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Exploring the efficacy of pulsed electric fields (PEF) in microbial inactivation during food processing: A deep dive into the microbial cellular and molecular mechanisms

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ABSTRACT

Pulsed electric field (PEF) is a food processing technology based on the phenomenon of electroporation for the inactivation of microorganisms with main advantage the minimal effect on the quality (nutritional, functional, and sensorial) characteristics of the food products. Despite the plethora of research literature on PEF-processed food safety, PEF's industrial application as an alternative of classical pasteurization is limited and mainly at industrial level is focused on high acid-liquid food products. Thus, the thorough assessment of the antimicrobial efficiency of PEF, coupled with the meticulous identification of key microbial resistance mechanisms is scientifically imperative. These efforts are essential for refining the process and exploring potential enhancements through synergistic integration and combination with other methods or/and hurdles. On this basis this manuscript aims to critically review and summarise: a) the antimicrobial mechanism of action, b) the microbial inactivation efficiency, and c) the effect of PEF at a microbial genomic/transcriptomic level.

Industrial application: Evaluating the effectiveness of inactivation and understanding the underlying resistance mechanisms can help on strategies to optimize PEF-mediated decontamination practices, and thereby enhancing the overall process efficiency.

1. Introduction

1.1. Mechanism of action; electroporation phenomenon and its effects

The underlying phenomenon to the application of an electric field to biological cells or tissues for the increase of membrane permeability is described as electroporation (or electropermeabilization) (Heinz et al., 2001; Wiktor et al., 2015). In general, the cell membrane is composed by lipids, which exhibit a structural duality with a polar (hydrophilic) head and a non-polar (hydrophobic) tail (Kotnik et al., 2012). These lipids are arranged into bilayer, where nonpolar tails align inward while polar heads face outward, interacting with the surrounding aqueous environment (Kotnik et al., 2012), After application of electric pulses, electroporation phenomenon is initiated by the aqueous molecules into the lipid bilayer of the membrane (a biological structure formed by amphilic lipids) and leads to reorientation of the lipids with the polar head groups towards these aqueous molecules (Kotnik et al., 2015; Müller et al., 2022). The theory of the mechanism of electroporation is mainly based on the formation of hydrophilic pores, however recent studies have revealed that other factors may contribute to the increased membrane's permeability, such as chemical changes to the lipids and alteration of the membrane's protein functions (Breton & Mir, 2018; Kotnik et al., 2019).

The application of short electric pulses of high voltages for short duration to biological cells and tissues to enhance membrane permeability, is becoming a pertinent method in different applications

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including applications in food technological (Mahnič-Kalamiza et al., 2014), medicinal (Batista Napotnik et al., 2021) biotechnological and environmental processes (Kotnik et al., 2015). However, depending on the research field, those processes/techniques appear in the scientific literature with different terms, namely electroporation, electropermeabilization, electropulsation or Pulsed electric field (PEF) treatment, and are characterized by subtle differences (Kotnik et al., 2019). More specifically, whilst electroporation focuses on the formation of aqueous pores in the lipid bilayer caused by the induced transmembrane voltage, electropermeabilization examines membrane permeability for molecules lacking the physiological mechanisms of transmembrane transport, and electropulsation/PEF generally comprise cell exposure to electric pulses, leading to membrane structural alterations and increased conductivity and/or permeability (Kotnik et al., 2019). The application of an electric field to a biological cell can lead to three different outcomes depending on (i) the electric field strength, (ii) the duration of exposure, and (iii) the membrane recovery rate (Mahnič-Kalamiza & Miklavčič, 2022). In case the electric field strength and the duration of exposure are insufficient to achieve pore formation, electroporation is unachievable, and there is no effect on the cell's permeability and viability (Kotnik et al., 2012; Mahnič-Kalamiza & Miklavčič, 2022). On the condition that the electric field strength exceeds the critical threshold value of the potential difference across a membrane (transmembrane potential), typically between 0.5 and 1.5 V, repulsion between the charge-carrying molecules induces pore formation in the cell membrane (Barba et al., 2015; Weaver & Chizmadzhev, 1996). Depending on the selected parameters of PEF treatment, reversible or irreversible electroporation can occur, with the intensity of treatment determining whether the electroporation can cause temporary or permanent injuries for microbial cells.(Jaeger et al., 2009; Weaver & Chizmadzhev, 1996). When reversible, it causes the cell membrane to discharge (Mohamed & Amer Eissa, 2012) and facilitates microorganism injuries (Yun, Zeng, et al., 2016). In this case and, after a short exposure to an electric field, the membrane resealing and recover, ensuring the viability of the biological cell (Kotnik et al., 2012). Recovery of the cell is an active cellular process involving intricate cellular machinery after reversible electroporation (Batista Napotnik et al., 2021). The occurrence of irreversible pores following appropriate PEF applications leads to microbial cell death as a result of the release of intracellular substances due to the increased permeability of the membrane, structural membrane changes, and osmotic or swelling phenomena (Golberg et al., 2010; Min et al., 2007). For example, Yang et al. (2008) have demonstrated that the peptidoglycan layer of *S. epidermis* cells experienced changes after exposure to PEF. Additionally, Wang et al. (2016) have shown that in *S. aureus* (cultivated at 37 °C) cells, following a PEF treatment at an electric field strength of 39.0 kV/cm, a pulse frequency of 1.0 kHz, for a 1.6 ms treatment time, and a maximum thermal effect of <30 °C with the cells undergoing shape distortions, exhibiting more oval phenotypes.

1.2. PEF as pasteurization alternative

The demand for more sustainable food production methods and the growing consumer interest in fresher and more nutritious food products in combination with the advancement of human knowledge and technology progress, have facilitated the development of new food processing technologies for food preservation and safety, in replacement of the more classical food processing technologies like heating (Chacha et al., 2021; Golberg et al., 2010; Nowosad et al., 2021; Ortega-Rivas & Salmerón-Ochoa, 2014). The term, "non-thermal", was coined to describe those alternative-to-temperature-based-pasteurization methods (Chacha et al., 2021; Morales-de la Peña et al., 2019; Ortega-Rivas & Salmerón-Ochoa, 2014; Pereira & Vicente, 2010). "Non-thermal" technologies can utilize ultrasound (US), high hydrostatic pressure (HPP), ultraviolet light, pulsed electric fields (PEF), non-thermal /cold plasma (NTP), and ozone. They can be classified as physical (US), HPP), UV, and PEF) or chemical (NTP, and ozone) (Chacha et al., 2021; Valdramidis & Koutsoumanis, 2016).

PEF, is described as one of the most promising technologies to inactivate microorganisms and achieve microbial inactivation equivalent with thermal treatments (Barba et al., 2015; Peng et al., 2020) with main advantages as a process being a) the lower treatment temperature, b) the shorter processing time, and c) the potential for its application in continuous flow treatments (Bhat et al., 2019). The PEF process is based on pulse intensities which vary from 0.5 to 1.5 kV/cm for the induction to stress responses and reversible electroporation and from 15 to 40 kV/cm intensities for microbial inactivation, and irreversible electroporation (Raso et al., 2016). Industrially, an electric field strength between (~ 10–20 kV/cm) has been employed as a prerequisite for PEF systems for the microbial inactivation to date (Toepfl, 2012). The exposure of microbial cells to a source of electrical field for a few microseconds is associated with structural changes of the cell membrane, ultimately leading to cell damage (Heinz et al., 2001; Roobab et al., 2018).

A variety of food products, from liquid or semi-liquid to solid, have been researched for microbial inactivation by PEF treatment (Abbas Syed, 2017). Most of the studies related to PEF process for microbial inactivation focus on the application of high voltage pulses to milk and dairy products, juices and a variety of liquids (Abbas Syed, 2017; Tanino et al., 2020). Depending on the type of the food product (liquid, semi-liquid and solid), the effectiveness of different PEF systems can be explored, that of the batch or the continuous (Mohamed & Amer Eissa, 2012; Niu et al., 2020; Nowosad et al., 2021). The batch PEF systems are primarily opted for handling static volumes of solid or semi-solid foods (Mohamed & Amer Eissa, 2012), whereas the continuous treatment PEF systems have a potential for industrial processing of liquid and semi-liquid foods, as they allow the continuous/steady flow of products (Niu et al., 2020). The first commercialized PEF system for fruit juices (apple, strawberry, and other flavors) preservation was used by Genesis Juices (Oregon,USA) in 2006 (Clark, 2006) while the first commercial PEF line for fruit juice preservation (1500 L/h) was reported in Europe back in 2009 (Siemer et al., 2014). According with Raso et al. (2016). The most common treatment chambers in use for continuous systems have their electrodes with parallel plate or colinear configuration. The parallel plate configuration is characterized by a large electrode surface and low intrinsic electrical resistance, with the main advantage the formation of a uniform electric field, but the limitation of creating electrode corrosion due its high current operation that may trigger undesired electrochemical phenomena. The colinear treatment chamber functions at lower current compared to the parallel plate configuration. It is known to be conducive, minimizing electrode reactions and enabling the parallel connection of multiple co-linear units from an electrical standpoint. Nevertheless, its main drawback lies in the uneven distribution of electric field strength and temperature within the treatment zone during PEF processing (Raso et al., 2016).

2. Microbial inactivation by PEF treatments

Food products harbour a variety of different microorganisms, several of which can be used in the food industry to drive fermentation processes. However, other food associated microbes can cause food spoilage and generate public health problems (Mosqueda-Melgar et al., 2008). Common food spoilage microorganisms include *Saccharomyces, Lactiplantibacillus, Leuconostoc, Brochothrix thermosphacta, Pseudomonas, Acinetobacter, Moraxella Penicillium*, *Cladosporium* spp. (Mermelstein, 2017) while common foodborne pathogens include *Salmonella, Campylobacter, Enterohaemorrhagic Escherichia coli, Listeria and Vibrio cholerae* (World Health Organization, 2020). Consequently, for the application of an effective PEF disinfection, and for safeguarding food stability and safety, the identification of PEF-resistant pathogenic and spoilage bacteria becomes pivotal (García, Gómez, Raso, et al., 2005). Over the last two decades, the impact of PEF on enzyme and microorganism inactivation, as well as on energy-saving has been heavily

researched (Nowosad et al., 2021). Significant PEF-mediated microbial inactivation have been reported for several pathogenic bacteria including *Staphylococcus aureus*, *Yersinia entercolitica, Escherichia coli, Listeria monocytogenes* and *Salmonella Enteritidis,* as well as for spoilage microorganisms, such as *Acetobacter* spp., *Bacillus subtillis, Lactiplantibacillus plantarum*, and *Saccharomyces cerevisiae* (as shown in Table 1) (García, Gómez, Mañas, et al., 2005; Katiyo et al., 2017; Niu et al., 2019; Wang et al., 2018).

