, utilizing the data on free fatty acids, conducted a detailed qualitative analysis of the fatty acids present. This allowed them to categorize the fatty acids into distinct groups, specifically: saturated fatty acids, monounsaturated fatty acids, and polyunsaturated fatty acids. This comprehensive classification not only provided insight into the overall composition but also helped in understanding the specific types of fatty acids present, which could have implications for assessing the nutritional profile and potential health benefits of *Hermetia illucens* (Bogusz, Bryś, *et al.*, 2023). In the extraction of the lipid fraction, the process that works at higher temperatures results in higher extraction yield but, the quality of lipids may change during the process. The freeze-drying instead gives a lower yield but with a higher quality overall nutrient wise. The tries on *Hermetia illucens* larvae show a reduction of moisture % in comparison with the control sample not pre-treated with PEF, from 16.80 ± 2.33 to 12.51 ± 0.51 (5kj/kg of energy consumption) and 16.77 ± 2.01 (20kj/kg). The higher energy consumption causing a lower moisture content may be caused by irreversible electroporation but needs further study (Bogusz, Pobiega, *et al.*, 2023). A similar study regarding the lipid content of *Acheta domesticus,* state that PEF pre-treatment combined with freeze-drying enhances the extraction yield by 41.75% with an energy consumption of 49kj/kg, in this case the extraction method was a cold extraction with the assistance of a centrifuge and rotavapor (Psarianos *et al.*, 2022). The technologies discussed facilitate significantly improved performance compared to treatments that do not use PEF as a pre-treatment. Depending on the specific requirements and intended use of the product, it is possible to adjust the types of processes employed and/or combine various methods to achieve optimized results. Nevertheless, this approach requires ongoing research and experimentation to fully understand the potential benefits and to refine the techniques for practical application (Mohd Zaini *et al.*, 2023).



	PEF0_CD	PEF5_CD	PEF20_CD	PEF40_CD	PEF0_IR-CD	PEF5_IR-CD	PEF20_IR-CD	PEF40_IR-CD
Capric acid (C10:0)	0.89 ± 0.01 d[1]	1.18 ± 0.01 e	0.83 ± 0.03 cd	0.88 ± 0.01 d	0.74 ± 0.01 a	0.80 ± 0.03 abc	0.76 ± 0.02 ab	0.82 ± 0.08 bc
Lauric acid (C12:0)	41.56 ± 0.86 a	47.15 ± 0.11 c	41.67 ± 1.25 a	44.85 ± 0.17 b	47.93 ± 0.03 c	44.98 ± 0.40 b	44.40 ± 0.52 b	46.87 ± 1.24 c
Myristic acid (C14:0)	11.90 ± 0.12 a	11.90 ± 0.02 a	11.90 ± 0.04 a	12.01 ± 0.08 a	13.35 ± 0.04 d	12.90 ± 0.09 c	12.55 ± 0.16 b	12.49 ± 0.23 b
Palmitic acid (C16:0)	16.92 ± 0.20 cd	14.72 ± 0.04 a	17.02 ± 0.31 d	16.45 ± 0.11 bc	17.02 ± 0.17 d	17.19 ± 0.16 d	17.14 ± 0.08 d	16.23 ± 0.56 b
Palmitoleic acid (C16:1)	3.12 ± 0.18 d	3.30 ± 0.03 de	3.33 ± 0.06 e	3.29 ± 0.02 de	2.54 ± 0.01 a	2.70 ± 0.04 ab	2.75 ± 0.17 bc	2.91 ± 0.11 c
Margaric acid (C17:0)	0.21 ± 0.04 cd	0.19 ± 0.01 cd	0.21 ± 0.01 d	0.18 ± 0.01 cd	0.11 ± 0.01 a	0.18 ± 0.01 cd	0.17 ± 0.03 bc	0.14 ± 0.02 ab
Stearic acid (C18:0)	3.00 ± 0.13 bc	2.32 ± 0.04 a	3.12 ± 0.13 c	2.93 ± 0.01 b	3.44 ± 0.04 d	3.05 ± 0.04 bc	3.03 ± 0.15 bc	2.89 ± 0.01 b
Oleic acid (C18:1 n-9c)	11.49 ± 0.24 e	9.93 ± 0.03 cd	11.40 ± 0.45 e	10.40 ± 0.01 d	8.26 ± 0.21 a	9.38 ± 0.04 b	9.94 ± 0.28 cd	9.50 ± 0.28 bc
Linoleic acid (C18:2 n-6c)	9.28 ± 0.08 f	7.94 ± 0.03 d	8.84 ± 0.28 e	7.55 ± 0.04 c	5.70 ± 0.01 a	7.53 ± 0.08 c	7.94 ± 0.06 d	6.99 ± 0.15 b
α-Linolenic acid (C18:3 n-3)	0.97 ± 0.06 d	0.84 ± 0.04 c	0.97 ± 0.06 d	0.84 ± 0.01 c	0.50 ± 0.01 a	0.77 ± 0.01 c	0.79 ± 0.06 c	0.67 ± 0.01 b
Arachidic acid (C20:0)	0.54 ± 0.06 cd	0.40 ± 0.01 b	0.57 ± 0.01 d	0.52 ± 0.01 c	0.32 ± 0.01 a	0.42 ± 0.01 b	0.43 ± 0.01 b	0.41 ± 0.04 b
Other acid	0.15 ± 0.01 de	0.15 ± 0.01 de	0.16 ± 0.01 e	0.14 ± 0.01 cde	0.10 ± 0.01 a	0.14 ± 0.01 cd	0.13 ± 0.01 bc	0.12 ± 0.01 b

Table 5-1composition post-treatment of the lipidic fraction of Hermetia illucens (Bogusz, Bryś, et al., 2023).

