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Pulsed electric field treatment for the stimulation of microorganisms: Applications in food production

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Abstract: The pulsed electric field (PEF) technology is a non-thermal processing technique usually used for microbial inactivation in food industries. The application of this technology at sub-lethal levels prior to or during the fermentation processes enhances the mass transfer and cell permeability. It could also cause changes in the genetic, metabolic, and physiological responses of microbial strains leading to an improvement in the fermentation process. Several studies reported the benefits of PEF on microorganisms including growth stimulation, an increase in the fermentation rates and product yields, and improvement in the metabolite extraction. All of these modifications could improve the organoleptic and nutritional properties of fermented food products. The purpose of this review is to summarise and discuss the main findings reported in the literature to date about the effect of PEFs applied at sub-lethal levels on microorganisms in the context of food processing.

Keywords: cell membrane; electroporation; fermentation; food products; microbial growth; sub-lethal level

A pulsed electric field (PEF) treatment consists of producing electric current through biological membranes for a short time (from a few nanoseconds to several milliseconds) with a pulsed electric field intensity varying between 100–300 $V \cdot cm^{-1}$ and 20–80 $kV \cdot cm^{-1}$ (Barba et al. 2015; Koubaa et al. 2015). Membrane electroporation induced by PEF treatments have received a wide range of applications in food science and in the food industry (Wang et al. 2018).

At moderate electric field strengths (500–1 000 $V \cdot cm^{-1}$), PEF treatments have been applied in the food industries for enhancing juice extraction, diffusion processes, drying and freezing (Ben Ammar et al. 2011). Pressing is usually used in food industries to produce juice from fruits and vegetables (Lebovka et al. 2003). Pre-treatment techniques, such as heating and grinding, are generally employed to facilitate the juice extraction from plant tissues (Bobinaitė et al. 2015). However, these techniques increase the turbidity of the juice and are usually followed by multi juice clarification steps. They also cause a decrease in the juice quality, a loss of vitamins, and modifications to the colour and flavour (Charles-Rodríguez et al. 2007). Many studies have demonstrated the efficacy of PEF applications before or in combination with pressing to increase the obtained juice yields from several products such as apples (Grimi et al. 2011; Turk et al. 2012), sugar beets (Jemai and Vorobiev 2006; Loginova et al. 2011), blueberries (Bobinaitė et al. 2015), citrus fruits (El Kantar et al. 2018), and grapes (Praporscic et al. 2007; Grimi et al. 2009). Moreover, the PEF technology decreases the resistance of the cell membranes to diffusion, which improves the extraction of bioactive compounds from the cells into the juice

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(Salehi 2020). Therefore, the antioxidant properties (Grimi et al. 2011), colour (Delsart et al. 2012) and flavour (Min and Zhang 2003) of the juice are improved if the PEF treatment is combined with the pressing process.

Freezing and drying are generally used as food preservation techniques. However, these techniques can affect the quality of food products and cause changes in their colour, flavour and nutritional properties (Parniakov et al. 2016). Previous studies have demonstrated the positive effects of PEF pre-treatments on freezing of different foods (Jalté et al. 2009; Ben Ammar et al. 2010; Wiktor et al. 2015; Liu et al. 2021). A PEF application prior to freezing shortened the freezing time and maintained the shape, texture and colour of the samples (Ben Ammar et al. 2010; Shayanfar et al. 2014). The PEF technique has also been proposed as a non-thermal pre-treatment preceding drying protocols that improves the moisture diffusion, thus enhancing the drying process of thermally sensitive foods (Lebovka et al. 2007). For example, a PEF pre-treatment at moderate electric field strengths (below 1 000 V·cm^{-1}) decreased the drying temperature of red beetroots by 20 °C (Shynkaryk et al. 2008) and the drying time of carrots and onions by 55% and 30%, respectively, (Ostermeier et al. 2018; Liu et al. 2020) compared to untreated products.

PEF treatments have shown to be an alternative efficient method to conventional pasteurisation and sterilisation processes for food preservation (Oziembłowski and Kopeć 2005). Successful microbial inactivation in food products by PEF at high electric field strengths (>10 kV·cm^{-1}) has been reported by several researchers, with the advantages of maintaining the nutritional and organoleptic properties of the treated products (Sampedro et al. 2007; Toepfl et al. 2007; Mosqueda-Melgar et al. 2008; Altuntas et al. 2010; Timmermans et al. 2014). In this case, the electric field results in the irreversible electroporation in the membranes of microorganisms and leads to cell death (Timmermans et al. 2014). However, one of the most interesting new areas of research in the field of food fermentation is PEF applications at sublethal levels. This electrical treatment could stimulate microorganisms during fermentation which could improve the desirable properties of the fermented products. Food fermentation involves the chemical conversion of complex organic compounds into simpler compounds by microorganisms including yeast and bacteria (Ojha et al. 2017). The major role of fermentation that has been associated to food preservation was through the formation of inhibitory metabolites such as organic acids, ethanol and bacteriocins (Paul Ross et al. 2002; Ivey et al. 2013). More recent applications of the fermentation technique involve the improvement of the nutritional value and organoleptic properties of food (Bourdichon et al. 2012).