2.1. Processing parameters

The efficiency of decontamination by PEF technology is dependent on different factors. These can mainly be grouped under three different categories: processing conditions, characteristics of microbes, and product parameters (Abbas Syed, 2017; Roobab et al., 2018). The main process parameters for PEF treatments are: the electric field strength (E), the treatment time (t), the pulse shape, the pulse width (τ), the number of pulses (n), the pulse repetition frequency, and the total specific energy input (W) and (Raso et al., 2016). Different treatment conditions (E = 12–50 kV/cm, 27–2000 μs) and a variety of food matrices are presented in this review (see Table 1).In general, changes in PEF processing conditions can impact the efficiency of microbial inactivation, with microbial inactivation generally increasing with higher electric field strength or longer treatment times (Wouters et al., 2001). However, an increase in the total specific energy causes an increase of the temperature in the treatment medium due to Joule's heating which is of great importance as heat sensitive compounds could be affected (Schottroff et al., 2019).

2.2. Microbial characteristics

The main microbial characteristics that play an important role in microbial inactivation by PEF are: the type of microorganism, the species, the strain and culture conditions (Raso et al., 2016). In a pilot study, Lee et al. (2015) have shown that PEF-treatments of low-fat milk with an electric field strength of 10 kV/cm, total specific energy input of 200 kJ/L and an inlet temperature of 30 °C enabled a 4.4-$\log_{10}$ CFU/mL, a 4.5-$\log_{10}$ CFU/mL, and a 6.0-$\log_{10}$ CFU/mL inactivation, for *L. brevis, E. coli*, and *S. cerevisiae,* respectively. Additionally, Huang et al. (2014) reported that PEF-treatment (24 kV/cm, 180 μs, inlet temperature of 30 °C and maximum temperature of 38.2 ± 0.8 °C) of grape juice resulted in a 2.69-$\log_{10}$ CFU/mL, a 3.6-$\log_{10}$ CFU/mL and a 6.01-$\log_{10}$ CFU/mL inactivation, for *Staphylococcus aureus, E. coli*, and *S. cerevisiae,* respectively. Consequently, the effectiveness of PEF disinfection is microbial species dependent (Niu et al., 2020) where in general the larger the cells the more susceptible are to the electrical fields (Heinz et al., 2014). Studies have also revealed that even different strains of the same bacterial species exhibit different sensitivities to the same PEF treatment (Raso et al., 2016). For example, Walter et al. (2016) showed that PEF treatment (35 kV/cm, 30 μs treatment time and an average temperature of 40 °C) of UHT whole-milk (4% fat), resulted in a > 2 $\log_{10}$ CFU/mL for *E. coli* strains ATCC 11775 and FSAW 1325, whereas strains of O157:H7 Sakai and FSAW 1326 appeared more PEF-resistant (<1.5 $\log_{10}$ inactivation; $p < 0.05$).

Other important factors affecting the PEF treatment efficiency include the bacterial growth environment and growth stage of the cells (Liu et al., 2017). For example, Niu et al. (2019) have shown that ethanol has an effect on the cell membrane properties of *Acetobacter* sp., as under the same PEF treatment (20.0 kV/cm,6.0 ms and maximum temperature < 35 °C) the reduction of the cells cultured with 9% (v/v) ethanol was higher (5.17-$\log_{10}$) than those without it (3.22-$\log_{10}$). Additionally, Álvarez et al. (2002) have shown that L. *monocytogenes* cells grown at 35 °C were more PEF-resistant than those grown at 4 °C, with PEF resistance increasing during the incubation time and reaching its maximum value at the stationary growth phase of the bacterium. More specifically, after PEF treatment (25 kV/cm800 μs and maximum temperature < 35 °C) for two suspensions (with an initial concentration of 10^8 cells/mL) grown at 4 °C and 35 °C, their maximal resistance reduced by 2.0 and 1.2 $\log_{10}$, respectively (Álvarez et al., 2002). Additionally, Ohshima et al. (2002) have shown that the culture temperature influences the resistance against PEF treatment. More specifically it was identified that in the optimum culture temperature, i.e., 37 °C, *E. coli* was more resistant under PEF treatment in comparison to culture temperatures of 20 or 42 °C. The sensitivity of *E. coli* under PEF treatment after cultivation at 20 °C is associated with an increased content of unsaturated fatty acids in phospholipids, which induces cell membrane fragility, whereas cultivation at 42 °C initiates cell destruction cascades, following protein unfolding and activation of bacteria and activates the heat shock proteins (Ohshima et al., 2002). Yun, Liu, et al. (2016) reported that under the same PEF treatment (25 kV/cm,1.2 ms) and initial inactivation temperature of 25 °C, *Salmonella Typhimurium* cells reaching their stationary phase exhibited 3.30-$\log_{10}$, 2.48-$\log_{10}$, 1.99-$\log_{10}$, 1.86-$\log_{10}$ and 1.63-$\log_{10}$ reductions when grown at 10, 20, 30, 37 and 45 °C, respectively. Additionally, Wouters et al. (1999), showed that *L. innocua* cells grown into stationary phase were more resistant to PEF treatments than cells in their log-growth phase. This phenomenon may be attributed to either the heightened susceptibility of membrane areas involved in cell division, or the larger size of bacterial cells in the exponential phase, leading to decreased resistance to PEF. The resistance of microorganisms grown to their stationary phase may result from alterations in the expression of stress-related genes, instigated by the alternative sigma factor, as well as different metabolic, structural and morphological changes relative to the exponentially grown cells (Somolinos et al., 2008).

2.3. Treatment medium properties

Food product parameters that can influence the microbial inactivation efficiency are: the conductivity, and the water activity (a_w) and the pH of the product (Abbas Syed, 2017; Chacha et al., 2021; García, Gómez, Raso, et al., 2005). The conductivity of the medium is related to the resistivity of the medium in a treatment chamber and influences current intensity that is needed for the generation of electric fields (Gachovska et al., 2013).

In a range of conductivities that do not influence the distribution of the electric field, lowering the conductivity increases the difference between the ionic concentration of the cytoplasm and the treatment medium and as a consequence leads to an increased flow of ions across the membrane. This phenomenon weakens the membrane and the membrane's resistance to pulses (Jayaram et al., 1993). Different studies have shown that the highest microbial inactivation was achieved at the lowest conductivity (Sensoy et al., 1997; Wouters et al., 1999). Although conductivity is considered a parameter that influences microbial inactivation, different researches have shown that there was no influence under the same total specific energy used (conductivities between from 0.05 up to 0.45 S/m) (Álvarez et al., 2003; Timmermans et al., 2019). Reduction of a_w has shown to increase the PEF resistance of microorganisms (Álvarez et al., 2002; Aronsson et al., 2001). Regarding the effect of the pH, media of pH 7.0 and 4.0 affected differently L. *monocytogenes*, with higher inactivation levels achieved at pH 4.0, suggesting that for specific microorganisms an acidic environment can be a hurdle (Saldaña et al., 2009). On the contrary, the opposite behavior was observed for PEF-treated *E. coli* with higher resistance at pH 4.0 than pH 7.0, indicating that different strategies need to be applied depending on the target microorganism (Saldaña et al., 2009). In another study, Jaeger et al. (2009) reported that the microbial inactivation of *Lactiplantibacillus rhamnosus* was influenced by increasing the protein content. However, this effect was not observed in L. *innocua* with increasing protein content under neutral pH condition (Schottroff et al., 2019), Thus, further research on a plethora of microorganisms related to the microbiota of each food matrix and the PEF equipment is important for optimization of the process with a final aim at achieving a 5-log microbial reduction minimum.

Table 1
Summary of processing parameters and decontamination efficiency of different PEF treatments.

Microorganism	Product	Treatment conditions	Reduction	Reference
Gram-negative bacteria				
Escherichia coli (ATCC 8739)	Green tea beverage pH: 6.0 at 20 °C Conductivity: 0.1 S/m at 20 °C	E: 38.4 kV/cm t: 160 μs τ: 2 μs n: * NS f: 667 pps Energy input: 236 × 10^3 J/L Inlet temperature: 20 °C Maximum temperature: * NS Pulse type: bipolar / square	5.6 $\log_{10}$	(Zhao et al., 2008)
Escherichia coli (ATCC 8739)	Soymilk pH: 7.22 ± 0.16 at 20 °C Conductivity: 0.21 ± 0.02 S/m at 20 °C	E: 40 kV/cm t: 547 μs τ: 2 μs n: * NS f: 400 Hz Energy input: * NS Flow rate: 1 mL/s Inlet temperature: 25 °C Maximum temperature: < 35 °C (in process) Pulse type: bipolar / square	5.2 $\log_{10}$	(Li et al., 2013)
Escherichia coli (ATCC26)	McIlvaine buffer solution pH: 3.8 at 25 °C Conductivity: 2 mS/cm at 25 °C	E: 12 kV/cm t: * NS τ: 4 μs n: * NS f: * NS Energy input:40 J/mL Flow rate:15 mL/min Inlet temperature: 25 °C Maximum temperature: 29 ± 1 °C Pulse type: monopolar/square	2.25 $\log_{10}$	(Pataro et al., 2014)
Escherichia coli (ATCC 8739)	Commercial low-fat milk pH: * NS Conductivity: * NS	E: 10 kV/cm t: * NS τ: 30 μs n: * NS f: * NS Energy input: 200 kJ/kg Flow rate:30 L/h Inlet temperature: **30** °C Maximum temperature: * NS Pulse type: bipolar/square	4.5 $\log_{10}$	(Lee et al., 2015)
Escherichia coli (ATCC 11775)	UHT whole milk with 4.0% fat (*w*/w) pH: * NS Conductivity: * NS	E: 35 kV/cm t: 124 μs τ: 2 μs n: * NS f: * NS Energy input: * NS Flow rate: 120 mL/min Inlet temperature: * NS Maximum temperature: 30 °C on average (in process) Pulse type: * NS / * NS	4.8 $\log_{10}$	(Walter et al., 2016)
Escherichia coli K12 *Escherichia coli* (35218)	Orange Juice pH: 3.4 ± 0.1 at * NS°C Conductivity: * NS	E: 20 kV/cm t: 70 μs τ: 2.6 μs n: * NS f: 740 Hz Energy input: * NS Flow rate: 120 mL/min Inlet temperature: * NS Maximum temperature: 55 °C (outlet) Pulse type: bipolar / square	5.06 $\log_{10}$ 2.02 $\log_{10}$	(Gurtler et al., 2010)
Escherichia coli DH5α (CGMCC 1.2170)	Grape Juice Soluble solid content: 15.8 ± 0.2°Brix at * NS°C pH: 5.98 ± 0.02 at * NS°C Conductivity: 0.086 ± 0.002 S/m at 20 °C & 0.098 ± 0.002 S/m at 30 °C	E: 24 kV/cm t:180 μs τ: 3 μs n: * NS f: 120 Hz Energy input: * NS Flow rate: 7.6 mL/min Inlet temperature: 30 °C Maximum temperature: 38.2 ± 0.8 °C Pulse type: monopolar / square	3.06 $\log_{10}$	(Huang et al., 2014)
Escherichia coli 1.107	Melon Juice Soluble solid content:	E: 35 kV/cm	3.91 $\log_{10}$	(Mosqueda-Melgar et al., 2007)

