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Pulsed electric field treatment of *Lacticaseibacillus rhamnosus* and *Lacticaseibacillus paracasei*, bacteria with probiotic potential

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ABSTRACT

Lactic acid bacteria play an important role in functional food and fermentation products for human and animal nutrition, as probiotics, paraprobiotics, postbiotics or high-lactic acid-producing strains in biorefineries. Pulsed electric field (PEF) treatment is gaining recognition in the food industry, but little is known about the effects of PEF treatment on the probiotic characteristics of lactic acid (LA) bacteria or its application for the production of paraprobiotics and postbiotics. Thus, we studied the inactivation kinetics and permeabilization of *Lacticaseibacillus rhamnosus* and *Lacticaseibacillus paracasei* as high LA-producing strains with probiotic characteristics by batch and continuous PEF treatment.

Significant linear correlations between the logN reduction and permeabilization of the studied bacteria and specific energy input and current were observed during PEF treatment. Sublethal PEF treatment (5 kV/cm, 8 × 1 ms, 1 Hz) induced 10% higher LA production in *L. rhamnosus*, as well as the release of proteins from both bacteria. Sublethal PEF treatment did not change the susceptibility to specific antibiotics in *L. rhamnosus*, while *L. paracasei* showed some decrease in susceptibility to antibiotics. The results obtained are valuable for PEF treatment of functional food with probiotics and the production of paraprobiotics and postbiotics to improve food safety and functionality.

1. Introduction

Lactic acid bacteria (LAB) are a taxonomically diverse group of microorganisms that produce lactic acid (LA) as a common characteristic of their glucose metabolism (König & Fröhlich, 2017). LA is an antimicrobial substance, and LA-producing microorganisms play an important role in food preservation, but are also exploited in biorefinery processes (Djukić-Vuković et al., 2013). LAB are present in fermented products and functional food as starter cultures for dairy products, sausages, beverages, etc.

LAB are generally recognized as safe, and some, because of their important role in the gut microbiota and human health, are also recognized as probiotics (Rajilić-Stojanović & de Vos, 2014). Probiotics are “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host” (WHO/FAO, 2002). Probiotics can be present in fermented food (Hill et al., 2014; Marco et al., 2021) or can be administered by other routes, but they should survive harsh

conditions in the gut; eliminate pathogens through the production of LA, H₂O₂ or bacteriocins; and attach to the intestinal mucosa. They also must have a favourable profile of antibiotic susceptibility to avoid the transfer of antibiotic resistance to microorganisms that are part of the human or animal microbiome (Lee et al., 2017; Sharma et al., 2014). LAB can influence host organisms when living, but some of their positive effects are present even when inactivated. LAB develop intracellular and extracellular mechanisms to survive in hostile conditions imposed by different stressors, such as oxidants (H₂O₂, pathogen-induced ROS, etc.) in the gut or O₂ during food storage (Feng & Microbes, 2020), low pH (hydrochloric acid, volatile fatty acids, acetic acid, benzoic acid, etc.) in the gut and food and other stressors, such as heat or high salt concentrations, during food processing (Tsakalidou & Papadimitriou, 2011). Enzymes such as superoxide dismutases, NADH oxidases, exopolysaccharides, those with metal-chelating abilities, etc. Enable the protection of LAB but can also be used in novel ways for health or technological purposes. For example, the antioxidant activity of milk or

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Table 1

The energy input of different batch PEF treatments of *L. rhamnosus* ATCC 7469 bacterial suspensions (table with the energy input for *L. paracasei* is given in suppl. file, Table 1S).

Treatment No.	Applied Voltage [V]	Distance between electrodes [mm]	E [kV/cm]	Number (n) and duration of pulses [μ s]	Current [A]	Sample volume [μ L]	Energy input [J]	Specific energy input [kJ/L]
1000	2	2	5	8 × 100	4.59	400	3.6	9
1500	2	2	7.5	8 × 100	7.78	400	9.34	23.35
800	1	1	8	8 × 100	0.824	90	0.53	5.89
1000	1	1	10	8 × 100	1.018	90	0.814	9.04
2000	1	1	20	8 × 100	2.37	90	3.79	42.1
2500	1	1	25	8 × 100	2.90	90	5.92	65.8
1000	2	2	5	20 × 100	5.01	400	10.2	25.05
1000	2	2	5	2 × 1000	5.07	400	20.28	50.7
500	1	1	5	8 × 1000	0.437	90	1.75	19.42
1000	1	1	10	8 × 1000	1.211	90	9.69	107.6