This review summarises the effect of the PEF technology on microorganisms during food fermentation processes. It provides an overview on the potential use of a PEF to stimulate microorganisms and improve the quality of fermented foods.

MECHANISM OF ACTION OF PEF

During a PEF treatment, samples are placed or circulated between electrodes in a treatment chamber connected to a pulse generator. The application of an external electric field (E) induces an accumulation of charges on both the membrane surfaces, and a transmembrane potential (u_m) is then created on the cell membranes. The potential difference across the membrane proportionally increases to the applied electric field. When the transmembrane potential exceeds a threshold value (typically 0.2–1 V), the electric field causes the temporary loss of membrane semi-permeability or cell damage. This phenomenon is called electroporation. Depending on the intensity of the applied electric field and the duration of the PEF treatment, the formation of pores could be reversible (temporary) or irreversible (permanent) (Vorobiev and Lebovka 2008) (Figure 1).

The efficiency of the PEF depends on the process parameters (Abbas Syed 2017). The electric field strength is an important parameter to control during the PEF treatment, depending on the intended application. It is defined as the voltage applied between the electrodes divided by the distance between them (McDonald et al. 2000). Besides the electric field strength, other parameters should also be controlled such as the number of pulses (n), pulse duration, pulse frequency (f), pulse width (t_p) and shape, and treatment time (t_{PEF}) (Galván-D'Alessandro and Carciochi 2018). The treatment time corresponds to the pulse width (μs) multiplied by the number of applied pulses ($t_{PEF} = t_p \times n$) (Oziembłowski and Kopeć 2005). The pulse frequency (Hz) corresponds to the number of pulses applied per second. The most utilised pulse shapes are exponential decay and a square waveform (Figure 2). Square waveform geometry is considered the ideal pulse shape since the electric field intensity is uniform for the whole pulse

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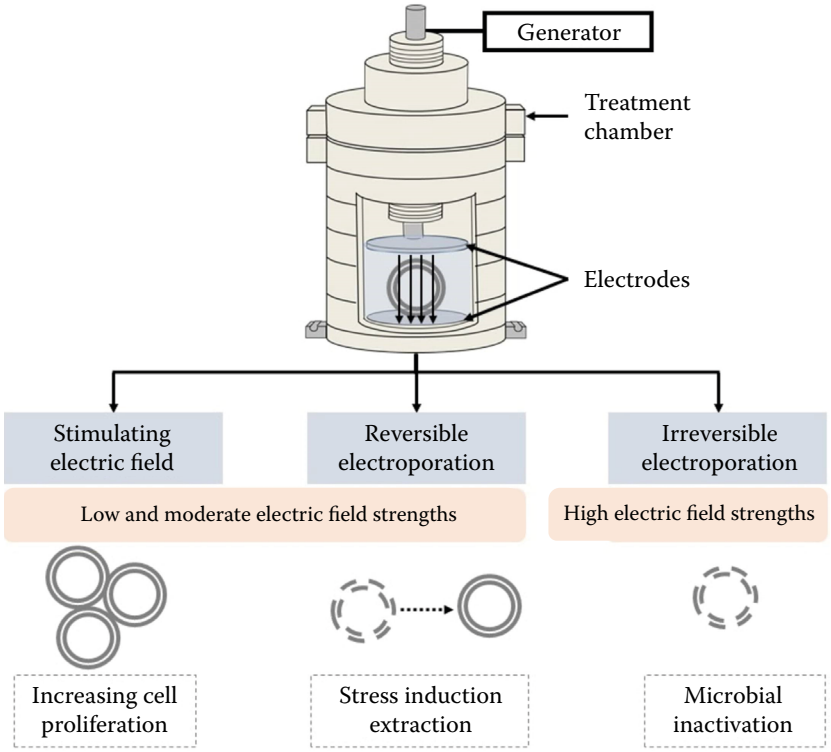

Figure 1. Schematic representation of the effect of a pulsed electric field on microbial cells depending on the applied PEF treatment conditions: Stimulating electric fields, reversible electroporation and irreversible electroporation

duration. Moreover, in a square waveform pulse, the pulse width is equal to the duration of the pulse. However, in the exponential decay geometry, the pulse width corresponds to the time required for the voltage to decay to 37% of its maximum value (Puértolas et al. 2012).