5.1.3 *Polyphenols and micronutrients*

The polyphenols present in edible insects are significant due to their potent antioxidant properties and can offer considerable health benefits. These compounds contribute to reducing oxidative stress and inflammation, potentially supporting overall well-being and mitigating various health risks (Bogusz *et al*., 2024). Additionally, the presence of polyphenols in edible insects may enhance their nutritional profile and functional attributes, making them a valuable component of sustainable food systems and providing a promising alternative to traditional protein sources. Further research into the specific types and concentrations of polyphenols in different insect species is essential to fully understand their impact and optimize their benefits for human consumption (Bogusz, Bryś, *et al*., 2023). The polyphenol content in edible insects can vary significantly depending on several factors, such as the species of insect, the developmental stage, and the diet they are fed. Typically, the concentration of polyphenols ranges between 100 and 200 mg per 100 grams of dry mass, indicating that these insects can serve as a valuable source of this important micronutrient. Given the growing interest in alternative and sustainable food sources, edible insects stand out as a promising candidate not only for their high protein content but also for their micronutrient profile, including polyphenols, which are known for their antioxidant properties and potential health benefits (Bogusz *et al*., 2024). In the various processing techniques that have been examined, the results have generally been encouraging in terms of preserving or enhancing polyphenol content, further supporting the nutritional value of edible insects. However, one notable exception is the combined process of PEF and electrohydrodynamic drying. The outcomes from this method have been less promising, suggesting that it may not be as effective in retaining polyphenols compared to other techniques. The result was 0.2 ± 0.06 g/100g wet mass less than the control sample, the researchers state that this specific process requires further research and development to optimize it for industrial applications. Continued investigation in this area could lead to advancements that would make edible insects an even more efficient and reliable source of polyphenols and other essential nutrients (Psarianos *et al.*, 2024). During the spectrophotometric analysis conducted using the Folin–Ciocalteu method on *Tenebrio molitor* subjected to PEF treatment followed by convective drying, a notable increase in polyphenol yield was observed when the PEF treatment was applied with an energy consumption of 40 kJ/kg. In contrast, lighter PEF treatments, using lower energy levels, resulted in insignificant or negligible changes in polyphenol extraction. These findings suggest that a more intense electroporation process, achieved through higher energy input, can significantly enhance the efficiency of polyphenol extraction from edible insects.

This observation is particularly important as it indicates that optimizing the intensity of PEF treatment could play a key role in maximizing the nutritional value of insect-based products, especially in terms of their polyphenol content (Bogusz *et al.*, 2024). In the same study, it was further noted that when Pulsed Electric Field (PEF) treatment was combined with infrared-convective drying, the polyphenol yields were consistently higher across all levels of energy consumption. This enhanced performance can likely be attributed to the lower temperature of the infrared-convective drying process. Unlike conventional drying methods, which often involve higher temperatures, this approach minimizes thermal degradation, thereby preserving the integrity of polyphenols that are highly sensitive to heat and prone to breakdown when exposed to elevated temperatures for extended periods.

The thermolabile nature of polyphenols makes them particularly vulnerable during food processing, and the fact that infrared-convective drying operates at gentler temperatures likely explains the improved retention and extraction of these valuable compounds. This finding suggests that the combination of PEF and infrared-convective drying represents a more effective and efficient method for processing insect-based products while maximizing their nutritional benefits, specifically in terms of preserving polyphenol content (Bogusz *et al.*, 2024). The same phenomenon is also observed in another study involving *Hermetia illucens*, which similarly employed the same combination of technologies. The results obtained are consistent with previous findings, showing that infrared-convective drying led to significantly higher yields compared to convective drying alone. This reinforces the idea that the addition of infrared drying, with its lower temperature profile, plays a crucial role in improving polyphenol extraction, preserving these thermolabile compounds better than traditional convective drying methods (Bogusz, Bryś, *et al.*, 2023).