why is significantly increased when fermented by selected probiotics (Rochat et al., 2006; Virtanen et al., 2007). Therefore, fractions, extracts or metabolites of probiotics called postbiotics or nonviable probiotics (paraprobiotics) (de Almada et al., 2016), could provide significant benefits for consumers. A meta-analysis of studies related to the health benefits of paraprobiotics was recently published (Andresen et al., 2020; Kazemi et al., 2020). Nevertheless, adequate technologies for the production of paraprobiotics and postbiotics are still needed. One of the strategies used to manipulate different bacteria, including LAB, is the application of a pulsed electric field (PEF) (Mahnić-Kalamiza et al., 2014), which acts as an abiotic stressor to cells (Galindo et al., 2009) and enables different biological responses in bacteria, from inactivation to stimulation (Peng et al., 2020).

The application of PEFs of adequate strength and duration to eukaryotic and prokaryotic cells causes an increase in cell membrane permeability if the induced transmembrane voltage surpasses a certain value (Kotnik et al., 2010). This phenomenon, known as electroporation, provides an increase in mass transfer across the cell membrane (Kotnik et al., 2019). Depending on the PEF treatment conditions and parameters, electroporation can be reversible, causing an increase in the permeability of cell membranes without lethal effects, while in the case of irreversible electroporation, cells are unable to recover after treatment (Rems & Miklavčić, 2016).

Irreversible electroporation of undesirable microorganisms is applied in food processing (Odriozola-Serrano et al., 2013; Sepulveda et al., 2005; Sharma et al., 2014). Additionally, PEF treatment can influence texture (Barba et al., 2015, pp. 773–798) or change drying and extraction kinetics from various foodstuffs (Mahnić-Kalamiza et al., 2014). In contrast, reversible electroporation has been used for drying LAB (Vaessen et al., 2018, 2020), electrotransformation and gene delivery (Yadav et al., 2017), the development of advanced probiotics for oral vaccines (Alimolaei et al., 2016; Lin et al., 2017) or other biotechnological purposes (Kotnik et al., 2015).

The effects of PEF on the probiotic characteristics of LAB have not yet been extensively studied. Other thermal (Andresen et al., 2020; Barros et al., 2021) or nonthermal technologies (Cuevas-González et al., 2020; de Almada et al., 2016) have been studied for the production of paraprobiotics and postbiotics, but not PEFs. Improvement of the functional characteristics of LAB, such as an increase in exopolysaccharide production (Ohba et al., 2016) or protease activity (Najim & Aryana, 2013), was reported as a consequence of electroporation. We were interested in examining the effects of electroporation on the probiotic strains *Lactocaseibacillus rhamnosus* ATCC 7469 and *Lactocaseibacillus paracasei* NRRL B-4564 (Djukić-Vuković et al., 2015) for food applications and the production of paraprobiotics and postbiotics.

We studied the effect of PEF treatment on LA production, viability, membrane permeabilization, protein extraction and susceptibility to antibiotics in batch and continuous mode, which is more convenient on an industrial scale where larger volumes need to be treated (Flisar et al., 2014; Sack & Mueller, 2016). Furthermore, their responses were

compared with inactivation and permeabilization kinetics of two model pathogen microorganisms, *Escherichia coli* and *Listeria innocua*.

2. Material and methods

2.1. Preparation of bacterial cells

Lactocaseibacillus rhamnosus ATCC 7469, *Listeria innocua* ATCC 33090 (American Type Culture Collection, LGC Standards GmbH, Germany), *Lactocaseibacillus paracasei* NRRL B-4564 (Northern Regional Research Laboratory, Peoria, USA) and *Escherichia coli* K12 Top10 with plasmid pEGFP-N1 (Clontech Laboratories Inc., CA, USA) were used in this study. *Lactocaseibacillus* spp. at a 1% (v/v) concentration were inoculated in Man Rogosa Sharpe (MRS) broth and incubated at 37 °C for 11 h (mid exponential phase, $1-3 \times 10^8$ CFU/ml). *E. coli* bacteria were inoculated in Luria broth (LB) medium with 50 μ g/ml kanamycin (Carl Roth GmbH, Germany) and agitated for 5 h (mid exponential phase). *Listeria innocua* was inoculated in nutrient broth (NB) and grown at 37 °C for 10 h (mid-exponential phase). MRS, LB and NB were purchased from Sigma-Aldrich Chemie GmbH, Germany.