Besides the PEF parameters, the effects of the PEF are also related to the treated matrix. For example, a PEF application on a fermentation medium with high electrical conductivities reduces the effect of the electrical treatment on the microorganisms. Therefore, a difference in the electrical conductivity between the microbial cytoplasm and the fermentation medium is required to observe the effect of the PEF on the treated microorganisms (Galván-D'Alessandro and Carciochi 2018). Moreover, the sensitivity of microorganisms to a PEF treatment depends on the cell size and shape, type of the microorganism and its growth stage (Abbas Syed 2017). For example, the presence of a complex arrangement of peptidoglycans in the cell wall of gram-positive bacteria makes them more resistant to PEF treatments than the gram-negative bacteria (Chaturongakul and Kirawanich 2013). Additionally, the transmembrane potential is proportional to the size of the cell and the cell damage induced by electroporation is higher for large cells than for smaller ones (Ben Ammar et al. 2011). Consequently, yeast cells could be more sensitive to PEF than bacteria because of their larger size (Abbas Syed 2017). Finally, the effectiveness of the PEF is affected by the growth stage of the microorganisms. Cells in a logarithmic (log) phase undergo division and their membrane is more sensitive to PEF treatments than cells in lag (adaptation) and stationary phases (Pothakamury et al. 1996). Therefore, an increase in the membrane permeability could improve the cell transport and nutrient uptake which increases the growth rate of microorganisms. Moreover, PEF treatments can possibly modify the genetic expression which enhances the production of the molecules of interests by microorganisms during fermentation.

IMPACT OF PEF ON THE FERMENTATIVE ACTIVITY OF MICROORGANISMS

A decrease in the PEF treatment energy, by modifying the electric field strength and/or the pulse width to sub-lethal levels could have a stimulatory effect on the cell growth. The effects of PEF treatments on the fermentative activity of microorganisms are summarised in Table 1. Many studies have investigat-

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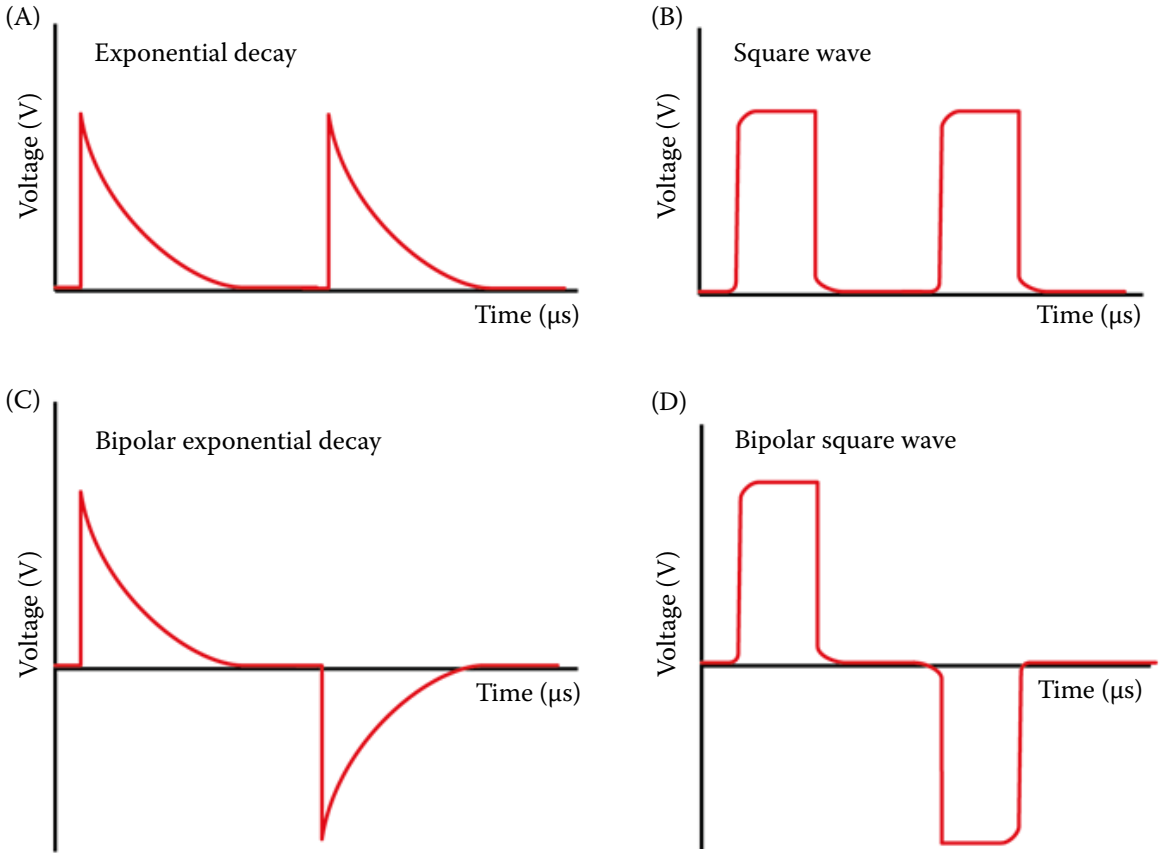