Another topic that has garnered significant interest in the field of insect-based edible food production is the potential allergenic nature of these products. Insects, as it is widely known, share certain biological and molecular characteristics with shellfish, which raises concerns about cross-reactivity in individuals with crustacean allergies (Bogusz, Bryś, *et al.*, 2023). Given this similarity, those who are allergic to shellfish may face substantial risks when consuming edible insects or products derived from them. This issue is particularly important to address, as it could pose a major challenge for the broader acceptance and safe consumption of insect-based foods in both domestic and global markets. Further research is therefore essential to fully understand the allergenic potential of insects and to develop appropriate guidelines for consumers with known food allergies (Bogusz, Bryś, *et al.*, 2023). During the of research on this topic, it was determined that the concentration of molecules responsible for triggering crustacean allergies in *Hermetia illucens* was initially measured at roughly 7,000 ppb. When PEF technology was applied as a pre-treatment, a significant reduction in these allergenic molecules was observed, bringing the concentration down to approximately 6,000 ppb. While this decrease is promising, it is clear that further studies are necessary to fully evaluate the effectiveness of PEF in mitigating allergenic risks and to explore whether even lower concentrations can be achieved. Continued investigation will also help determine the safety and practicality of using PEF as a standard processing method for insect-based food products, particularly regarding consumers with known allergies (Bogusz, Bryś, *et al.*, 2023). Other studies, however, have focused on different molecules that could potentially trigger allergic reactions, such as histamine and chitin. While regulations and guidelines regarding acceptable histamine levels in fish already exist (Reg UE 2073/2005), the researchers still lack comprehensive research on chitin. The experiments conducted on *Acheta domesticus* using PEF pre-treatment combined with electrohydrodynamic drying resulted in a significant reduction of histamine levels, from 127.27 ± 17.49 g/100 g dried mass to 94.35 ± 5.83 g/100 g dried mass. This outcome represents a highly positive result, demonstrating the potential effectiveness of these processing techniques in reducing allergenic compounds in edible insect products (Psarianos *et al.*, 2024). On the other hand, the test on *Acheta domesticus* that involved the use of PEF and freeze-drying to examine the concentration of chitin yield, resulted in no significant variation ($p>0.05$), maintaining a level of 10.10 ± 1.50 g chitin/100 g dried weight. However, the experiment has opened new hypotheses regarding the isolation of chitin through alkaline removal methods. Despite these insights, further research is necessary to explore this potential and to refine the process for more effective chitin extraction and reduction in insect-based food products (Psarianos *et al*., 2022)**.**

Application of PEF combined with technologies and nutritional values.

insect	stage	Nutrient evaluated	Method/ technology	Method of analysis	Control sample	Results	Major findings	references
Tenebrio molitor	larvae	Lipid content	PEF, convective drying	Soxhlet, Behrotest ET2 Control Unit	22.09 ± 0.07 g /100 g dried mass (0kj/kg)	24.7 g /100 g dried mass (20kj/kg)	Higher yield	(Bogusz *et al*., 2024)
Tenebrio molitor	larvae	Lipid content	PEF, infrared-convective drying	Soxhlet, Behrotest ET2 Control Unit	16.01 ± 0.23 g/100 g dried mass (0kj/kg)	17.7 g/100 g dried mass (5kj/kg)	Higher yield	(Bogusz *et al*., 2024)
Hermetia illucens	larvae	Lipids % based on moisture %	PEF, freeze drying	Soxhlet	16.80 ± 2.33 (M%) for 34.60 ± 1.96 (L%) (0kj/kg)	12.51 ± 0.51 (M%) for 34.52 ± 1.83 (L%) (5kj/kg) 16.77 ± 2.01 (M%) for 33.33 ± 2.47 (L%) (20kj/kg)	Higher lipid percentage	(Bogusz, Pobiega, *et al*., 2023)
Acheta domesticus	adult	Lipid content	PEF, freeze drying	n-hexane, centrifuge, rotavapor.	18.19 ± 0.63 g/100g dried weight	41.75% increase (49kj/kg)	Higher yield	(Psarianos *et al*., 2022)

Hermetia illucens	larvae	Lipid content	PEF, convective drying	PN-EN ISO 12966:2017	NS	0.992 Partial η^2 $p<0.001$ ANOVA variation system	Higher yield	(Bogusz, Bryś, *et al.*, 2023)
Hermetia illucens	Larvae	Lipid content	PEF, infrared-convective drying	PN-EN ISO 12966:2017	NS	0.992 Partial η^2 $p<0.001$ ANOVA variation system	Lower yield	(Bogusz, Bryś, *et al.*, 2023)
Tenebrio molitor	larvae	Protein content	PEF, convective drying	Kjeldahl, KjelFlex K-360	47.5 g/100 g dried mass (0kj/kg)	+0.05g (5kj/kg) <47.5g (20kj/kg) <47.5g (40kj/kg)	Higher yield at 5kj/kg PEF energy intake, lower yield at 20kj/kg and 40kj/kg Increase in EEAs	(Bogusz *et al.*, 2024)
Tenebrio molitor	larvae	Protein content	PEF, infrared-convective drying	Kjeldahl, KjelFlex K-360	48.7 g/100 g dried mass (0kj/kg)	-0.05g (5kj/kg) ±0.05g (20kj/kg) ±0.05g (40kj/kg)	Lower yield at 5kj/kg, Increase in EEAs	(Bogusz *et al.*, 2024)
Hermetia illucens	larvae	Protein % based on moisture %	PEF, freeze drying	Kjeldahl	16.80 ± 2.33 (M%) for 35.80 ±	12.51 ± 0.51 (M%) for 35.73 ±	Higher protein percentage	(Bogusz, Pobiega, *et al.*, 2023)