(*continued on next page*)

Table 1 (*continued*)

Microorganism	Product	Treatment conditions	Reduction	Reference
	11.1°Brix at *NS°C pH: 5.82 ± 0.04 at *NS°C Conductivity: 5.23 ± 0.03 mS/cm at *NS°C	t: 1250 μs τ: 4 μs n: *NS f: 250 Hz Energy input: 7662.23 J/cm^3 Flow rate: 100 mL/min Inlet temperature: *NS Maximum temperature: 39.1 ± 0.1 °C (outlet) Pulse type: bipolar / square		
Escherichia coli 1.107	Watermelon juice Soluble solid content: 6.5°Brix at *NS°C pH: 5.46 ± 0.11 at *NS°C Conductivity: 3.66 ± 0.05 mS/cm at *NS°C	E: 35 kV/cm t: 2000 μs τ:4 μs n: *NS f: 250 Hz Energy input: 7541.18 J/cm^3 Flow rate: 100 mL/min Inlet temperature: *NS Maximum temperature: 30.3 ± 0.2 °C (outlet) Pulse type: bipolar / square	4.01 $\log_{10}$	(Mosqueda-Melgar et al., 2007)
Escherichia coli (ATCC 11775)	Strawberry Juice (Model solution) Soluble solid content: 7.9 ± 0.28°Brix at *NS°C pH: 3.39 ± 0.01 at *NS°C Conductivity: 3.90 ± 0.14 mS/cm at *NS°C	E: 35 kV/cm t: 27 μs τ: 2 μs n: *NS f: 155 Hz Energy input: *NS Flow rate: 350 mL/min Inlet temperature: 22.7 ± 0.07 °C Maximum temperature: 46.0 °C Pulse type: monopolar / square	5.53 $\log_{10}$	(Yildiz et al., 2019)
Escherichia coli O157:H7 (ATCC 43894)	Apple cider Soluble solid content: 12.5°Brix at *NS°C pH: 3.83 at *NS°C Conductivity: 2088 μS/cm at *NS°C	E: 30 kV/cm t: *NS τ: *NS n: *NS f: 1500pps Energy input:408 J/mL Flow rate: 60 mL/min Inlet temperature: Maximum temperature: <55 °C (in process) Pulse type: bipolar / square	6.38 $\log_{10}$	(Mendes-Oliveira et al., 2020)
Salmonella enteritidis 1.82 (NCTC 9001)	Melon Juice Soluble solid content: 11.1°Brix at *NS°C pH: 5.82 ± 0.04 at *NS °C Conductivity: 5.23 ± 0.03 mS/cm at *NS°C	E: 35 kV/cm t: 1250 μs τ: 4 μs n: *NS f: 175 Hz Energy input: 7662.23 J/cm^3 Flow rate: 100 mL/min Inlet temperature: NS* Maximum temperature: 39.1 ± 0.1 °C (outlet) Pulse type: bipolar / square	3.75 $\log_{10}$	(Mosqueda-Melgar et al., 2007)
Salmonella enteritidis 1.82 (NCTC 9001)	Watermelon juice Soluble solid content: 6.5°Brix at *NS°C pH: 5.46 ± 0.11 at *NS°C Conductivity: 3.66 ± 0.05 mS/cm at *NS°C	E: 35 kV/cm t: 2000 μs τ: 4 μs n: *NS f: 100 Hz Energy input: 7541.18 J/cm^3 Inlet temperature: *NS Maximum temperature: 30.3 ± 0.2 °C (outlet) Pulse type: bipolar / square	4.27 $\log_{10}$	(Mosqueda-Melgar et al., 2007)
Salmonella Typhimurium (ATCC 14028)	Apple cider Soluble solid content: 12.5°Brix at *NS°C pH: 3.83 at *NS°C Conductivity: 2088 μS/cm at *NS°C	E: 30 kV/cm t: *NS τ: *NS n: *NS f: 1500pps Energy input:408 J/mL Flow rate: 60 mL/min Maximum temperature: <55 °C (in process) Pulse type: bipolar / square	6.34 $\log_{10}$	(Mendes-Oliveira et al., 2020)
Salmonella Typhimurium χ3985 *Salmonella Typhimurium χ4096* *Salmonella Typhimurium*	Orange Juice pH: 3.4 ± 0.1 at *NS°C Conductivity: *NS	E: 20 kV/cm t: 70 μs τ: 2.6 μs	1.62 $\log_{10}$ 2.36 $\log_{10}$	(Gurtler et al., 2010)

(*continued on next page*)

Table 1 (*continued*)

Microorganism	Product	Treatment conditions	Reduction	Reference
χ3751 *Salmonella Choleraesuis χ8442*		n: * NS f: 740 Hz Energy input: * NS Flow rate: 120 mL/min Inlet temperature: * NS Maximum temperature: 55 °C (outlet) Pulse type: bipolar / square	4.05 log_{10} 5.45 log_{10}	
Pseudomonas fluorescens (ATCC 948)	UHT whole milk with 4.0% fat (*w*/w) pH: * NS Conductivity:* NS	E: 35 kV/cm t: 124 μs τ: 2 μs n: * NS f: * NS Energy input: Flow rate: 120 mL/min Inlet temperature: * NS Maximum temperature: 30 °C on average (in process) Pulse type: * NS / * NS	2.4 log_{10}	(Walter et al., 2016)
Cronobacter sakazakii (29,544 ATCC)	Infant milk formula pH: 6.8 at 25 °C Conductivity: 0.278 S/m at 25 °C	E: 35 kV/cm t: 700 μs τ: 2.5 μs n: * NS f: * NS Energy input: * NS Flow rate: 30 mL/min Inlet temperature: 4 °C Maximum temperature: 25 ± 3 °C Pulse type: bipolar / square	1.1 ± 0.03 log_{10}	(Pina-Pérez et al., 2013)
Gram-positive bacteria				
Bacillus cereus (131)	Pasteurized skim Milk pH: 6.69 ± 0.03 at 25 °C Conductivity: 5.77 ± 0.10 mS/cm at 25 °C Mixture of pasteurized skim milk and liquid whole egg (80/20 *v*/v) pH: 6.18 ± 0.05 at 25 °C Conductivity: 7.23 ± 0.03 mS/cm at 25 °C	E: 35 kV/cm t: 200 μs τ: 2.5 μs n: * NS f: * NS Energy input: * NS Flow rate: 30 mL/min Inlet temperature: * NS Maximum temperature: 20 °C (in process) Pulse type: * NS / * NS	3.05 ± 0.02 log_{10} 3.03 ± 0.02 log_{10}	(Pina-Pérez et al., 2009)
Lactiplantibacillus plantarum (49445) *Lactiplantibacillus lactis* (114545) *Lactiplantibacillus fermentum* (9338) *Lactiplantibacillus casei* 393	Orange Juice pH: 3.4 ± 0.1 at * NS°C Conductivity: * NS	E: 20 kV/cm t: 70 μs τ: 2.6 μs n: * NS f: 740 Hz Energy input: * NS Flow rate: 120 mL/min Inlet temperature: * NS Maximum temperature: 55 °C (outlet) Pulse type: bipolar / square	3.07 log_{10} 4.53 log_{10} 3.22 log_{10} 0.60 log_{10}	(Gurtler et al., 2010)
Lactiplantibacillus brevis (ATCC13648)	Commercial low-fat milk pH: * NS Conductivity: * NS	E: 10 kV/cm t: * NS τ: 30 μs n: * NS f: * NS Energy input: 200 kJ/kg Flow rate:30 L/h Inlet temperature: **30** °C Maximum temperature: * NS Pulse type: bipolar/square	4.4 log_{10}	(Lee et al., 2015)
Listeria monocytogenes 1.131	Melon Juice Soluble solid content: 11.1°Brix at * NS°C pH: 5.82 ± 0.04 at * NS°C Conductivity: 5.23 ± 0.03 mS/cm at * NS°C	E: 35 kV/cm t: 2000 μs τ: 4 μs n: * NS f: 100 Hz Energy input: 7662.23 J/cm^3 Flow rate: 100 mL/min Inlet temperature: * NS Maximum temperature: 39.1 ± 0.1 °C (outlet) Pulse type: bipolar / square	4.27 log_{10}	(Mosqueda-Melgar et al., 2007)
Listeria monocytogenes 1.131	Watermelon juice Soluble solid content: 6.5°Brix at * NS °C	E: 35 kV/cm t: 2000 μs τ: 4 μs	3.77 log_{10}	(Mosqueda-Melgar et al., 2007)

(*continued on next page*)

Table 1 (*continued*)