Figure 2. Main pulse shapes applied during the pulsed electric field treatment

(A) exponential decay, (B) square wave, (C) bipolar exponential decay, and (D) bipolar square wave

ed the effect of PEF treatments on lactic acid bacteria that are widely used in the food industry to produce fermented products (Peng et al. 2020). For example, *Lactobacillus acidophilus* LA-K and *Lactobacillus delbrueckii* ssp. *bulgaricus* LB-12 exposed to PEF at an electric field strength of 1 kV·cm^{-1}, a pulse width of 3 μs, and a pulse period of 0.5 s reached the log growth phase an hour before the control (Najim and Aryana 2013). Similarly, the growth rate of *Lactobacillus acidophilus* BT 1088 treated with PEF at 7.5 kV·cm^{-1} for 3.5 ms increased by 4.49–21.25% compared with that of the untreated cells (Lye et al. 2012). The effect of a PEF treatment on the growth of lactic acid bacteria to produce a sweet cherry fermented juice was also investigated by Sotelo et al. (2018). The lactic acid bacterial growth increased in the cherry samples immediately after the PEF treatment at electric field strengths between 0.30 and 1.70 kV·cm^{-1}. This could be associated with the release of nutrients necessary for bacterial growth from the treated samples. However, the bacterial growth decreased when the treated cherry samples were stored for 24 h at 4 °C because of the interactions between the inhibitory compounds (phenolic compounds and organic acids) released from the PEF treated samples (Sotelo et al. 2018). Electrostimulation of yeast cells by PEF has also been investigated as a potential tool for improving the performance of yeast cells during fermentation. Al Daccache et al. (2020) evaluated the effect of a PEF treatment on *Hanseniaspora* sp., a yeast involved in the alcoholic fermentation of apple juice and cider production during the fermentation process. PEF assisted-fermentation at an electric field strength of 285 V·cm^{-1} applied during the lag and log phases (10 pulses of 100 μs) led to an increase in the growth rate and glucose consumption, and a decrease in the fermentation time (Al Daccache et al. 2020). The growth stimulation of yeast and bacteria by PEF treatments could be explained by the activation of enzymatic systems and metabolic pathways, leading to cell proliferation and differentiation (Hunt et al. 2009).

The production of metabolites including acetic acid, ethanol, enzymes, exopolysaccharides, aroma mol-

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Table 1. Summary of the effect of PEF application at different conditions on the fermentative activity of microorganisms

PEF conditions	Type of microorganisms	Main results	Reference
E: 1 kV·cm^{-1}; square unipolar pulse shape t_p: 3 µs Pulse period: 0.5 s Delay time: 20 µs PEF applied prior to fermentation	*Lactobacillus acidophilus* LA-K *Lactobacillus delbrueckii* ssp. *bulgaricus* LB-12	PEF allowed the bacteria to reach the logarithmic phase of growth an hour before the control	Najim and Aryana 2013
E: 7.5 kV·cm^{-1} for 3 ms PEF applied prior to fermentation	*Lactobacillus acidophilus* BT 1088	PEF pre-treatment of the cells enhanced growth rate by 4.49–21.25% compared to untreated cells	Lye et al. 2012
E: between 0.3 and 2.5 kV·cm^{-1}; square bipolar pulse shape f: 100 Hz t_p: 20 µs n: from 385 to 10 000 PEF applied on cherries and the effect of the treatment on bacterial growth was evaluated	*Lactobacillus acidophilus*	PEF treatment of cherries samples at mild electric field intensities of 0.30 kV·cm^{-1} and 1.70 kV·cm^{-1} stimulated bacterial growth due to the release of nutrients	Sotelo et al. 2018
E: 72 to 285 V·cm^{-1}; rectangular bipolar pulse shape n: 10 t_p: 100 µs PEF applied during fermentation (lag and log phases)	*Hanseniaspora* sp.	PEF increased growth rate and sugar consumption and decreased the fermentation time	Al Daccache et al. 2020
E: 100 and 6 000 V·cm^{-1}; near-rectangular monopolar pulse shape n: 1 000 Pulse duration: 100 µs PEF applied prior to fermentation	*Saccharomyces cerevisiae* Actiflore F33	PEF increased fructose consumption by 2.33 times at 100 V·cm^{-1} and 3.98 times at 6 000 V·cm^{-1}	Mattar et al. 2015
E: 20 to 2 000 V·cm^{-1}; near-rectangular bipolar pulse shape n: 1 to 10 000 t_{PEF}: 10^{-5} to 1 s PEF applied after fermentation	*Saccharomyces cerevisiae* Actiflore F33	PEF increased the viability of yeast cells PEF induced the extraction of ionic compounds from yeast at high electric field strengths (1 000 and 2 000)	Mattar et al. 2014
E: 40 to 60 kV·cm^{-1} n: 20 or 100 t_p: 35 ns PEF applied during fermentation (log phase)	*Lactobacillus plantarum* DSM 9843	PEF (5.0 kV, 700 pulses) increased L-lactic acid by 19% PEF (4.5 kV, 700 pulses) increased D-lactic acid by 6.8% PEF (4.5 kV, 1 000 pulses) increased acetic acid by 15%	Kanafusa et al. 2021
E: 0.15, 0.3, 0.45 and 0.6 V·cm^{-1} t_{PEF}: 3, 3.5 and 4 ms PEF applied during fermentation	*Weissella cibaria*	The increase in PEF intensity decreased the production of lactic acid and ethanol and favoured the production of acetic acid	Joo et al. 2013
Voltage: 3 and 4 V PEF applied during fermentation	*Saccharomyces bayanus* EC 1118	PEF improved malate consumption and ethanol production	Min et al. 2009