					1.04 (P%) (0kj/kg)	1.00 (P%) (5kj/kg) 16.77 ± 2.01 (M%) for 37.38 ± 0.87 (P%) (20kj/kg)		
Tenebrio molitor	larvae	Protein % based on moisture %	PEF, freeze drying	Kjeldahl	6.69 ± 0.25 (M%) for 48.02 ± 0.54 (P%) (0kj/kg)	4.19 ± 1.05 (M%) for 48.03 ± 0.49 (P%) (5kj/kg) 4.15 ± 0.11 (M%) for 45.40 ± 0.59 (P%) (20kj/kg)	Higher protein percentage	(Bogusz, Pobiega, *et al.*, 2023)
Acheta domesticus	adult	Protein content	PEF, freeze drying	NaOH, 0.5M, centrifuge.	28.4 g/100g dried weight (15 min)	39.55% increase (49kj/kg) (60 min)	Higher yield	(Psarianos *et al.*, 2022)
Hermetia illucens	Larvae	Protein content	PEF, convective drying	Kjeldahl	NS	0.842 Partial η^2 $p<0.001$ ANOVA variation system	Higher yield at 5kj/kg Lower yield at 20-40kj/kg	(Bogusz, Bryś, *et al.*, 2023)
Hermetia illucens	Larvae	Protein content	PEF, infrared-	Kjeldahl	NS	0.842 Partial η^2	Higher yield at 5kj/kg	(Bogusz, Bryś, *et al.*, 2023)

			convective drying			$p<0.001$ ANOVA variation system	Lower yield at 20-40kj/kg	
Acheta domesticus	NS	Total polyphenol content	PEF, EHD drying	12 ml ethanol/acetic acid/water solvent, centrifuge	1.0± 0.06 g/100 g Wet mass	0.98 ± 0.06g/100g wet mass	Lower yield	(Psarianos *et al.*, 2024)
Tenebrio molitor	larvae	Total polyphenol content	PEF, convective drying	Folin–Ciocalteu spectro-photometric	(124.3 ± 0.4 mg chlorog. acid/100 g dried mass	±0.05g (5kj/kg) ±0.05g (20kj/kg) >124.3g (40kj/kg)	Higher TPC at 40kj/kg	(Bogusz *et al.*, 2024)
Tenebrio molitor	larvae	Total polyphenol content	PEF, infrared-convective drying	Folin–Ciocalteu spectro-photometric	(124.3 ± 0.4 mg chlorog. acid/100 g dried mass	>124.3g (5kj/kg) >124.3g (20kj/kg) >124.3g (40kj/kg)	Higher TPC	(Bogusz *et al.*, 2024)
Hermetia illucens	Larvae	Total polyphenol content	PEF, convective drying	Folin–Ciocalteu spectro-photometric	NS	0.947 Partial η^2 $p<0.001$ ANOVA variation system	Higher yield	(Bogusz, Bryś, *et al.*, 2023)
Hermetia illucens	larvae	Total polyphenol content	PEF, infrared-convective drying	Folin–Ciocalteu spectro-photometric	NS	0.947 Partial η^2 $p<0.001$ ANOVA	Higher yield	(Bogusz, Bryś, *et al.*, 2023)

						variation system		
Acheta domesticus	adult	Chitin content	PEF, freeze drying	NaOH, 1 M, centrifuge.	10.10 ± 1.50 g chitin / 100 g dried weight	10.10 ± 1.50 g chitin/ 100 g dried weight	No significant variations	(Psarianos *et al.*, 2022)
Acheta domesticus	NS	Histamine	PEF, EHD drying	2 g with 20 ml 0.85% NaCl solution, centrifuge	127.27 ± 17.49 g/100 g dried mass	94.35 ± 5.83 g/100 g dried mass	Lower yield	(Psarianos *et al.*, 2024)

Table 5-2 post-treatment results on nutrients and main findings

5.2 **Post-treatment techno-functional properties**

The PEF treatment enhances the permeability of cell membranes through electroporation, modifying and improving the functional characteristics, including increased emulsifying capacity, enhanced foaming properties, and improved hygroscopicity and rehydration capacity (Psarianos *et al.*, 2022).

Enhanced hygroscopicity allows insect-based ingredients to better absorb moisture from the environment, making them suitable for dry formulations, while improved rehydration capacity is particularly beneficial for dried products (Bogusz, Pobiega, *et al.*, 2023).

These improvements could make edible insects highly versatile for a wide range of applications in the food industry. Furthermore, the enhanced water binding capacity and rehydration properties are critical in maintaining the desired texture and stability of processed food products (Psarianos *et al.*, 2022).

The studies highlighted that the treatment performed on insect flour had a significant and measurable effect on its oil-holding capacity. This enhancement in oil retention suggests that the process could improve the insect flour's ability to incorporate and stabilize fats within various food products (Queiroz *et al.*, 2023).