Microorganism	Product	Treatment conditions	Reduction	Reference
	pH: 5.46 ± 0.11 at * NS°C Conductivity: 3.66 ± 0.05 mS/cm at * NS°C	n: * NS f: 250 Hz Energy input: 7541.18 J/cm^3 Flow rate: 100 mL/min Inlet temperature: * NS Maximum temperature: 30.3 ± 0.2 °C (outlet) Pulse type: bipolar / square		
Staphylococcus aureus (CECT 240)	Homogenized UHT skim-milk pH of 6.68 ± 0.02 at 25 °C Conductivity: 6.03 ± 0.01 mS/cm at 25 °C	E:35 kV/cm t: * NS n: 150 pulses τ: 8 μs f: 100 Hz Energy input: * NS Inlet temperature: * NS Maximum temperature: 25 °C (in process) Pulse type: bipolar / square E: 35 kV/cm t: * NS n: 150 pulses τ: 8 μs f: 100 Hz Energy input: * NS Inlet temperature: * NS Maximum temperature: 25 °C (in process) Pulse type: monopolar / square	4.5 $\log_{10}$ 3.6 $\log_{10}$	(Sobrino-López et al., 2006)
Staphylococcus aureus (ATCC 6538)	Green tea beverage pH: 6.0 at 20 °C Conductivity: 0.1 S/m at 20 °C	E: 38.4 kV/cm t: 160 μs τ: 2 μs n: * NS f: 667 pps Energy input: 236 × 10^3 J/L Inlet temperature: 20 °C Maximum temperature: * NS Pulse type: bipolar / square	4.9 $\log_{10}$	(Zhao et al., 2008)
Staphylococcus aureus (ATCC 6538)	Soymilk pH: 7.22 ± 0.16 at 20 °C Conductivity: 0.21 ± 0.02 S/m at 20 °C	E: 40 kV/cm t: 547 μs τ: 2 μs n: * NS f: 400 Hz Energy input: * NS Flow rate: 1 mL/s Inlet temperature: 25 °C Maximum temperature: < 35 °C (in process) Pulse type: bipolar / square	3.51 $\log_{10}$	(Li et al., 2013)
Staphylococcus aureus (CICC 21648)	Grape Juice Soluble solid content: 15.8 ± 0.2°Brix at * NS°C pH: 5.98 ± 0.02 at * NS°C Conductivity: 0.086 ± 0.002 S/m at 20 °C & 0.098 ± 0.002 S/m at 30 °C	E: 24 kV/cm t:180 μs τ: 3 μs n: * NS f: 120 Hz Energy input: * NS Flow rate: 7.6 mL/min Inlet temperature: 30 °C Maximum temperature: 38.2 ± 0.8 °C (outlet) Pulse type: monopolar / square	2.69 $\log_{10}$	(Huang et al., 2014)
Yeast				
Candida stellate (NRRL Y- 1446) *Saccharomyces cerevisiae* (ATCC 16664)	Sterilized deionized water pH: 5.6 at * NS°C Conductivity: 68 μS/cm at * NS°C Sterilized deionized water pH: 5.2 at * NS°C Conductivity: 15 μS/cm at * NS°C	E: 12.5 kV/cm t: NS* τ: 0.3 ms n: 5 pulses f: * NS Energy input: * NS Inlet temperature: * NS Maximum temperature: < 30 °C Pulse type: * NS / exponential	3.5 ± 0.2 $\log_{10}$ 3.3 ± 0.6 $\log_{10}$	(Geveke & Kozempel, 2003)
Saccharomyces cerevisiae (CICC 1374)	Grape Juice Soluble solid content: 15.8 ± 0.2°Brix at * NS°C pH: 5.98 ± 0.02 at * NS°C Conductivity:	E: 24 kV/cm t:180 μs τ: 3 μs n: * NS f: 120 Hz	6.01 $\log_{10}$	(Huang et al., 2014)

(*continued on next page*)

Table 1 (*continued*)

Microorganism	Product	Treatment conditions	Reduction	Reference
	0.086 ± 0.002 S/m at 20 °C & 0.098 ± 0.002 S/m at 30 °C	Energy input: * NS Flow rate: 7.6 mL/min Inlet temperature: 30 °C Maximum temperature: 38.2 ± 0.8 °C (outlet) Pulse type: monopolar / square		
Saccharomyces cerevisiae (ATCC 26603)	Commercial low-fat milk pH: * NS Conductivity: * NS	E: 10 kV/cm t: * NS τ: 30 μs n: * NS f: * NS Energy input: 200 kJ/kg Flow rate:30 L/h Inlet temperature: **30** °C Maximum temperature: * NS Pulse type: bipolar/square	6.0 $\log_{10}$	(Lee et al., 2015)
Saccharomyces cerevisiae ascospores (DSMZ 1848)	Lager Beer ≤0.05% alc/vol Conductivity: 2.20 mS/cm at 23 °C Dark Ale beer 5.0% alc/vol Conductivity: 1.97 mS/cm at 23 °C Ale beer 7.0% alc/vol Conductivity: 2.76 mS/cm at 23 °C	E: 45 kV/cm t: 70 μs τ: 1.5 μs n: 46.3 pulses f: 700 Hz Energy input: * NS Flow rate: 4.34 mL/s Inlet temperature: * NS Maximum temperature: < 43 °C Pulse type: bipolar/square	0.2 $\log_{10}$ 0.7 $\log_{10}$ 2.2 $\log_{10}$	(Milani et al., 2015)
Brettanomyces bruxellensis (AWRI 1608)	Red Wine (Cabernet Sauvignon) 13.5% alc/vol pH: * NS Conductivity: 0.789 mS/cm at 5 °C	E: 50 kV/cm t: 42 μs τ: 1.7 μs n: * NS f: 100 Hz Energy input: 119.3 ± 3.5 kJ/kg Flow rate: 0.23 mL/s Inlet temperature: 4.0 ± 0.2 °C Maximum temperature: 31.8 ± 0.7 °C (outlet temperature) Pulse type: bipolar / square	0.9 $\log_{10}$	(van Wyk et al., 2018)
Brettanomyces bruxellensis (AWRI 1499)	Red Wine (Cabernet Sauvignon) 13.5% alc/vol pH: * NS Conductivity: 0.789 mS/cm at 5 °C	E: 50 kV/cm t: 42 μs τ: 1.7 μs n: * NS f: 100 Hz Energy input: 117.5 ± 1.4 kJ/kg Flow rate: 0.23 mL/s Inlet temperature: 3.8 ± 0.1 °C Maximum temperature: 31.1 ± 0.5 °C (outlet temperature) Pulse type: bipolar / square	3.0 $\log_{10}$	(van Wyk et al., 2018)
Brettanomyces bruxellensis (AWRI 1613)	Red Wine (Cabernet Sauvignon) 13.5% alc/vol pH: * NS Conductivity: 0.789 mS/cm at 5 °C	E: 50 kV/cm t: 42 μs τ: 1.7 μs n: * NS f: 100 Hz Energy input: 120.8 ± 3.7 kJ/kg Flow rate: 0.23 mL/s Inlet temperature: 3.4 ± 0.1 °C Maximum temperature: 31.3 ± 0.6 °C (outlet temperature) Pulse type: bipolar / square	3.7 $\log_{10}$	(van Wyk et al., 2018)

E: electric field strength; t: is total treatment time; τ: is pulse width; n: number of pulses; f: pulse repetition rate.
* NS: non-specified.

3. Effect of PEF processing treatment on food-borne microorganisms

PEF treatments, similar to most of the environmental stresses do not lead to a “all or nothing “effect to a microbial population (Wang et al., 2015). Numerous studies have demonstrated that the electroporation phenomena observed in microbial cells after PEF application are not proportionally linked to cell death and there is a clear difference between electroporated and dead cells (Golberg et al., 2010). Consequently, insufficient PEF treatments can generate sub-lethally injured cells that raise food safety and security challenges (Jaeger et al., 2009). The occurrence of reversible pores after PEF treatment supports microbial membrane re-sealing, to a greater or lesser extent, enabling cell survival (Fig. 1) (García, Gómez, Mañas, et al., 2005; Golberg et al., 2010).For example, 45% of sublethally injured cells of *C. sakazakii* resealed their cytoplasmic membranes immediately after PEF treatment (Arroyo et al., 2010). However, Zhao et al. (2011) have demonstrated that PEF applications of >25 kV/cm field electric strength

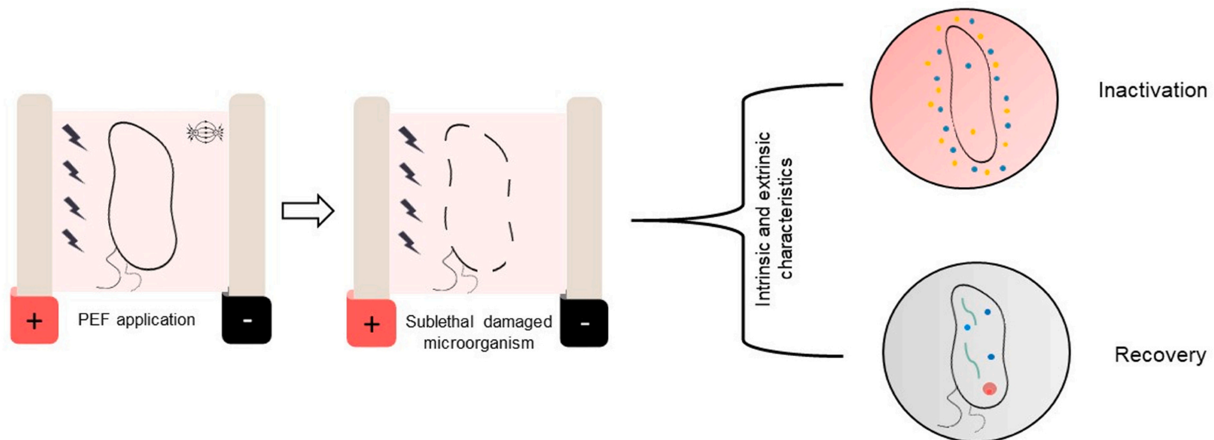

Fig. 1. Sublethal damaged microorganisms after PEF application.

lead to permanent membrane damage to *E. coli* cells due to irreversible permeabilization, whilst at lower field strengths reversible electroporation manifested up to 20% and 10% of the cells, respectively. Chueca et al. (2015) have also shown that a PEF treatment of 50 pulses at 20 kV/cm lead to 40% inactivation and 40% injured *E. coli* cells. Furthermore, Zand et al. (2022) showed that under PEF treatments of 8 kV/cm and 18 kV/cm, 38% and 63% of the initial *E. coli* population were in intermediate cellular states, respectively, whereas PEF treatment of 25 kV/cm irreversibly injured 93 ± 1% of *E. coli* cells.

In general, under environmental stresses, including temperature and osmotic changes, potential alterations in the organization and structure of the membrane lipids can lead to consecutive modulation of cellular activities (Los & Murata, 2004). Zhao et al. (2014a) noted that *S. cerevisiae* PEF treatments at 20 kV/cm for 100, 200, 300, 400 and 500 μs achieved lower microbial reductions in the presence of non-selective media (0.8, 1.2, 1.9, 2.5 and 3.1 log_{10}) than in the presence of selective media (2.1, 3.0, 3.8 and 4.1 log_{10}), indicating that the salt composition of the selective media sensitised a large cell fraction to membrane damage. Additionally, Aronsson et al. (2005) showed that exposure of *S. cerevisiae* cells to a PEF treatment of 20 kV/cm and 2 μs resulted in a 1.8 log_{10} inactivation and a 3.8 log_{10} permeabilized cells, while under the same conditions, *E. coli* cells exhibited a 0.5 log_{10} inactivation and a 0.3 log_{10} membrane integrity reduction. These results show that cells of *S. cerevisiae* did not lose their ability to multiply albeit their membranes were permeabilized (Aronsson et al., 2005). Thus, the cytomembrane is a key target for PEF treatment-induced damage and only under favourable conditions sublethal populations can repair their membrane injuries (Zhao et al., 2014b).