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Table 1 to be continued

PEF conditions	Type of microorganisms	Main results	Reference
E: 1 to 3.67 kV·cm^{-1} Exponential decay pulses n: 5 to 50 f: 0.5 to 4 Hz PEF applied prior to fermentation	*Streptococcus thermophilus* DIL 5218 *Lactobacillus delbrueckii* subsp. *bulgaricus* DSMZ 20081T	PEF induced an earlier acidification of the medium PEF (3.67 kV·cm^{-1}, 50 pulses) decreased the oxidation reduction potential	Chanos et al. 2020
E: 8 kV·cm^{-1} n: 200 PEF applied during fermentation (log phase)	*Lactococcus cremoris*	PEF (one-pass treatment) increased EPS by 32% PEF (circular treatment) increased EPS by 94%	Ohba et al. 2016
E: 0.3 to 25 kV·cm^{-1}; square wave pulse shape t_p: 100 µs to 1 ms f: 1 Hz n: 8 PEF applied during fermentation	*Lacticaseibacillus rhamnosus* ATCC 7469 *Lacticaseibacillus paracasei* NRRL B-4564	PEF at sublethal conditions (5 kV·cm^{-1}, 8 × 1 ms, 1 Hz) increased lactic acid production from *Lacticaseibacillus rhamnosus* and increased the release of proteins in the same conditions	Djukić-Vuković et al. 2021
E: 7.5 kV·cm^{-1} for 3.5 ms PEF applied prior to fermentation	*Lactobacillus. fermentum* BT 8219	PEF increased intracellular and extracellular β-glucosidase activities which allowed the bioconversion of isoflavones to bioactive aglycones	Ewe et al. 2012a
E: 2.5–7.5 kV·cm^{-1}; exponential decay pulses t_{PEF}: 3.0–4.0 ms PEF applied prior to fermentation	*Bifidobacterium* sp. FTDC 8943 *Bifidobacterium longum* FTDC 8643 *Lactobacillus casei* FTDC 2113 *Lactobacillus casei* BT 1268	PEF treatment (4 ms at 5.0 and 7.5 kV·cm^{-1}) increased intracellular β-glucosidase activity by 12.5–80.0% and extracellular β-glucosidase activity by 23.8%	Yeo and Liong 2013
E: 2.5, 5.0, or 7.5 kV·cm^{-1} t_{PEF}: 3, 3.5, or 4 ms PEF applied prior to fermentation	*Lactobacillus acidophilus* BT 1088 *Lactobacillus acidophilus* FTCC 0291 *Lactobacillus bulgaricus* FTCC 0411 *Lactobacillus bulgaricus* FTDC 1311 *Lactobacillus casei* BT 1268	PEF (7.5 kV·cm^{-1} for 3.5 ms) increased cell growth and cholesterol assimilation by 127.2%	Lye et al. 2011
E: 10 to 60 kV·cm^{-1} n: 100 to 1 600 t_p: 100 ns PEF applied prior to fermentation	*Streptomyces avermitilis* ATCC 31267	PEF (20 kV·cm^{-1}, 20 pulses) increased cell growth up to 233%; PEF (15 kV·cm^{-1}, 20 pulses) increased avermectins production by 42%	Guo et al. 2016
E: 2.0 kV·cm^{-1} t_p: 20 µs t_{PEF}: 15 min PEF applied prior fermentation	*Lactobacillus rhamnosus* B 442	PEF increased magnesium uptake by 220%	Góral and Pankiewicz 2017
E: 2.0 kV·cm^{-1} t_p: 20 µs t_{PEF}: 15 min PEF applied prior fermentation	*Lactobacillus rhamnosus* B 442	PEF increased zinc accumulation by 164%	Góral et al. 2019

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Table 1 to be continued

PEF conditions	Type of microorganisms	Main results	Reference
Voltage: 1 500 V *n*: 1 200 Pulse width: 10 µs Treatment time: 20 min PEF applied after 8, 12, 16, 20, and 24 h of cultivation.	*Saccharomyces cerevisiae* 11 B1	PEF increased iron accumulation by 157%	Nowosad et al. 2021
Voltage: 50 to 3 000 V t_p: 10 to 150 µs t_{PEF}: 5 to 20 min PEF applied after 20 h of cultivation	*Saccharomyces cerevisiae* 11 B1	PEF (1 500 V, pulse width 10 µs and treatment duration 20 min) increased zinc accumulation by 63%	Pankiewicz and Jamroz 2011
Voltage: 50 to 2 500 V t_p: 10 to 150 µs t_{PEF}: 5 to 20 min PEF applied after 20 h of cultivation	*Saccharomyces cerevisiae*	PEF (2 000 V, pulse width 20 µs and treatment duration 15 min) permitted the accumulation of 40% of the amount of magnesium present in the medium	Pankiewicz and Jamroz 2010