A cell pellet was collected by centrifugation (4248×g, 30 min, 4 °C) and suspended in sterile distilled water to attain a conductivity of 0.4–0.7 mS/cm and viable cell number of approximately 5×10^7 CFU/ml. Cell density was determined by the plate count method using serial dilutions, and 100 μ l of the dilution was plated into MRS (*Lactocaseibacillus* spp.), LB kanamycin (*E. coli*) or nutrient (*L. innocua*) agar medium. Plates with inoculated bacteria were incubated at 37 °C for 24 h and counted manually.

2.2. Batch PEF treatment

Batch PEF treatment experiments were performed in sterile aluminium cuvettes with built-in electrodes (VWR International, Austria, cat. no.: 732–1136). The suspension of bacterial cells (Section 2.1.) was transferred into the cuvettes and exposed to electric pulses using a HVP-VG square wave electric pulse generator (IGEA s.r.l., Italy). A new cuvette was used for each treatment. Different pulse amplitudes (in the range from 300 V to 2500 V, resulting in electric field strengths from 0.3 to 25 kV/cm) as estimated according to equation (1) were applied while other treatment parameters were kept constant (Table 1). The pulse repetition rate was 1 Hz in all experiments.

Immediately after the treatment, 100 μ l of treated bacterial suspension was withdrawn, and the number of viable bacteria was determined using the pour plate counting method (Section 2.1.). The viability is presented as the log (N/N₀), where N represents the CFU/ml in the sample exposed to electric pulses and N₀ represents the CFU/ml in the control (untreated bacterial suspension). All experiments were performed at room temperature (22 °C). The applied electric field (E) was estimated as follows:

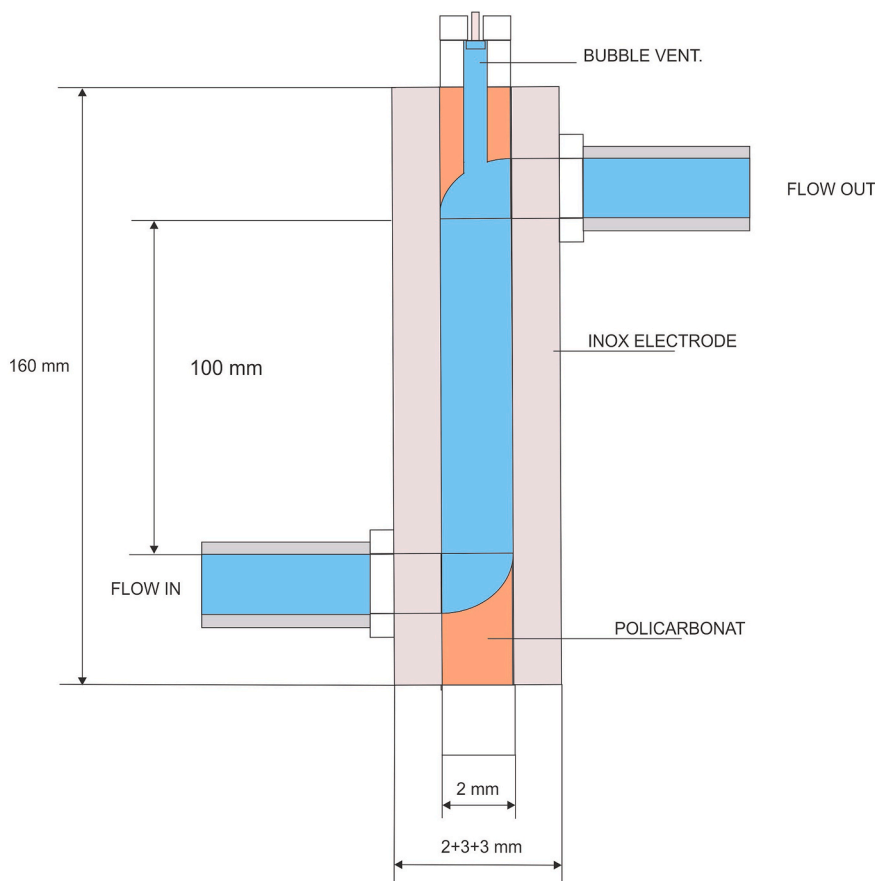


Fig. 1. Flow-through treatment chamber with built-in electrodes used in the study.