Chapter 6
FOOD SAFETY AND REGULATORY ASPECTS

6.1 **Regulations on the consume of edible insects**

6.1.1 *Novel food overview*

Edible insects fall under the category of novel foods, defined as "any food that was not consumed to a significant extent by humans within the European Union before May 15, 1997. This date marks the introduction of the first regulation on "novel foods," Regulation (EU) N. 258/1997, which was later repealed and replaced by the currently active Regulation (EU) N. 2283/2015.

The term "novel" goes beyond just describing something as innovative, unusual, or unconventional. It also carries a temporal significance. Foods are classified as "novel" if they were not widely consumed or introduced into the market before May 15, 1997. This means that any food product with which there was no significant prior contact or consumption within the European Union before that date is considered "new" under the regulatory framework. This definition highlights not only the uniqueness of the food but also its relative recent introduction into the human diet in terms of historical consumption patterns.

Regulation (EU) 2015/2283 on novel foods plays a key role in facilitating the introduction of innovative food products by businesses within the food industry into the European Union market. It provides a structured framework that not only helps companies navigate the approval process for novel foods but also ensures that these new products meet stringent safety standards. By doing so, the regulation safeguards consumer health and well-being, ensuring that any novel food entering the EU market has undergone rigorous evaluation. This balance between fostering innovation and maintaining high safety standards benefits both food producers and European consumers, promoting trust in the safety of new food products while encouraging advancements in the food industry.

6.1.2 *Edible insect as novel food*

The topic of insect consumption has been explicitly regulated through Regulation (EU) 2015/2283 on novel foods. This regulation, adopted in 2015 and fully implemented in 2018, was designed to provide a clear legal framework for the introduction and use of novel food sources, including edible insects, within the European Union. By addressing insect consumption under this regulation, the EU has set guidelines to ensure that these new food products meet stringent safety and quality standards before they are made available to consumers. This regulatory framework has allowed for the structured integration of edible insects into the food market while prioritizing consumer protection and promoting innovation within the food industry.

The Food and Agriculture Organization (FAO) has estimated that approximately 2 billion people globally engage in entomophagy, or the consumption of insects. The FAO has been instrumental in bringing attention to the advantages of incorporating edible insects into diets. Through its "Edible Insects" program, the FAO has actively promoted the use of insects both as a food source for humans and as animal feed. This initiative seeks to advance understanding of how edible insects can contribute to better health outcomes and offer environmental benefits (Sogari & Mora, 2016). By advocating for the broader adoption of edible insects, the FAO aims to address global food security challenges and encourage sustainable practices within the food industry.

edible insects on the market

Edible insect species	Common name	stage	Product	Regulation implementing Reg (EU) 2015/2283
Tenebrio molitor	Yellow mealworm	Adult Larvae	Dried	Regulation (EU) 2021/882
			Frozen Dried powdered	Regulation (EU) 2022/169
Acheta domesticus	House Cricket	Adult Larvae	Dried Ground frozen	Regulation (EU) 2022/188
			Partially defatted powder	Regulation (EU) 2023/5
Locusta migratoria	Migratory Locust	Adult Larvae	Dried frozen	Regulation (EU) 2021/1975
Alphitobius diaperinus	Lesser Mealworm	larvae	Frozen Paste Dried powdered	Regulation (EU) 2023/58

Table 6-1 List of regulated edible insects as novel food (IPPIF INFO SHEET COMMERCIALISATION OF EDIBLE INSECTS IN THE EU, n.d.)

6.2 **Microbiological risks and studies on safety**

There are two types of microorganisms to consider as potential microbiological hazards in insects: those that are inherently associated with the insect biomass itself and those introduced from external sources during the farming and processing stages.

The diversity of microorganisms present in insects is influenced by their lifestyle, whether they are living in their natural environment or controlled farming conditions. In their natural habitats, insects can be exposed to a wide range of microorganisms from soil, plants, and other environmental sources. Instead, in farming settings, the types and quantities of microorganisms can vary based on factors such as hygiene practices, feed quality, and overall management of the rearing environment. This variation in microorganism presence underscores the importance of understanding and managing microbial risks throughout both natural and controlled insect production systems ("Risk Profile Related to Production and Consumption of Insects as Food and Feed," 2015).

CONCLUSIONS

The central objective of this thesis was to conduct a comprehensive analysis of the effects of PEF technology on edible insect processing. The aim was to gain a deeper understanding of how this innovative method influences the biological and mechanical processes involved, to improve the overall quality and characteristics of the final product. Through this research, the intention was to explore the potential benefits of PEF in enhancing not only the nutritional and sensory properties of edible insects, but also their processing efficiency and sustainability, thereby contributing to the advancement of this emerging field.

The research provided valuable insights into the significant role that PEF technology plays in improving the production processes of various edible insect species (*Tenebrio molitor*, *Hermetia illucens*, and *Acheta domesticus)*. Among the most important findings was the observation that nearly all the drying treatments tested exhibited improvements, particularly in terms of reduced processing times. In some cases, such as with freeze drying, there were notable enhancements in the preservation of nutritional quality. However, this method also proved to be more costly compared to other approaches, posing a challenge in balancing quality and economic feasibility.