E – electric field strength; n – number of pulses; f – pulse frequency; t_p – pulse width; t_{PEF} – treatment time

ecules and proteins by microorganisms during fermentation, contributes to the organoleptic properties of the final food product (Leroy and De Vuyst 2004). Mattar et al. (2015) applied a PEF treatment to *Saccharomyces cerevisiae* cells at electric field strengths of 100 and 6 000 V·cm^{-1} (1 000 pulses with a pulse duration of 100 µs) prior to batch fermentation for 150 h at 30 °C. As a result, an enhancement in the fermentation characteristics was observed, such as an acceleration in the sugar consumption in the lag phase and an increase in the protein synthesis (Mattar et al. 2015). Improvement in the yeast performance during fermentation after PEF electrostimulation or during PEF-assisted fermentation is associated with the activation of the oxidative stress response or modification of the membrane protein activity resulting in the assimilation of larger amounts of substrates (Mattar et al. 2014). Similarly, Kanafusa et al. (2021) applied a PEF treatment to *Lactobacillus plantarum* DSM 9843 in fermented watermelon juice during the log phase of the bacteria. The electric field strength was varied from 40 kV·cm^{-1} to 60 kV·cm^{-1} and the number of pulses was varied from 100 to 1 600 with a pulse duration of 35 nanoseconds. The PEF application induced an increase in the production of metabolites without affecting the cell growth. The increase in the L-lactate, D-lactate, and acetate production depended on the applied voltage and resulted from the reactive oxygen species production that regulated the gene transcription and enzyme activity, thus redirecting the metabolic fluxes in the bacteria (Kanafusa et al. 2021). Joo et al. (2013) studied the effect of the PEF on the quality and sensorial properties of kimchi, which is a Korean side dish made from fermented vegetables. Kimchi fermentation is mainly induced by lactic acid bacteria that use the nitrogen and carbon present in the vegetables to produce various metabolites including lactic acid, ethanol, and acetic acid. The authors varied the intensity of the electric pulses from 0.15 V·cm^{-1} to 0.6 V·cm^{-1} applied at intervals of 5 s to a culture of *Weissella cibaria* SKkimchi1 in a de Man, Rogosa and Sharpe (MRS) medium and a kimchi-producing culture (KMC). The increase in the PEF intensity had no effect on the lactic acid bacteria present in the KMC, but decreased the growth of *Weissella cibaria* SKkimchi1. Moreover, the PEF application could modify the redox potential of the bacterial cultures which affect the production of metabolites. Therefore, the production of lactic acid and ethanol was favoured at low PEF intensities, while the production of acetic acid increased at a high PEF intensity (Joo et al. 2013).

Electroporation has been widely used in the wine making industry to enhance the juice yields obtained from pressing grapes (Praporscic et al. 2007), to improve the quality of wine such as its colour (El Darra et al. 2016) and aroma (Maza et al. 2019), and to inhibit microbial growth in wine (Delsart et al. 2016). Min et al. (2009) tested the effect of a low intensity PEF treatment on the activa-

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tion of the yeast *Saccharomyces bayanus* (EC-1118) in winemaking cultures. An electrochemical bioreactor equipped with two electrodes was used for the fermentation and a direct current electricity was applied at intervals of 30 s on the winemaking culture. The obtained results showed that the stimulation of the yeast with a low intensity PEF at 3 V or 4 V improved the malate consumption and ethanol production. Therefore, PEF can be used as an effective tool to modify the yeast metabolism and shorten the fermentation period (Min et al. 2009). In a similar study, a mixture of *Streptococcus thermophilus* DIL 5218 and *Lactobacillus delbrueckii* subsp. *bulgaricus* DSMZ 20081T in yoghurt starter cultures were treated with PEF prior to fermentation in a skim milk medium. The treatment of the culture with 50 pulses of PEF at 3.67 kV·cm^{-1} resulted in a fast decrease in the oxidation/reduction potential, which reflects the activation of the yeast metabolism. Moreover, an earliest pH decrease in the media was observed due the production of lactic acid during fermentation, which is a desired characteristic for the industry (Chanos et al. 2020).

The application of PEF treatments at sub-lethal levels could induce stress in cells (reversible electroporation) and initiate a conductive channel across the membrane without inactivating the microorganisms. Ohba et al. (2016) investigated the impact of a PEF treatment on the synthesis of extracellular metabolites known as exopolysaccharides (EPS) by the lactic acid bacterial strain *Lactococcus lactis*. The production of EPS was increased by 32% when PEF was applied as a one-pass treatment (200 pulses, 8 kV·cm^{-1}) on the cells during the exponential growth phase, compared to the control fermentation. However, the effect of the PEF was better when it was applied as a circular treatment for 4 h and led to a 94% increase in the EPS production compared to the control. Following the PEF treatment, the permeability of the cells increased by 7–10%, which could explain the improvement in the EPS production. Moreover, the chemical composition of the EPS did not change after the PEF treatment, but their molecular weight was lower than those obtained from the control fermentation (Ohba et al. 2016). The increase in the EPS yields in fermented milk improves its textural properties and confers health benefits to the host. For example, the oral administration of milk fermented with EPS-producing *Lactococcus lactis* subsp. *cremoris* (*L. cremoris*) to host animals exerted protective effects against influenza virus infections (Maruo et al. 2012).