Table 2

The energy input of different continuous mode PEF treatments of *L. rhamnosus* ATCC 7469 bacterial suspensions (in Tables 2S, 3S, 4S for other microorganisms in suppl. file).

Treatment No.	Applied Voltage [V]	E [kV/cm]	Number (n) and duration of pulses [μ s]	Current [A]	Sample volume [μ L]	Energy input [J]	Specific energy input [kJ/L]
500	2.5	2.5	8 \times 100	0.77	500	0.36	0.72
1000	5.0	5.0	8 \times 100	1.60	500	1.44	2.88
1200	6.0	6.0	8 \times 100	2.00	500	2.10	4.20
1500	7.5	7.5	8 \times 100	2.67	500	3.36	6.72
2500	12.5	12.5	8 \times 100	9.00	500	9.50	19.00

$$E = U/d \quad (1)$$

where U denotes the applied voltage and d is the distance between the electrodes, i.e., electrode gap. The energy input delivered is reported in Table 1. Energy input is electrical energy received by the treated product (J) and specific energy is electrical energy received per volume of the treated product (J/L). The specific energy was calculated as follows:

$$W = U \times I \times (n \times T)/V \quad (2)$$

where U denotes the applied voltage, I is the measured current, n is the number of applied pulses, T is the pulse duration and V is the sample volume (Raso et al., 2016).

During the treatments, temperature was monitored using a fibre optic sensor system (opSens, Québec, CAN) that consisted of a ProSens signal conditioner and an OTG-M170 fibre optic temperature sensor.

2.3. Continuous mode PEF treatment

Continuous mode PEF treatment experiments were performed in a

flow-through chamber with built-in electrodes ($d = 2$ mm, volume 0,5 ml), as presented in Fig. 1. The suspension of bacterial cells (Section 2.1.) was run through the chamber to deliver the desired number of pulses (Table 2) (Pataro et al., 2011). The flow rate was set at 3.8 ml/min (Flisar et al., 2014), and 5 ml of bacterial cell suspension was exposed to electric pulses. The field strength ranged from 2.5 to 12.5 kV/cm according to Eq. (1). The conductivity of the samples was between 0.4 and 0.7 mS/cm and calculated using U and I for the lowest and the highest amplitudes, respectively. This shows that conductivity is a function of the applied electric field (Park et al., 2009). A viable cell number of approximately 5×10^7 CFU/ml was observed in all samples. The square wave prototype pulse generator described earlier was used for treatment (Flisar et al., 2014).

Immediately after the treatment, 100 μ l of treated bacterial suspension was withdrawn, and the number of viable bacteria was determined using the pour plate counting method (Section 2.1.). The viability was calculated as explained in Section 2.2.