Zhao et al. (2014a) have shown that after PEF treatment at 20 kV/cm with a process temperature < 30 °C, *S. cerevisiae* cells had an increase in their fluorescence anisotropy value (identified by measuring fluorescence anisotropy in intact whole cells by using hydrophobic 1,6-diphenyl-1,3,5-hexatriene (DPH) as a probe) indicating a decrease in the cytoplasmatic membrane fluidity. Additionally, Zhao et al. (2014b) following PEF treatment of 20 kV/cm of *S. cerevisiae* cells for 100, 200, 300, 400, and 500 μs with a process temperature < 30 °C have shown that there was an important increase in the membrane's micro-viscosity value *n* (i.e., 116.7%, 130.0%, 133.3%, 141.3% and 161.3%, respectively to the treatment times), indicating that the longer the PEF treatment the more rigid the cytomembrane was. In general, the response of the cells after environmental stresses is dependent on both the cytoplasmic membrane fluidity and the membrane fatty acid composition (Yun, Zeng, et al., 2016). Yun et al. (2017) have shown that an increase in growth temperature from 10 to 45 °C, increased both the C—C bond and lamellar packing order of phospholipid chains leading to decreased membrane fluidity. Furthermore, Yun et al. (2017) have demonstrated that the PEF treatment time under the same electric field strength (29.33 kV/cm) for the inactivation of 90% of *S. typhimurium* cells was almost 4 times higher when cells were cultured at 10 °C than at 45 °C which indicates a PEF-efficiency dependence on membrane lipid composition.

Additionally, Wang et al. (2016) have shown that different growth temperatures lead to different percentages of injured *S. aureus* cells, following PEF treatments. Specifically, they found that *S. aureus* cells cultivated at 10 and 25 °C prior to a PEF treatment at 52.0 kV/cm, exhibited sublethal injuries to 12.26% and 20.75%, respectively. Those differences were attributed to changes in membrane structural integrity and fluidity, as a result of altered fatty acid membrane composition at different temperatures. *S. aureus* cells cultivated at 10 °C were more PEF sensitive because of their increased branched chain fatty acid (BCFAs) membrane content (such as anteiso C15:0 and anteiso C17:0) and their resulting membrane fluidity, whereas cells cultivated at 25 °C were more PEF resistant due to their reduced BCFA membrane content, increased straight chain fatty (such as C16:0, C17:0 and C18:0) and their reduced membrane fluidity (Wang et al., 2016). In line with the previous studies, the ratio of unsaturated and saturated fatty acids in the membrane lipids of *S. cerevisiae* changed after PEF treatment (20 kV/cm and 500 μs,) from 71.14% and 23.6% to 60.56% and 30.27%, respectively, confirming once again the tight relationship between membrane fluidity and effective microbial inactivation (Los & Murata, 2004; Zhao et al., 2014a).

Rivas et al. (2013) demonstrated that after PEF application of 15 kV/cm, 700 μs and five inlet temperatures (7, 16, 24, 30 and 38 °C) the percentage of sub-lethally damaged *E. coli* DH5α cells reached a 16% maximum with increasing temperature from 7 to 30 °C, whereas a decrease in sub-lethal damages was observed at 38 °C. Zhao et al. (2022) have shown that depending on different water bath temperatures (4, 15, 35, 55 °C) in combination with a PEF treatment (20 kV/cm for 200 μs) different inactivation rates of *S. cerevisiae* BY4742 were identified (i.e., at 55 °C – 6.1 log_{10}, at 4 °C – 4.3 log_{10}, at 35 °C – 3.9 log_{10} and at 15 °C – 3.9 log_{10})). Additionally, after storage at 4 °C for 5 h different sublethal populations were reported at 15 °C and 55 °C. Garcia, et al., (2005) showed that Gram-positive in comparison to Gram- negative bacteria were more resistant to PEF treatments at pH 7.0, whereas Gram-negative bacteria were more resistant in comparison to Gram-positive to PEF treatments at pH 4.0. In harmony with the previous study, Arroyo et al. (2010) found *C. sakazakii* to sustain sublethal injuries over the 3.5–4.0 pH range, where the strain exhibited its maximum resistance to the

treatment.

Finally, Zhao et al. (2014a) have shown that injured cells of *S. cerevisiae* BY4742 treated with PEF (20 kV/cm) fully recovered after 70 min of incubation in 20 mmol/L glucose solution at 37 °C, suggesting that the repair mechanism is glucose-energy dependent and the mitochondria are one of the putative PEF treatment targets. Additionally, Garcia et al. (2006) have shown that the sublethally membrane injured population of *E. coli* (NCTC 5934) after PEF treatment was heterogeneous, with a small proportion (<5%) repairing their membranes immediately after PEF treatment and a remaining proportion (95%) requiring energy and lipid synthesis for membrane repair. This suggests that positive responses to the biosynthetic requirements of cytoplasmatic membrane leads to repair of the sub-lethally injured cells (García et al., 2006).

4. Effect of PEF treatment at a microbial genomic/transcriptomic level

Gene expression alterations and differential protein synthesis before and after PEF treatments can reveal useful information for elucidating PEF-microbial inactivation and resistance mechanisms (Table 2)(Chueca et al., 2015). Recently, Yun, Liu, et al. (2016) reported that following PEF application of 25 kV/cm and 1.2 ms to *S. typhimurium* cultivated at various temperatures (10, 20, 30, 37 and 45 °C), the transcription levels of the alternative sigma factor genes *rpoS*, *rpoE* and *rpOH* (genes which play an important role in protecting cells from environmental stress and repairing damages) were altered. More specifically: *rpoS* (RNA polymerase, Sigma S) transcription level was higher for cells cultivated at 10 and 20 °C, whereas the transcription levels for *rpoE* (RNA polymerase, extracytoplasmic E) and *rpoH* (RNA polymerase, Sigma H) were higher at 45 °C. Additionally, at higher cultivation temperatures (37,45 °C), the transcription levels of the fatty acid biosynthesis related genes *cfa* (encoding Cyclopropane-fatty-acyl-phospholipid synthase involved with the formation of cyclopropane fatty acid,(CFA)) and *fabD* (encoding malonyl CoA:ACP transacylase and associated with the formation of malonyl ACP transformed by malonyl CoA) which may contribute to the alteration of PEF resistance to *S. typhimurium,* were higher, whereas the transcription level of fatty acid biosynthesis gene, *fabA* was lower (Yun, Zeng, et al., 2016). Studies by Chueca et al. (2015) showed that after PEF treatment (E:25 kV/cm, τ: 2 μs, n:50, <35 °C), targeting almost 90% of *E. coli* MG1655 cells, 20 genes were up-regulated and 27 were down-regulated. Some of the up-regulated genes included: cytochrome *bo*

Table 2
Genes/Proteins investigated under PEF treatments and their effect to microorganism's survival.

Microorganism	PEF parameters & Medium characteristics	Genes/ Proteins	Effect	Reference
Gram negative				
S. typhimurium	PEF parameters (E:25 kV/cm, t:1.2 ms, τ:40 μs,n:50 bipolar square pulses) Medium characteristics (pH: 6.9 ± 0.1, 180 ± 1 μS/cm)	• alternative sigma factor S, (*rpoS*) • alternative sigma factor E, *(rpoE)* • alternative sigma factor H, (*rpoH)* • fatty acid biosynthesis, *(cfa, fabD, fabA)*	Upregulation dependent on culture temperature (10, 20, 30, 37, 45 °C)	(Yun, Liu, et al., 2016)
E. coli MG1655	PEF parameters (E:25 kV/cm, τ: 2 μs, n:50 exponential pulses, maximum temperature <35 °C) Medium characteristics (pH: 4.0, 2 mS/cm)	• cytochrome *bo* oxidase, *(cyoB,cyoC, cyoD)* • hemeO synthase, *(cyoE)* • transcriptional repressors of bet genes, *(betI)* • succinate dehydrogenase, (sdhC, sdhD, sdhA, sdhB) • chromosomal ars operon, *(arsR)*	Upregulation	(Chueca et al., 2015)
E. coli DH5α	PEF parameters (E:15 kV/cm, t: 700 μs, bipolar square pulses maximum temperature <25 °C) Medium characteristics (pH: close to 7.0, *†NS mS/cm)	• protein of the outer membrane, (OmpA) • phosphoheptose isomerase, (gmhA) • protein involved in the degradation of unfolded or abnormal proteins, (ClpA) • ribosomal protein S6 in the 30S subunit of ribosome, (RS6) • enzymedeoxyuridine 5′-triphosphate nucleotidohydrolase, (Dut) • ferritine, (FtnA)	Decreased level (affected by treatment) Increased level after treatment (recovery related)	(Rivas et al., 2013)
Gram positive				
S. aureus Newman	PEF parameters (E:19–31 kV/cm, exponential pulses, maximum temperature <35 °C) Medium characteristics (pH: 7.0, 2 mS/cm)	• alternative sigma factor B, (*sigB*)	Importance for resistance	(Cebrián et al., 2009)
L. monocytogenes EGD-e	PEF parameters (E:30 kV/cm, τ:3 μs, f:1 Hz, square pulses, <35 °C maximum temperature) Medium characteristics (pH: 4.0 and 7.0, 1 mS/cm)	• alternative sigma factor B, (*sigB*)	No effect	(Somolinos et al., 2010)
Yeast				
S. cerevisiae BY4742	PEF parameters (voltage:2 and 4 kV, t: 30 min) Medium characteristics (sterilized distilled water)	• heat-shock-protein encoding gene, (*HSP104*) • genes encoding enzymes for superoxide removal, *(SOD1, SOD2)* • glutathione synthesis genes, (*GLR1, GSH1*)	Slightly induced expression Induced expression	(Tanino et al., 2012)
S. cerevisiae BY4742	PEF parameters (E:20 kV/cm, t: 200 μs, τ:2 μs) Medium characteristics (pH: 7.2, *NS mS/cm)	• heat-shock-protein encoding gene, (*HSP104*)	Importance for resistance	(Zhao et al., 2022)

E: electric field strength; t: is total treatment time; τ: is pulse width; n: number of pulses; f: pulse repetition rate.
* NS: non-specified.
† ND: not determined.

oxidase (one of the 3 major terminal oxidases in the aerobic respiratory of *E.coli*) genes (*cyoB, cyoC, cyoD*) and hemeO synthase (*cyoE*) which are included in functioning of cytochrome bo oxidase, succinate dehydrogenase (sdhCDAB) operon and transcriptional repressors of bet genes (*betI*) and chromosomal ars operon (*arsR*) (Chueca et al., 2015). Cebrián et al. (2009) showed that absence of the alternative Sigma factor (sigB) regulator, in *S. aureus* (Newman and IK184, an isogenic *DrsbUVW-sigB* knockout mutant) lowered the resistance against PEF treatments. However, in the study by Somolinos et al. (2010) the *SigB* gene was dispensable to PEF, as both strains of *Listeria monocytogenes* (EGD-e and its isogenic deletion mutant *ΔsigB*) showed the same resistance under the same conditions, independently of the treatment's pH. However, under heat treatment, the genes regulated by SigB appeared to contribute to heat resistance at pH 7.0 (Somolinos et al., 2010). Also, Tanino et al. (2012) showed differences in the transcription level of the heat stress response gene *HSP104* and oxidation stress response gene *GLR1* after thermal (42 °C for 30 min) and PEF treatments (2 kV and 30 min, 4 kV and 30 min), respectively. Additionally, expression of different stress response genes, such as *GSH1* and *GLR1* (responsible for glutathione synthesis enzymes) and *SOD1* and *SOD2* (encoding enzymes for superoxide removal) were induced following only PEF treatments. Interestingly, the same study suggested that glutathione-dependent biological defense mechanisms against oxidation stress appear to be important in the *S. cerevisiae* resistance against PEF treatments.