The effect of a PEF treatment on the probiotic properties of lactic acid bacteria has been evaluated. The viability of *Lactobacillus rhamnosus* and *Lacticaseibacillus paracasei* bacteria was maintained after a batch PEF application at an electric field strength of 8 kV·cm^{-1} with an increase in the cells permeabilization. Additionally, the production of lactic acid by electroporated *Lactobacillus rhamnosus* at 5 kV·cm^{-1} with 8 pulses (duration of 1 pulse = 1 ms) was 10% higher after 24 h of fermentation compared to the untreated bacteria. At these PEF conditions, the release of proteins from *Lactobacillus rhamnosus* was higher than the control. Note that the electroextraction of proteins from lactic acid bacteria during fermentation could have an important application for the production of food rich in post-biotics (Djukić-Vuković et al. 2021). For example, a PEF treatment increased the release of the β-glucosidase protein from lactic acid bacteria, which led to the production of bioactive aglycones from isoflavones in fermented soymilk (Ewe et al. 2012a; Yeo and Liong 2013). Isoflavones present in soy-based foods have been extensively considered for their health benefits and biological activities such as anti-cancer (Pudenz et al. 2014), anti-obesity (Wang et al. 2017), anti-inflammation (Yu et al. 2016) and anti-diabetes (Gilbert and Liu 2013). They exist in two forms: a high percentage of glucosides and a small part of aglycones, which are biologically active and rapidly absorbed in human intestines. The addition of β-glucosidase-possessing lactobacillus to soy-based products resulted in a fermentation favouring the production of high amounts of aglycones as a result of the hydrolysis of the glucose moiety from the glucosides (Ewe et al. 2012b). Ewe et al. (2012a) studied the effect of electroporation on the β-glucosidase activity of *Lactobacillus fermentum* BT 8219 in biotin-supplemented soymilk. The bacterial cells were pre-treated with PEF at 7.5 kV·cm^{-1} for 3.5 ms prior to fermentation in soymilk and the effect of electroporation on the enzymatic activity was monitored through three subsequent cultures. The formation of pores in the membranes of the pre-treated parental cells increased the release of β-glucosidase to the extracellular medium, which improved the bioconversion of the isoflavones to bioactive aglycones. However, this trend was not observed in the medium containing the first, second and third subcultures, suggesting a non-inheritance effect of the PEF treatment on the following subcultures. Moreover, electroporation worsened the probiotic characteristics of the parental cells (anti-

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microbial activity against pathogens, tolerance low pH and bile), but promoted these properties in the subsequent subcultures (Ewe et al. 2012a). Similarly, Yeo et al. (2013) applied a PEF treatment on lactobacilli and bifidobacteria cell cultures (2.5–7.5 kV·cm^{-1} for 3.0–4.0 ms) prior to their addition in mannitol-soymilk for fermentation. The growth of the cells significantly decreased at the highest electric field due to membrane lipid peroxidation resulting from the production of reactive oxygen radicals during the PEF treatment. However, cell viability was maintained due to their aptitude to restore the membrane damage induced by electroporation. Both the intracellular and extracellular β-glucosidase activities significantly increased by 12.5–80.0% and 23.8%, respectively, for a treatment duration of 4.0 ms at high electric field strengths (5.0 and 7.5 kV·cm^{-1}) compared to the control. As a result, a greater bioconversion of isoflavones to bioactive aglycones in mannitol-soymilk was observed (Yeo and Liong 2013).

Reversible membrane electroporation induced by a PEF pre-treatment improves the membrane permeability and the further nutrient uptake by the cells. Previous studies have shown the potential use of PEF-treated lactobacilli cells as cholesterol-reducing supplements in dairy products. Lye et al. (2011) treated lactobacilli cells with a PEF treatment at 2.5, 5 or 7.5 kV·cm^{-1} for 3, 3.5 or 4 ms prior to fermentation at 37 °C for 24 hours. As a consequence, a significant increase in cell growth and cholesterol assimilation was observed. Fluorescence anisotropy revealed that the assimilated cholesterol was incorporated into the cellular membrane and was followed by an increase in the cholesterol to phospholipids ratio (50.0–59.6%) at a field strength of 7.5 kV·cm^{-1} (Lye et al. 2011).