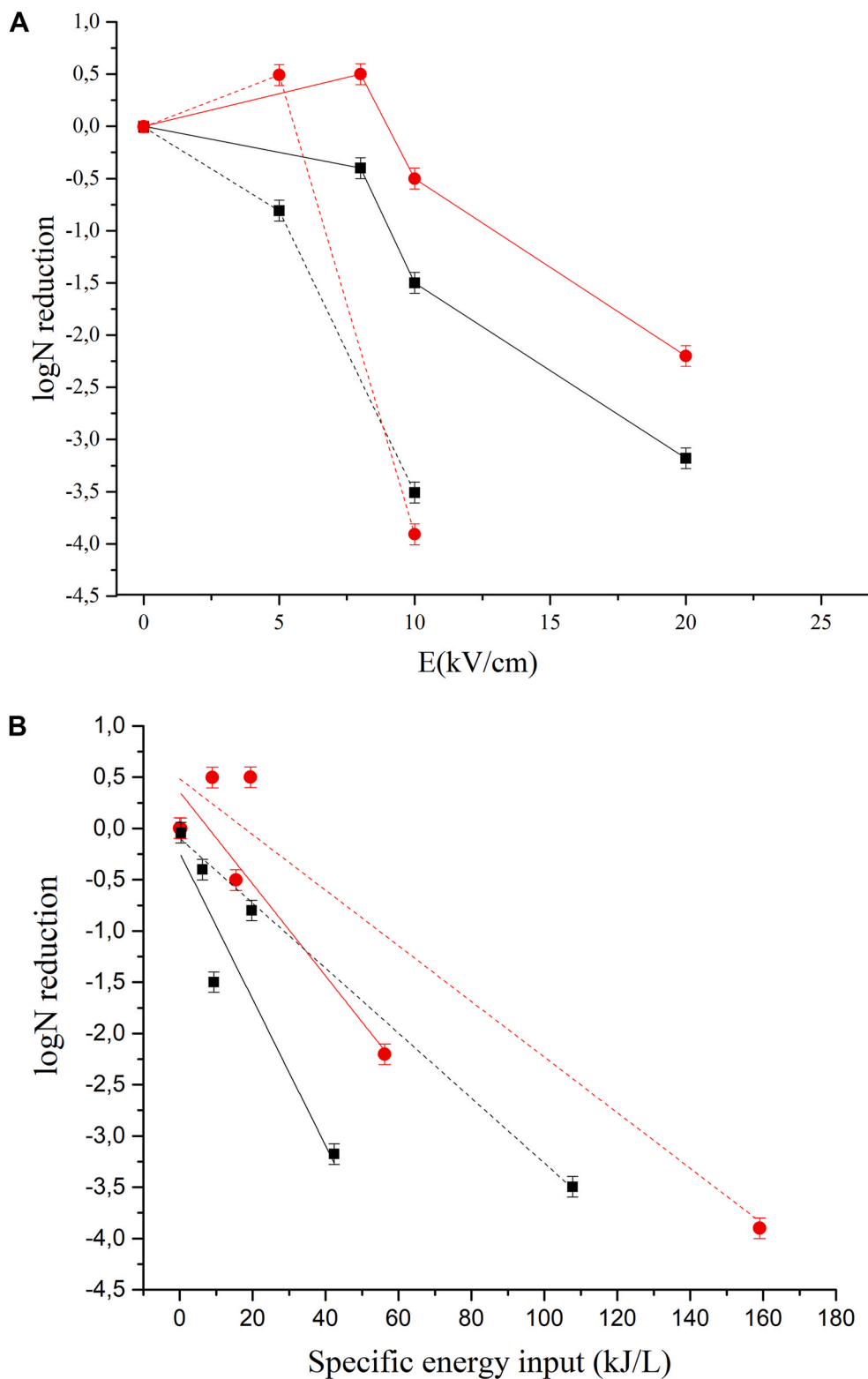


Fig. 2. Inactivation curves (A) and correlations of logN reduction and specific energy input (B) for *L. rhamnosus* and *L. paracasei* in batch PEF treatment mode for different electric field strengths and pulse durations. Experimental conditions: batch treatment in cuvettes, 8 pulses, 1 Hz frequency. Symbols: dashed line – 1 ms pulse duration; solid line – 100 μs pulse duration, black line and square symbols – *L. rhamnosus*, red line and circle symbols – *L. paracasei*. Experiments were repeated three times on different days to prove repeatability. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

2.4. Lactic acid production by *Lactocaseibacillus* spp.

The parameters of lactic acid fermentation (LAF) by electroporated *Lactocaseibacillus* spp. and control were compared. Immediately after the continuous mode PEF treatment, bacterial suspensions were used as inoculum at a concentration of 5% (v/v) for LAF in MRS broth, while untreated bacterial suspensions were used as controls. The

fermentations were performed at 37 °C with shaking (100 rpm) for 24 h. The LA concentration in fermentation broth was determined as titratable acidity using 0.1 M NaOH titration (Salmerón et al., 2015).