Liu et al. (2019a) found 29 out of the 175 differentially expressed proteins of *E. coli* CGMCC44102 after PEF treatment, were located at the cell membrane, with 16 of the identified proteins playing a role in the transmembrane transport of various small metabolites and macromolecules, including phosphate ions, zinc, glycine betaine, trehalose, as well as short peptides, carbohydrates and lipids. The study of Rivas et al. (2013) identified 7 common proteins differentially produced following PEF treatment of *E. coli* DH5α (15 kV/cm, for 700 μs at various temperatures (7, 16, 24,and 38 °C). More specifically, outer membrane protein A (ompA), had levels that were lower at 7 °C and 16 °C than the control or even non-existent at 24 °C and 38 °C (Rivas et al., 2013). In contrast, the levels of phosphoheptose isomerase (GmhA; involved in the biosynthesis of cell wall lipopolysaccharide), cytosolic phosphorylase A (ClpA;involved in n the degradation of unfolded or abnormal proteins), ribosomal protein S6 in the 30S ribosomal subunit (RS6;involved in the translation stage of protein biosynthesis), enzyme deoxyuridine 5′-triphosphate nucleotidohydrolase (Dut; responsible of the production of dUMP and involved in nucleic acid metabolism) and ferritin A (FtnA; involved in storing iron) were connected with the recovery of the microorganism (Rivas et al., 2013).

Furthermore, Zhao et al. (2014a) reported changes in the activity of cellular enzymes of *S. cerevisiae* by PEF. More specifically, following a PEF treatment at 20 kV/cm for 500 μs, *S. cerevisiae* lipase C14, cystine arylamidase, naphthol-AS-BI-phosphohydrolase and a-glucosidase activities were undetectable, whereas esterase (C4), esterase lipase (C8), leucine arylamidase, valine aryl-amidase and phosphatase acid were markedly reduced, indicating a reduced rate of lipolytic processes (Zhao et al., 2014b). Additionally, Zhao et al. (2022) have identified that after a combination of mild heating and PEF treatment there were 8 significantly up regulated proteins (such as cystathionine beta-synthase, intracellular glyceraldehyde-3-phosphate dehydrogenase, phosphopyruvate hydratase ENO2 etc.) and 11 down regulated spots (such as translation machinery-associated protein 17, thioredoxin peroxidase, dihydroorotate dehydrogenase etc.). Among the up regulated proteins, the intracellular glyceraldehyde-3-phosphate dehydrogenase is an enzyme playing an important role in glycolysis and glucogenesis pathways and is important in the cellular energy metabolism. Additionally, due to emerging thermal effects, heat shock proteins, such as heat shock protein 70 (Heat shock protein of Hsp70 family and stress-seventy subfamily A protein) were also upregulated (Zhao et al., 2022). In line with the aforementioned observations, Zhao et al. (2014a) showed compromised recovery from *S. cerevisiae* sublethal injuries under PEF-induced mitochondrial damage. Thus, the production of different proteins related to the cell membrane functioning and repair, glycolysis and heat shock can play an important role in the resistance against PEF treatment (Liu et al., 2019b; Zhao et al., 2022).

In conclusion, the investigation into gene expression alterations and differential protein synthesis after PEF treatments have provided valuable insights into microbial inactivation and resistance mechanisms. Studies on *S. typhimurium* have highlighted temperature-dependent variations in the transcription levels of key genes involved in stress response and damage repair, emphasizing the complexity of microbial responses to PEF. Additionally, research on *S. aureus* has implicated the regulatory alternative sigma factor B, in determining resistance to PEF treatments. Analysis of protein expression profiles in *E. coli* and *S. cerevisiae*, has revealed the involvement of membrane-associated proteins and enzymes related to cellular metabolism in the resistance mechanisms against PEF. Notably, the combination of mild heating and PEF treatment has been shown to induce changes in the expression of key proteins associated with glycolysis, cellular energy metabolism, and heat stress response. These findings collectively underscore the multifaceted nature of microbial responses to PEF and provide a foundation for enhancing our understanding of PEF-based microbial control strategies. Further investigations into the transcriptomics, proteomics, and metabolomics of different microorganisms after PEF treatments it is vital for gaining a more comprehensive understanding of the resistance mechanisms and exploring the potential optimization of PEF, whether applied alone or in combination with other hurdles.

An overview on recent investigations of microbial disinfection of food products following PEF-treatments is presented in the current manuscript. In this review, it was shown that 5-log reduction of microorganisms was achieved in specific experimental conditions. Additionally, it was shown that the PEF-inactivation efficiency (5-log reduction) varies with the nature of the food products, the characteristics of the microorganisms and the PEF system/set parameters employed. For example, the threshold of 5-log reduction was achieved for *E. coli* and high liquid acid products such as orange juice and strawberry juice. In parallel, this review underlined the different mechanisms that can contribute to the microbial PEF resistance (i.e.,alterations in the synthesis profile of proteins involved in cell membrane function/transport, cell membrane repairment, oxidative stress adaptation and cellular energy metabolism). However, further investigations should be conducted in this area of research. A deeper understanding and identification of the underlying mechanisms of microbial PEF resistance can aid in optimizing the technology and potentially integrating it with other hurdles.

5. Conclusion

PEF technology is a promising method and has shown its potential to inactivate bacterial vegetative cells, yeasts and moulds, and to achieve the food safety requirements, especially for high acid liquid food products, such as fruit juices. However, the attainment of the goal of food safety is contingent upon the specific targeted microorganism, the nature of the food product, and the applied PEF parameters. In certain instances, achieving high-intensity treatments necessary for the antimicrobial efficacy may pose drawbacks, including increased process costs and potential impacts on the organoleptic and nutritional quality of the food. In parallel, PEF process due to its dependence on specific application parameters, may result in reversible electroporation especially in lower intensity treatments, allowing a partial recovery of the initial population, potentially posing serious food safety concerns. This reversible nature means that PEF's effectiveness is not absolute, and partial recovery could lead to significant food safety issues. Therefore, PEF is a promising technology with limitations on the applicability for microbial inactivation depending on the aforementioned parameters. In conclusion, advancing the industrialization of PEF process for microbial inactivation and enhancing its efficiency can progress along two distinct paths: optimizing the PEF process and integrating the technology with

additional hurdles.

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CRediT authorship contribution statement

Fotios Lytras: Conceptualization, Investigation, Visualization, Writing – original draft, Writing – review & editing. **Georgios Psakis:** Conceptualization, Supervision, Writing – review & editing. **Ruben Gatt:** Conceptualization, Supervision, Funding acquisition, Writing – review & editing. **Guillermo Cebrián:** Writing – review & editing. **Javier Raso:** Writing – review & editing. **Vasilis Valdramidis:** Conceptualization, Supervision, Resources, Project administration, Funding acquisition, Writing – review & editing.

Declaration of competing interest

None.

Data availability

Data will be made available on request.

References

Abbas Syed, Q. (2017). Pulsed electric field technology in food preservation: A review. *Journal of Nutritional Health & Food Engineering, 6*(6), 168–172. https://doi.org/10.15406/jnhfe.2017.06.00219

Álvarez, I., et al. (2002). Environmental factors influencing the inactivation of *Listeria monocytogenes* by pulsed electric fields. *Letters in Applied Microbiology, 35*(6), 489–493. https://doi.org/10.1046/j.1472-765X.2002.01221.x

Álvarez, I., et al. (2003). The influence of process parameters for the inactivation of *Listeria monocytogenes* by pulsed electric fields. *International Journal of Food Microbiology, 87*(1–2), 87–95. https://doi.org/10.1016/S0168-1605(03)00056-4

Aronsson, K., Rönner, U., & Borch, E. (2005). Inactivation of *Escherichia coli*, *Listeria innocua* and *Saccharomyces cerevisiae* in relation to membrane permeabilization and subsequent leakage of intracellular compounds due to pulsed electric field processing. *International Journal of Food Microbiology, 99*(1), 19–32. https://doi.org/10.1016/j.ijfoodmicro.2004.07.012

Aronsson, K., et al. (2001). Inactivation of microorganisms using pulsed electric fields: the influence of process parameters on *Escherichia coli*, *Listeria innocua*, *Leuconostoc mesenteroides* and Saccharomyces cerevisiae. *Innovative Food Science & Emerging Technologies, 2*(1), 41–54. https://doi.org/10.1016/S1466-8564(01)00021-2

Arroyo, C., et al. (2010). Pulsed electric fields cause sublethal injuries in the outer membrane of *Enterobacter sakazakii* facilitating the antimicrobial activity of citral. *Letters in Applied Microbiology, 51*(5), 525–531. https://doi.org/10.1111/j.1472-765X.2010.02931.x

Barba, F. J., et al. (2015). Current applications and new opportunities for the use of pulsed electric fields in food science and industry. *Food Research International, 77* (February 2018), 773–798. https://doi.org/10.1016/j.foodres.2015.09.015

Batista Napotnik, T., Polajžer, T., & Miklavčič, D. (2021). Cell death due to electroporation – A review. *Bioelectrochemistry, 141*. https://doi.org/10.1016/j.bioelechem.2021.107871

Bhat, Z. F., et al. (2019). Current and future prospects for the use of pulsed electric field in the meat industry. *Critical Reviews in Food Science and Nutrition, 59*(10), 1660–1674. https://doi.org/10.1080/10408398.2018.1425825

Breton, M., & Mir, L. M. (2018). Investigation of the chemical mechanisms involved in the electropulsation of membranes at the molecular level. *Bioelectrochemistry, 119*, 76–83. https://doi.org/10.1016/j.bioelechem.2017.09.005

Cebrián, G., et al. (2009). Role of the alternative sigma factor σb on *Staphylococcus aureus* resistance to stresses of relevance to food preservation. *Journal of Applied Microbiology, 107*(1), 187–196. https://doi.org/10.1111/j.1365-2672.2009.04194.x

Chacha, J. S., et al. (2021). Revisiting non-thermal food processing and preservation methods—Action mechanisms, pros and cons: A technological update (2016–2021). *Foods, 10*(6). https://doi.org/10.3390/foods10061430

Chueca, B., Pagán, R., & García-Gonzalo, D. (2015). Transcriptomic analysis of *Escherichia coli* MG1655 cells exposed to pulsed electric fields. *Innovative Food Science and Emerging Technologies, 29*, 78–86. https://doi.org/10.1016/j.ifset.2014.09.003

Clark, J. P. (2006). *Pulsed electric field processing* (Vol. 60). Food Technology Magazine | Article. No. 1. Available at: https://www.ift.org/news-and-publications/food-technology-magazine/issues/2006/january/columns/processing (Acessed 06 April 2024).