Infrared drying, on the other hand, garnered substantial attention due to its relatively lower cost, while still delivering results that, in some instances, surpassed expectations, making it a promising candidate for broader application in the edible insect industry.

Electrohydrodynamic drying, however, remains a subject of ongoing investigation. Though it shows potential, additional research is required to thoroughly evaluate its functionality and determine whether it can be effectively integrated into the production process. Overall, the study highlighted the diverse impacts of various drying technologies, emphasizing the need to balance quality improvements with cost-efficiency for sustainable industrial-scale insect production.

Another significant outcome that emerged from the research is that PEF pre-treatment frequently resulted in higher nutrient yields when compared to samples that did not undergo this process. This suggests that PEF not only optimizes the production process but also enhances the nutritional value of insect-based food products. Furthermore, the benefits of PEF have been extensively documented in various studies, which have explored its positive effects on improving key techno-functional properties. For instance, PEF has been shown to enhance the rehydration capacity of the insects, a critical factor in maintaining the quality and texture of the final product. These findings underline the broader potential of PEF technology in advancing the efficiency and quality of edible insect production.

The use of PEF as a pre-treatment in the production of food derived from edible insects has been confirmed as a highly impactful and promising technology. This method has shown significant potential in optimizing various aspects of the production process, enhancing both the efficiency and the overall quality of the final product. By improving key factors such as texture, nutrient retention, and processing time, PEF has demonstrated its value as an innovative approach in the development of sustainable, insect-based food products. Its effectiveness highlights the growing importance of advanced technologies in shaping the future of food production.

BIBLIOGRAPHY

Alkhafaji, S. R., & Farid, M. (2007). An investigation on pulsed electric fields technology using new treatment chamber design. *Innovative Food Science and Emerging Technologies*, *8*(2), 205–212. https://doi.org/10.1016/j.ifset.2006.11.001

Baltacıoğlu, C., Yetişen, M., Baltacıoğlu, H., Karacabey, E., & Buzrul, S. (2023). Impacts of Pulsed Electric Fields (PEF) Pre-treatment on the Characteristics of Fried Yellow- and Purple-Fleshed Potatoes: a Chemometric-Assisted FTIR Study. *Potato Research*. https://doi.org/10.1007/s11540-023-09673-1

Bogusz, R., Bryś, J., Onopiuk, A., Rybak, K., Witrowa-Rajchert, D., & Nowacka, M. (2023). Effect of Pulsed Electric Field Technology on the Composition and Bioactive Compounds of Black Soldier Fly Larvae Dried with Convective and Infrared–Convective Methods. *Molecules*, *28*(24). https://doi.org/10.3390/molecules28248121

Bogusz, R., Pobiega, K., Kowalczewski, P. Ł., Onopiuk, A., Szulc, K., Wiktor, A., Rybak, K., & Nowacka, M. (2024). Nutritional Value and Microbiological Aspects of Dried Yellow Mealworm (Tenebrio molitor L.) Larvae Pretreated with a Pulsed Electric Field. *Applied Sciences (Switzerland)*, *14*(3). https://doi.org/10.3390/app14030968

Bogusz, R., Pobiega, K., Rybak, K., Wiktor, A., Parniakov, O., Smetana, S., & Nowacka, M. (2023). The Pulsed Electric Field Treatment Effect on Drying Kinetics and Chosen Quality Aspects of Freeze-Dried Black Soldier Fly (Hermetia illucens) and Yellow Mealworm (Tenebrio molitor) Larvae. *Applied Sciences (Switzerland)*, *13*(18). https://doi.org/10.3390/app131810251

Bogusz, R., Smetana, S., Wiktor, A., Parniakov, O., Pobiega, K., Rybak, K., & Nowacka, M. (2022). The selected quality aspects of infrared-dried black soldier fly (Hermetia illucens) and yellow mealworm (Tenebrio molitor) larvae pre-treated by pulsed electric field. *Innovative Food Science and Emerging Technologies*, *80*. https://doi.org/10.1016/j.ifset.2022.103085

Casti, P., Salmeri, M., Mencattini, A., Bonfà, F., Ferrari, S., & Bertini, I. (n.d.). *Sviluppo di un sistema (semi)automatico di progettazione di apparecchiature per la conservazione di* *alimenti attraverso campi elettrici pulsati, dotate di opportuni sistemi di monitoraggio e controllo. MINISTERO DELLO SVILUPPO ECONOMICO*.

Coban, M., & Fidan, M. (n.d.). *High Voltage Pulse Generator for Pulsed Electric Field Treatments*.