Guo et al. (2016) tested a PEF application to improve the fermentation productivity of avermectins, a group of pesticides usually used in agricultural, veterinary, and medical fields in *Streptomyces avermitilis.* Spores of *Streptomyces avermitilis* were pre-treated with a PEF treatment (10 kV·cm^{-1} to 60 kV·cm^{-1}, 20 or 100 pulses of 100 ns) prior to fermentation. The results showed that the proliferation rate of the cells significantly increased up to 233% when 20 PEF pulses were applied at 20 kV·cm^{-1}. The production of avermectins increased by 42% with a reduction in the required time to reach a plateau in the fermentation process by 2 days when 20 pulses were applied at 15 kV·cm^{-1}. The improvement in the avermectin biosynthesis was shown to be associated with the overexpression of the *aveR* and *malE* genes in *Streptomyces avermitilis* in addition to the reduction of the oxidation-reduction potential and temperature increase of the PEF-treated liquids (Guo et al. 2016).

The increase in the cell membrane permeability by electroporation may also improve the transport of ions inside the microbial cells. Ions, such as iron, zinc and magnesium, are involved in metabolic and physiological processes in the human body (Lee 2017). They are important regulators to maintain human health, and their deficiency may increase the risks of depression, anaemia, immune systems defects and cardiovascular diseases (Chakraborti et al. 2002; Jacka et al. 2009; Fukada et al. 2011; Derom et al. 2013; Short and Domagalski 2013; Lee 2017). Previous studies have shown the positive effect of PEF treatments in increasing the ion accumulation in bacteria. A PEF treatment was applied after 20 h of growing on a culture of *Lactobacillus rhamnosus* B 442 for 15 min at 2.0 kV·cm^{-1} with a pulse duration of 20 μs in a medium enriched with magnesium. As a result, the accumulation of magnesium increased by 220% in the treated cells compared to the untreated ones (Góral and Pankiewicz 2017). Similarly, the accumulation of zinc in the same PEF bacteria treated under the same conditions, but at different electric field strength (3.0 kV·cm^{-1}), was 164% higher than the control (Góral et al. 2019). In both studies, the PEF treatments did not affect the cell viability and did not reduce the total number of cells in the medium. Therefore, bacteria enriched with ions can be used as food supplements to increase the ion absorption in the human body and reduce their deficiency. For example, the addition of probiotic lactic acid bacteria replete with magnesium to ice cream did not change the freezing properties or the organoleptic characteristics of the ice cream (Góral et al. 2018).

The effect of a PEF treatment on the ion accumulation in the yeast *S. cerevisiae* has also been investigated for their use as food supplements. After 20 h of cultivation in a media containing iron salts, the cultures were treated with a PEF for 20 min at 3 kV·cm^{-1} with 1 200 pulses of 10 microseconds. Under these optimal conditions, the accumulation of iron ions (200 μg·mL^{-1}) in the cells was 157% higher than the control (without the PEF) with a maximum value of 48.01 mg·g^{-1} dry mass (Nowosad et al. 2021). Similar results were obtained when a *S. cerevisiae* biomass was exposed to a PEF treatment to improve the zinc accumulation in this

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yeast. The authors found that the maximum value of zinc accumulation (15.57 $mg \cdot g^{-1}$ dry mass) was reached when the culture grown for 20 h containing 100 $\mu g \cdot mL^{-1}$ of zinc was treated with a PEF for 20 min at 3 $kV \cdot cm^{-1}$ with a pulse duration of 10 microseconds. Compared to the untreated cultures, the PEF treatment improved the zinc accumulation by 63% (Pankiewicz and Jamroz 2011). Pankiewicz et al. (2010) found that the optimal conditions for the maximum magnesium accumulation in *S. cerevisiae* were: 15 min at 2 000 V with a pulse width of 20 microseconds. At these conditions and with an initial magnesium concentration of 100 $mg \cdot mL^{-1}$, 40% accumulation of that element was reached (Pankiewicz and Jamroz 2010). The difference in the PEF conditions between the three studies is due to the assimilation of the different elements by the *S. cerevisiae* cells.

CONCLUSION

This review highlighted the potential of microbial cells with PEF treatments at sub-lethal levels to improve the quality of fermented foods. PEF treatments could be applied to microbial cells prior to or during the fermentation process. The effectiveness of this technology depends on several factors including the type of microorganism, the medium composition and the process parameters (electric field strength, pulse number and width). The electrostimulation of microbial cells with a PEF treatment at electric field strengths varying from 100 $V \cdot cm^{-1}$ to 3 000 $V \cdot cm^{-1}$ enhanced the cell growth and proliferation and decreased the fermentation time. A few studies also showed that a higher electric field strength (e.g. >3 000 $V \cdot cm^{-1}$) did not affect the cell viability and improved the fermentation characteristics of microorganisms. The PEF treatments increased the metabolite production by yeast and bacteria during fermentation, which contributed to the enhanced organoleptic and nutritional properties of the final food product. The PEF application also improved the assimilation of ions by the microbial cells, which can be used as food supplements to remedy ion deficiencies. Therefore, PEF technologies could promote benefits to fermentation, but the optimisation of the PEF parameters is needed to avoid cell damage and any loss of viability. In addition, the scaling up PEF systems is necessary for their implementation in the food industry.

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