Gachovska, T. K., et al. (2013). Inactivation of *E. coli* affected by medium conductivity in pulsed electric field. In *Digest of Technical Papers-IEEE International Pulsed Power Conference* (pp. 1–7). https://doi.org/10.1109/PPC.2013.6627703

García, D., Gómez, N., Mañas, P., et al. (2005). Occurrence of sublethal injury after pulsed electric fields depending on the micro-organism, the treatment medium ph and the intensity of the treatment investigated. *Journal of Applied Microbiology, 99* (1), 94–104. https://doi.org/10.1111/j.1365-2672.2005.02611.x

García, D., Gómez, N., Raso, J., et al. (2005). Bacterial resistance after pulsed electric fields depending on the treatment medium pH. *Innovative Food Science and Emerging Technologies, 6*(4), 388–395. https://doi.org/10.1016/j.ifset.2005.04.003

Garcia, D., Manas, P., Gomez, N., Raso, J., & Pagan, R. (2006). Biosynthetic requirements for the repair of sublethal membrane damage in *Escherichia coli* cells after pulsed electric fields. *Journal of Applied Microbiology, 100*, 428–435. https://doi.org/10.1111/j.1365-2672.2005.02795.x

García, D., et al. (2006). Biosynthetic requirements for the repair of sublethal membrane damage in *Escherichia coli* cells after pulsed electric fields. *Journal of Applied Microbiology, 100*(3), 428–435. https://doi.org/10.1111/j.1365-2672.2005.02795.x

Geveke, D. J., & Kozempel, M. F. (2003). Pulsed electric field effects on bacteria and yeast cells. *Journal of Food Processing and Preservation, 27*(1), 65–72. https://doi.org/10.1111/j.1745-4549.2003.tb00501.x

Golberg, A., Fischer, J., & Rubinsky, B. (2010). 'The use of irreversible electroporation in food preservation', series. *Biomedical Engineering*, 273–312. https://doi.org/10.1007/978-3-642-05420-4_13

Gurtler, J. B., et al. (2010). Selection of surrogate bacteria in place of *E. coli* O157:H7 and *Salmonella typhimurium* for pulsed electric field treatment of orange juice. *International Journal of Food Microbiology, 139*(1–2), 1–8. https://doi.org/10.1016/j.ijfoodmicro.2010.02.023

Heinz, V., et al. (2001). Preservation of liquid foods by high intensity pulsed electric fields - basic concepts for process design. *Trends in Food Science and Technology, 12* (3–4), 103–111. https://doi.org/10.1016/S0924-2244(01)00064-4

Heinz, V., et al. (2014). *Overview of pulsed electric fields processing for food* (pp. 93–114). https://doi.org/10.1016/B978-0-12-411479-1.00006-1

Huang, K., et al. (2014). A comparison of pulsed electric field resistance for three microorganisms with different biological factors in grape juice via numerical simulation. *Food and Bioprocess Technology, 7*(7), 1981–1995. https://doi.org/10.1007/s11947-014-1272-3

Jaeger, H., et al. (2009). Protective effect of milk constituents and sublethal injuries limiting process effectiveness during PEF inactivation of Lb. rhamnosus. *International Journal of Food Microbiology, 134*(1–2), 154–161. https://doi.org/10.1016/j.ijfoodmicro.2009.06.007

Jayaram, S., Castle, G. S. P., & Margaritis, A. (1993). The effects of high field DC pulse and liquid medium conductivity on survivability of *Lactobacillus brevis*. *Applied Microbiology and Biotechnology, 40*(1), 117–122. https://doi.org/10.1007/BF00170439

Katiyo, W., Yang, R., & Zhao, W. (2017). Effects of combined pulsed electric fields and mild temperature pasteurization on microbial inactivation and physicochemical properties of cloudy red apple juice (*Malus pumila* Niedzwetzkyana (Dieck)). *Journal of Food Safety, 37*(4), 1–9. https://doi.org/10.1111/jfs.12369

Kotnik, T., et al. (2012). Cell membrane electroporation - Part 1: The phenomenon. *IEEE Electrical Insulation Magazine, 28*(5), 14–23. https://doi.org/10.1109/MEI.2012.6268438

Kotnik, T., et al. (2015). Electroporation-based applications in biotechnology. *Trends in Biotechnology, 33*(8), 480–488. https://doi.org/10.1016/j.tibtech.2015.06.002

Kotnik, T., et al. (2019). Membrane electroporation and electropermeabilization: mechanisms and models. *Annual Review of Biophysics, 48*, 63–91. https://doi.org/10.1146/annurev-biophys-052118-115451

Lee, G. J., et al. (2015). Inactivation of *Escherichia coli*, *Saccharomyces cerevisiae*, and lactobacillus brevis in low-fat Milk by pulsed electric field treatment: A pilot-scale study. *Korean Journal for Food Science of Animal Resources, 35*(6), 800–806. https://doi.org/10.5851/kosfa.2015.35.6.800

Li, Y. Q., et al. (2013). Effects of pulsed electric field processing on quality characteristics and microbial inactivation of soymilk. *Food and Bioprocess Technology, 6*(8), 1907–1916. https://doi.org/10.1007/s11947-012-0868-8

Liu, Z., et al. (2019a). Proteomics-based mechanistic investigation of *Escherichia coli* inactivation by pulsed electric field. *Frontiers in Microbiology, 10*. https://doi.org/10.3389/fmicb.2019.02644

Liu, Z., et al. (2019b). Proteomics-based mechanistic investigation of *Escherichia coli* inactivation by pulsed electric field. *Frontiers in Microbiology, 10*(November), 1–16. https://doi.org/10.3389/fmicb.2019.02644

Liu, Z. W., et al. (2017). Effect of cell membrane fatty acid composition of *Escherichia coli* on the resistance to pulsed electric field (PEF) treatment. *LWT - Food Science and Technology, 76*, 18–25. https://doi.org/10.1016/j.lwt.2016.10.019

Los, D. A., & Murata, N. (2004). Membrane fluidity and its roles in the perception of environmental signals. *Biochimica et Biophysica Acta - Biomembranes, 1666*(1–2), 142–157. https://doi.org/10.1016/j.bbamem.2004.08.002

Mahnič-Kalamiza, S., & Miklavčič, D. (2022). The phenomenon of electroporation. *Food Engineering Series.*. https://doi.org/10.1007/978-3-030-70586-2_3

Mahnič-Kalamiza, S., Vorobiev, E., & Miklavčič, D. (2014). Electroporation in food processing and biorefinery. *Journal of Membrane Biology, 247*(12), 1279–1304. https://doi.org/10.1007/s00232-014-9737-x

Mendes-Oliveira, G., Jin, T. Z., & Campanella, O. H. (2020). Modeling the inactivation of *Escherichia coli* O157:H7 and *Salmonella typhimurium* in juices by pulsed electric fields: The role of the energy density. *Journal of Food Engineering, 282*. https://doi.org/10.1016/j.jfoodeng.2020.110001

Mermelstein, N. H. (2017). *The Ick Factor: Microbial Spoilage*. Food Technology Magazine | Article. Available at: https://www.ift.org/news-and-publications/food-tech

nology-magazine/issues/2017/november/columns/food-safety-and-quality-microbial-spoilage.

Milani, E. A., Alkhafaji, S., & Silva, F. V. M. (2015). Pulsed electric field continuous pasteurization of different types of beers. *Food Control, 50*, 223–229. https://doi.org/10.1016/j.foodcont.2014.08.033

Min, S., Evrendilek, G. A., & Zhang, H. Q. (2007). Pulsed electric fields: Processing system, microbial and enzyme inhibition, and shelf life extension of foods. *IEEE Transactions on Plasma Science, 35*(1), 59–73. https://doi.org/10.1109/TPS.2006.889290

Mohamed, M. E. A., & Amer Eissa, A. H. (2012). Pulsed electric fields for food processing technology. *Structure and Function of Food Engineering*. https://doi.org/10.5772/48678

Morales-de la Peña, M., Welti-Chanes, J., & Martín-Belloso, O. (2019). Novel technologies to improve food safety and quality. *Current Opinion in Food Science, 30*, 1–7. https://doi.org/10.1016/j.cofs.2018.10.009

Mosqueda-Melgar, J., Raybaudi-Massilia, R. M., & Martín-Belloso, O. (2007). Influence of treatment time and pulse frequency on *Salmonella Enteritidis*, *Escherichia coli* and *Listeria monocytogenes* populations inoculated in melon and watermelon juices treated by pulsed electric fields. *International Journal of Food Microbiology, 117*(2), 192–200. https://doi.org/10.1016/j.ijfoodmicro.2007.04.009

Mosqueda-Melgar, J., et al. (2008). Effects of pulsed electric fields on pathogenic microorganisms of major concern in fluid foods: A review. *Critical Reviews in Food Science and Nutrition, 48*(8), 747–759. https://doi.org/10.1080/10408390701691000

Müller, W. A., et al. (2022). Molecular dynamics insights on temperature and pressure effects on electroporation. *Biochimica et Biophysica Acta - Biomembranes, 1864*(12), Article 184049. https://doi.org/10.1016/j.bbamem.2022.184049

Niu, D., et al. (2019). Effect of ethanol adaption on the inactivation of Acetobacter sp. by pulsed electric fields. *Innovative Food Science and Emerging Technologies, 52*(June 2018), 25–33. https://doi.org/10.1016/j.ifset.2018.11.009

Niu, D., et al. (2020). Review of the application of pulsed electric fields (PEF) technology for food processing in China. *Food Research International, 137*. https://doi.org/10.1016/j.foodres.2020.109715

Nowosad, K., et al. (2021). The application of PEF technology in food processing and human nutrition. *Journal of Food Science and Technology, 58*(2), 397–411. https://doi.org/10.1007/s13197-020-04512-4

Ohshima, T., Okuyama, K., & Sato, M. (2002). Effect of culture temperature on high-voltage pulse sterilization of *Escherichia coli*. *Journal of Electrostatics, 55*, 227–235. https://doi.org/10.1016/s0304-3886(01)00206-6

Ortega-Rivas, E., & Salmerón-Ochoa, I. (2014). Nonthermal food processing alternatives and their effects on taste and flavor compounds of beverages. *Critical Reviews in Food Science and Nutrition, 54*(2), 190–207. https://doi.org/10.1080/10408398.2011.579362