Demir, E., Tappi, S., Dymek, K., Rocculi, P., & Gómez Galindo, F. (2023). Reversible electroporation caused by pulsed electric field – Opportunities and challenges for the food sector. In *Trends in Food Science and Technology* (Vol. 139). Elsevier Ltd. https://doi.org/10.1016/j.tifs.2023.104120

Donsì, F., Ferrari, G., & Pataro, G. (2010). Applications of pulsed electric field treatments for the enhancement of mass transfer from vegetable tissue. *Food Engineering Reviews*, *2*(2), 109–130. https://doi.org/10.1007/s12393-010-9015-3

Fabroni, S., Platania, G. M., Amenta, M., Ballistreri, G., Galvano, F., Nges, I. A., & Timpanaro, N. (2024). Pulsed Electric Field as a Mild Treatment for Extended Shelf-Life and Preservation of Bioactive Compounds in Blood Orange Juice. *Applied Sciences (Switzerland)*, *14*(16). https://doi.org/10.3390/app14167275

Ferraz, L. P., & Silva, E. K. (2025). Pulsed electric field-assisted extraction techniques for obtaining vegetable oils and essential oils: Recent progress and opportunities for the food industry. In *Separation and Purification Technology* (Vol. 354). Elsevier B.V. https://doi.org/10.1016/j.seppur.2024.128833

Gazda, P., & Glibowski, P. (2024). Advanced Technologies in Food Processing—Development Perspective. In *Applied Sciences (Switzerland)* (Vol. 14, Issue 9). Multidisciplinary Digital Publishing Institute (MDPI). https://doi.org/10.3390/app14093617

Genovese, J., Kranjc, M., Serša, I., Petracci, M., Rocculi, P., Miklavčič, D., & Mahnič-Kalamiza, S. (2021). PEF-treated plant and animal tissues: Insights by approaching with different electroporation assessment methods. *Innovative Food Science and Emerging Technologies*, *74*. https://doi.org/10.1016/j.ifset.2021.102872

IPPIF INFO SHEET COMMERCIALISATION OF EDIBLE INSECTS IN THE EU. (n.d.).

Jeyamkondan, S., Jayas, D. S., & Holley, R. A. (1999). Pulsed Electric Field Processing of Foods: A Review. In *Journal of Food Protection* (Vol. 62, Issue 9).

Kotnik, T., Rems, L., Tarek, M., & Miklavčič, D. (2024). *BB48CH04_Miklavcic ARjats.cls Annual Review of Biophysics Membrane Electroporation and Electropermeabilization: Mechanisms and Models BB48CH04_Miklavcic ARjats.cls*. *23*, 56. https://doi.org/10.1146/annurev-biophys-052118

Lange, K. W., & Nakamura, Y. (2021). Edible insects as future food: chances and challenges. In *Journal of Future Foods* (Vol. 1, Issue 1, pp. 38–46). Beijing Academy of Food Sciences. https://doi.org/10.1016/j.jfutfo.2021.10.001

Li, A., Dewettinck, K., Verheust, Y., Van de Walle, D., Raes, K., Diehl, B., & Tzompa-Sosa, D. A. (2024). Edible insects as a novel source of lecithin: Extraction and lipid characterization of black soldier fly larvae and yellow mealworm. *Food Chemistry*, *452*. https://doi.org/10.1016/j.foodchem.2024.139391

Lumanlan, J. C., Williams, M., & Jayasena, V. (2022). Edible insects: environmentally friendly sustainable future food source. In *International Journal of Food Science and Technology* (Vol. 57, Issue 10, pp. 6317–6325). John Wiley and Sons Inc. https://doi.org/10.1111/ijfs.16006

Mannozzi, C., Foligni, R., Mozzon, M., Aquilanti, L., Cesaro, C., Isidoro, N., & Osimani, A. (2023). Nonthermal technologies affecting techno-functional properties of edible insect-derived proteins, lipids, and chitin: A literature review. In *Innovative Food Science and Emerging Technologies* (Vol. 88). Elsevier Ltd. https://doi.org/10.1016/j.ifset.2023.103453

Mohd Zaini, N. S., Lim, E. J., Ahmad, N. H., Gengatharan, A., Wan-Mohtar, W. A. A. Q. I., & Abd Rahim, M. H. (2023). The Review of Cooking, Drying, and Green Extraction Methods on General Nutritional Properties of Mealworms and Locusts. In *Food and Bioprocess Technology* (Vol. 16, Issue 9, pp. 1904–1918). Springer. https://doi.org/10.1007/s11947-023-03020-5

Nowosad, K., Sujka, M., Pankiewicz, U., & Kowalski, R. (2021). The application of PEF technology in food processing and human nutrition. In *Journal of Food Science and Technology* (Vol. 58, Issue 2, pp. 397–411). Springer. https://doi.org/10.1007/s13197-020-04512-4

Ojha, S., Bußler, S., Psarianos, M., Rossi, G., & Schlüter, O. K. (2021). Edible insect processing pathways and implementation of emerging technologies. *Journal of Insects as Food and Feed*, *7*(5), 877–900. https://doi.org/10.3920/JIFF2020.0121

Olivadese, M., & Dindo, M. L. (2023). Edible Insects: A Historical and Cultural Perspective on Entomophagy with a Focus on Western Societies. In *Insects* (Vol. 14, Issue 8). Multidisciplinary Digital Publishing Institute (MDPI). https://doi.org/10.3390/insects14080690

Ostermeier, R., Hill, K., Dingis, A., Töpfl, S., & Jäger, H. (2021). Influence of pulsed electric field (PEF) and ultrasound treatment on the frying behavior and quality of potato chips.