Pataro, G., et al. (2014). Microbial inactivation of *E. coli* cells by a combined PEF-HPCD treatment in a continuous flow system. *Innovative Food Science and Emerging Technologies, 22*, 102–109. https://doi.org/10.1016/j.ifset.2013.12.009

Peng, K., et al. (2020). Effect of pulsed electric fields on the growth and acidification kinetics of *Lactobacillus delbrueckii* Subsp. bulgaricus. *Foods, 9*(9), 1–11. https://doi.org/10.3390/foods9091146

Pereira, R. N., & Vicente, A. A. (2010). Environmental impact of novel thermal and non-thermal technologies in food processing. *Food Research International, 43*(7), 1936–1943. https://doi.org/10.1016/j.foodres.2009.09.013

Pina-Pérez, M. C., Martínez-López, A., & Rodrigo, D. (2013). Cocoa powder as a natural ingredient revealing an enhancing effect to inactivate *Cronobacter sakazakii* cells treated by pulsed electric fields in infant milk formula. *Food Control, 32*(1), 87–92. https://doi.org/10.1016/j.foodcont.2012.11.014

Pina-Pérez, M. C., et al. (2009). Synergistic effect of pulsed electric fields and CocoanOX 12% on the inactivation kinetics of *Bacillus cereus* in a mixed beverage of liquid whole egg and skim milk. *International Journal of Food Microbiology, 130*(3), 196–204. https://doi.org/10.1016/j.ijfoodmicro.2009.01.021

Raso, J., et al. (2016). 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

Rivas, A., et al. (2013). Sublethally damaged cells of *Escherichia coli* by pulsed electric fields: The chance of transformation and proteomic assays. *Food Research International, 54*(1), 1120–1127. https://doi.org/10.1016/j.foodres.2013.01.014

Roobab, U., et al. (2018). The impact of nonthermal technologies on the microbiological quality of juices: A review. *Comprehensive Reviews in Food Science and Food Safety, 17*(2), 437–457. https://doi.org/10.1111/1541-4337.12336

Saldaña, G., et al. (2009). Comparing the PEF resistance and occurrence of sublethal injury on different strains of *Escherichia coli*, *Salmonella Typhimurium*, *Listeria monocytogenes* and *Staphylococcus aureus* in media of pH 4 and 7. *Innovative Food Science and Emerging Technologies, 10*(2), 160–165. https://doi.org/10.1016/j.ifset.2008.11.003

Schottroff, F., et al. (2019). Pulsed electric field preservation of liquid whey protein formulations – Influence of process parameters, pH, and protein content on the inactivation of Listeria innocua and the retention of bioactive ingredients. *Journal of Food Engineering, 243*(September 2018), 142–152. https://doi.org/10.1016/j.jfoodeng.2018.09.003

Sensoy, I., Zhang, Q. H., & Sastry, S. K. (1997). Inactivation kinetics of Salmonella Dublin by pulsed electric field. *Journal of Food Process Engineering, 20*(5), 367–381. https://doi.org/10.1111/j.1745-4530.1997.tb00428.x

Siemer, C., et al. (2014). Application of pulsed electric fields in liquid processing. In *Conventional and advanced food processing technologies* (pp. 645–672). https://doi.org/10.1002/9781118406281.ch26

Sobrino-López, Á., Raybaudi-Massilia, R., & Martín-Belloso, O. (2006). High-intensity pulsed electric field variables affecting *Staphylococcus aureus* inoculated in milk. *Journal of Dairy Science, 89*(10), 3739–3748. https://doi.org/10.3168/jds.S0022-0302(06)72415-8

Somolinos, M., et al. (2008). Effect of environmental factors and cell physiological state on pulsed electric fields resistance and repair capacity of various strains of *Escherichia coli*. *International Journal of Food Microbiology, 124*, 260–267. https://doi.org/10.1016/j.ijfoodmicro.2008.03.021

Somolinos, M., et al. (2010). SigB absence decreased *Listeria monocytogenes* EGD-e heat resistance but not its pulsed electric fields resistance. *International Journal of Food Microbiology, 141*(1–2), 32–38. https://doi.org/10.1016/j.ijfoodmicro.2010.04.023

Tanino, T., et al. (2012). Analysis of the stress response of yeast *Saccharomyces cerevisiae* toward pulsed electric field. *Journal of Electrostatics, 70*(2), 212–216. https://doi.org/10.1016/j.elstat.2012.01.003

Tanino, T., et al. (2020). Engineering of pulsed electric field treatment using carbon materials as electrode and application to pasteurization of sake. *Journal of Electrostatics, 104*(January), Article 103424. https://doi.org/10.1016/j.elstat.2020.103424

Timmermans, R. A. H., et al. (2019). Moderate intensity pulsed electric fields (PEF) as alternative mild preservation technology for fruit juice. *International Journal of Food Microbiology, 298*(January), 63–73. https://doi.org/10.1016/j.ijfoodmicro.2019.02.015

Toepfl, S. (2012). *Stewart postharvest review commercial applications* (September). https://doi.org/10.2212/spr.2012.2.4.

Valdramidis, V. P., & Koutsoumanis, K. P. (2016). Challenges and perspectives of advanced technologies in processing, distribution and storage for improving food safety. *Current Opinion in Food Science, 12*, 63–69. https://doi.org/10.1016/j.cofs.2016.08.008

Walter, L., et al. (2016). Kinetic models for pulsed electric field and thermal inactivation of *Escherichia coli* and Pseudomonas fluorescens in whole milk. *International Dairy Journal, 57*, 7–14. https://doi.org/10.1016/j.idairyj.2016.01.027

Wang, L. H., et al. (2016). Temperature-mediated variations in cellular membrane fatty acid composition of *Staphylococcus aureus* in resistance to pulsed electric fields. *Biochimica et Biophysica Acta - Biomembranes, 1858*(8), 1791–1800. https://doi.org/10.1016/j.bbamem.2016.05.003

Wang, M., et al. (2015). LWT - food science and technology quantitative analysis of sublethally injured *Saccharomyces cerevisiae* cells induced by pulsed electric fi elds. *LWT - Food Science and Technology, 60*(2), 672–677. https://doi.org/10.1016/j.lwt.2014.09.028

Wang, Q., et al. (2018). Enhancing food processing by pulsed and high voltage electric fields: Principles and applications. *Critical Reviews in Food Science and Nutrition, 58*(13), 2285–2298. https://doi.org/10.1080/10408398.2018.1434609

Weaver, J. C., & Chizmadzhev, Y. A. (1996). Theory of electroporation: A review. *Bioelectrochemistry and Bioenergetics, 41*(2), 135–160. https://doi.org/10.1016/S0302-4598(96)05062-3

Wiktor, A., et al. (2015). The effect of pulsed electric field treatment on immersion freezing, thawing and selected properties of apple tissue. *Journal of Food Engineering, 146*, 8–16. https://doi.org/10.1016/j.jfoodeng.2014.08.013

World Health Organization. (2020). Food safety. Available at https://www.who.int/news-room/fact-sheets/detail/food-safety (Acessed:10 May 2020).

Wouters, P. C., Alvarez, I., & Raso, J. (2001). Critical factors determining inactivation kinetics by pulsed electric field food processing. *Trends in Food Science and Technology, 12*(3–4), 112–121. https://doi.org/10.1016/S0924-2244(01)00067-X

Wouters, P. C., et al. (1999). Effects of pulsed electric fields on inactivation kinetics of *Listeria innocua*. *Applied and Environmental Microbiology, 65*(12), 5364–5371. https://doi.org/10.1128/aem.65.12.5364-5371.1999

van Wyk, S., Silva, F. V. M., & Farid, M. M. (2019). Pulsed electric field treatment of red wine: Inactivation of Brettanomyces and potential hazard caused by metal ion dissolution. *Innovative Food Science and Emerging Technologies, 52*(June 2018), 57–65. https://doi.org/10.1016/j.ifset.2018.11.001

Yang, L., et al. (2008). Atomic force microscopy study of the effect of pulsed electric field on *Staphylococcus epidermidis*. *Analytical Chemistry, 80*(16), 6222–6227. https://doi.org/10.1021/ac800556f

Yildiz, S., et al. (2019). Identification of equivalent processing conditions for pasteurization of strawberry juice by high pressure, ultrasound, and pulsed electric fields processing. *Innovative Food Science and Emerging Technologies, 57*(July), Article 102195. https://doi.org/10.1016/j.ifset.2019.102195

Yun, O., Liu, Z. W., et al. (2016). *Salmonella typhimurium* resistance on pulsed electric fields associated with membrane fluidity and gene regulation. *Innovative Food Science and Emerging Technologies, 36*, 252–259. https://doi.org/10.1016/j.ifset.2016.06.013

Yun, O., Zeng, X. A., et al. (2016). Effect of pulsed electric field on membrane lipids and oxidative injury of *Salmonella typhimurium*. *International Journal of Molecular Sciences, 17*(8). https://doi.org/10.3390/ijms17081374

Yun, O., et al. (2017). Original article temperature alters the structure of membrane lipids and pulsed electric field (PEF) resistance of *Salmonella typhimurium*. *International Journal of Food Science and Technology*, 424–430. https://doi.org/10.1111/ijfs.13297

Zand, E., et al. (2022). Single-staining flow cytometry approach using SYTOXTM green to describe electroporation effects on *Escherichia coli*. *Food Control, 132*(June 2021), Article 108488. https://doi.org/10.1016/j.foodcont.2021.108488

Zhao, M., Zhao, W., & Li, L. (2022). Proteomics-based mechanistic study of sub-lethally injured *Saccharomyces cerevisiae* by pulsed electric fields. *Food Bioscience, 50*(August). https://doi.org/10.1016/j.fbio.2022.101989

Zhao, W., Yang, R., Gu, Y., et al. (2014a). Effects of pulsed electric fields on cytomembrane lipids and intracellular nucleic acids of *Saccharomyces cerevisiae*. *Food Control, 39*(1), 204–213. https://doi.org/10.1016/j.foodcont.2013.11.015

Zhao, W., Yang, R., Gu, Y.-j., et al. (2014b). LWT - food science and technology assessment of pulsed electric fields induced cellular damage in *Saccharomyces cerevisiae*: Change in performance of mitochondria and cellular enzymes. *LWT - Food Science and Technology, 58*(1), 55–62. https://doi.org/10.1016/j.lwt.2014.03.009

Zhao, W., et al. (2008). Effect of PEF on microbial inactivation and physical-chemical properties of green tea extracts. *LWT - Food Science and Technology, 41*(3), 425–431. https://doi.org/10.1016/j.lwt.2007.03.020

Zhao, W., et al. (2011). Quantitative and real time detection of pulsed electric field induced damage on *Escherichia coli* cells and sublethally injured microbial cells using flow cytometry in combination with fluorescent techniques. *Food Control, 22*(3–4), 566–573. https://doi.org/10.1016/j.foodcont.2010.10.006