Innovative Food Science and Emerging Technologies, *67*. https://doi.org/10.1016/j.ifset.2020.102553

Parniakov, O., Mikhrovska, M., Wiktor, A., Alles, M., Ristic, D., Bogusz, R., Nowacka, M., Devahastin, S., Mujumdar, A., Heinz, V., & Smetana, S. (2022). Insect processing for food and feed: A review of drying methods. *Drying Technology*, *40*(8), 1500–1513. https://doi.org/10.1080/07373937.2021.1962905

Psarianos, M., Dimopoulos, G., Ojha, S., Cavini, A. C. M., Bußler, S., Taoukis, P., & Schlüter, O. K. (2022). Effect of pulsed electric fields on cricket (Acheta domesticus) flour: Extraction yield (protein, fat and chitin) and techno-functional properties. *Innovative Food Science and Emerging Technologies*, *76*. https://doi.org/10.1016/j.ifset.2021.102908

Psarianos, M., Iranshahi, K., Rossi, S., Gottardi, D., & Schlüter, O. (2024). Quality evaluation of house cricket flour processed by electrohydrodynamic drying and pulsed electric fields treatment. *Food Chemistry*, *441*. https://doi.org/10.1016/j.foodchem.2023.138276

Puteri, B., Oehlmann, M., & Jahnke, B. (2024). Who has an appetite for insects? Identifying segments of early adopters of insect-based food and their product attribute preferences: Insights from a choice experiment study in Germany. *Food Research International*, *196*. https://doi.org/10.1016/j.foodres.2024.114994

Queiroz, L. S., Silva, N. F., de Carvalho, A. F., & Casanova, F. (2023). Impact of emerging technologies on colloidal properties of insect proteins. In *Current Opinion in Food Science* (Vol. 49). Elsevier Ltd. https://doi.org/10.1016/j.cofs.2022.100958

Raso, J., Frey, W., Ferrari, G., Pataro, G., Knorr, D., Teissie, J., & Miklavčič, D. (2016a). Recommendations guidelines on the key information to be reported in studies of application of PEF technology in food and biotechnological processes. *Innovative Food Science and Emerging Technologies*, *37*, 312–321. https://doi.org/10.1016/j.ifset.2016.08.003

Raso, J., Frey, W., Ferrari, G., Pataro, G., Knorr, D., Teissie, J., & Miklavčič, D. (2016b). Recommendations guidelines on the key information to be reported in studies of application of PEF technology in food and biotechnological processes. *Innovative Food Science and Emerging Technologies*, *37*, 312–321. https://doi.org/10.1016/j.ifset.2016.08.003

Risk profile related to production and consumption of insects as food and feed. (2015). *EFSA Journal*, *13*(10). https://doi.org/10.2903/j.efsa.2015.4257

Santiago-Mora, P., Skinner, M., Hendricks, A., Rimkus, T., Meyer, B., Gratzek, J., Pu, S., Woodbury, L., Bond, L., & McDougal, O. (2024). Pulsed electric field effect on acrylamide reduction and quality attributes of continuous-style Lamoka potato chips. *Heliyon*, *10*(11). https://doi.org/10.1016/j.heliyon.2024.e31790

Saulis, G. (2010). Electroporation of cell membranes: The fundamental effects of pulsed electric fields in food processing. *Food Engineering Reviews*, *2*(2), 52–73. https://doi.org/10.1007/s12393-010-9023-3

Sharma, B., Yadav, D. K., Malakar, S., Singh, S., Sharma, M., Suri, S., & Sridhar, K. (2024). Insect proteins – Production technologies, bio-functional, and food applications: A perspective. In *Food Bioscience* (Vol. 61). Elsevier Ltd. https://doi.org/10.1016/j.fbio.2024.104560

Sogari, G., & Mora, C. (2016). *L'entomofagia: tra curiosità e sostenibilità*. https://www.researchgate.net/publication/299603521

Tanga, C. M., & Ekesi, S. (2024). *Dietary and Therapeutic Benefits of Edible Insects: A Global Perspective*. *12*(10). https://doi.org/10.1146/annurev-ento-020123

Vinci, G., Prencipe, S. A., Masiello, L., & Zaki, M. G. (2022). The Application of Life Cycle Assessment to Evaluate the Environmental Impacts of Edible Insects as a Protein Source. *Earth (Switzerland)*, *3*(3), 925–938. https://doi.org/10.3390/earth3030054

Zafar, A., Shaheen, M., Tahir, A. Bin, Gomes da Silva, A. P., Manzoor, H. Y., & Zia, S. (2024). Unraveling the nutritional, biofunctional, and sustainable food application of edible crickets: A comprehensive review. In *Trends in Food Science and Technology* (Vol. 143). Elsevier Ltd. https://doi.org/10.1016/j.tifs.2023.104254