2.5. Membrane permeabilization

Membrane permeabilization was evaluated using propidium iodide

Table 3

Correlation of logN reduction and specific energy input and current in studied bacterial suspensions during applied batch PEF treatments.

logN reduction (y)	Specific energy input (x) [kJ/L]		Current (x) [A]	
	Pearson's correlation coefficient	Linear fitting	Pearson's correlation coefficient	Linear fitting
<i>L. rhamnosus</i> , 8 × 100 μs	$r = -$ 0.95826, $p =$ 0.042 ^a	$y =$ -0.26216- 0.07139x, r^2 = 0.877	$r = -0.86645$, $p = 0.133$	$y =$ 0.54095- 1.03509x, $r^2 = 0.626$
<i>L. rhamnosus</i> , 8 × 1000 μs	$r = -$ 0.99918, $p =$ 0.026 ^a	$y =$ -0.03168- 0.10517x, r^2 = 0.997	$r = -0.9882$, $p = 0.098$	$y =$ 0.16294- 2.93011x, $r^2 = 0.953$
<i>L. paracasei</i> , 8 × 100 μs	$r = -$ 0.94538, $p =$ 0.055	$y = 0.34263$ - 0.04487, $r^2 = 0.841$	$r = -0.8374$ $p = 0.163$	$y =$ -0.75684 + 0.59535, $r^2 = 0.552$
<i>L. paracasei</i> , 8 × 1000 μs	$r = -$ 0.97696, $p =$ 0.137	$y = 0.48193$ - 0.02712x $r^2 = 0.909$	$r = -0.94295$ $p = 0.216$	$y =$ -2.43502 + 0.67559, $r^2 = 0.778$

^a Significant, $p < 0.05$.

(PI) after PEF treatment of bacterial cells in accordance with the procedure described earlier (Haberl-Meglić et al., 2016). PI enters the cell if its membrane is permeabilized (Batista Napotnik & Miklavčič, 2018). Bacterial cells were prepared as described in Section 2.1. For batch treatments, immediately before electric pulse application, PI was added (final concentration of PI was 100 μg/ml), and 200 μl of bacterial suspension with PI was placed in a cuvette with built-in aluminium electrodes. In flow-through chamber experiments, a concentrated solution of PI was mixed with a bacterial suspension (Section 2.1) immediately before PEF treatment (final concentration of PI was 100 μg/ml), and a total of 5 mL of cell suspension with PI was placed in the chamber for continuous PEF treatment using a square wave prototype pulse

generator (Flisar et al., 2014). A HVP-VG square wave electric pulse generator (IGEA s.r.l., Italy) was used for batch treatment to deliver PI into the cells. The same PEF treatment parameters were applied to study membrane permeabilization and bacterial inactivation (Tables 1 and 2, Tables in suppl. files). After pulses were applied, bacterial cells were incubated for 22 min in the dark at room temperature (22 °C) to allow PI to enter the cell through the permeabilized membrane and then centrifuged for 4 min at 8000×g at 22 °C to remove extracellular PI and determine the amount of PI within cells. The pellet was resuspended in 400 μl of sterile distilled water, and the uptake of PI was evaluated with a spectrofluorometer (Tecan infinite M200, Tecan GmbH, Austria) at 617 nm. The permeabilization (P, uptake of PI) was defined as follows:

$$P(\%) = \frac{F_{\text{sample}} - F_{E=0}}{F_{\text{positive control}} - F_{E=0}} \times 100 \quad (3)$$

where F_{sample} denotes the fluorescence intensity of cells subjected to electric pulses, $F_{E=0}$ is the fluorescence intensity of cells at $E = 0$, i.e., control cells, and $F_{\text{positive control}}$ is the maximal fluorescence intensity, i.e., where saturation fluorescence was achieved and cells were completely permeabilized (cells were exposed to electric field strength of 25 kV/cm, 8 × 100 μs).

2.6. Extraction of proteins by means of electroporation and determination of total protein content

Bacterial suspensions prepared as described in Section 2.1. were subjected to PEF treatment (Table 1., Table S1 Table 2.) and analysed with respect to the amount of extracted proteins using a similar procedure as reported (Haberl-Meglić et al., 2016). In brief, after the PEF treatment, samples were left to stand at room temperature for 10 min and then filtered through a 0.22 μm filter to remove the bacteria (Millex-GV; Millipore Corporation, MA, USA). The protein concentration was determined with Bradford's assay (Bradford, 1976), where bovine serum albumin (BSA, Sigma-Aldrich Chemie GmbH, Germany) was used as the standard. The concentration of extracted proteins ($C_{\text{extracted}}$) was

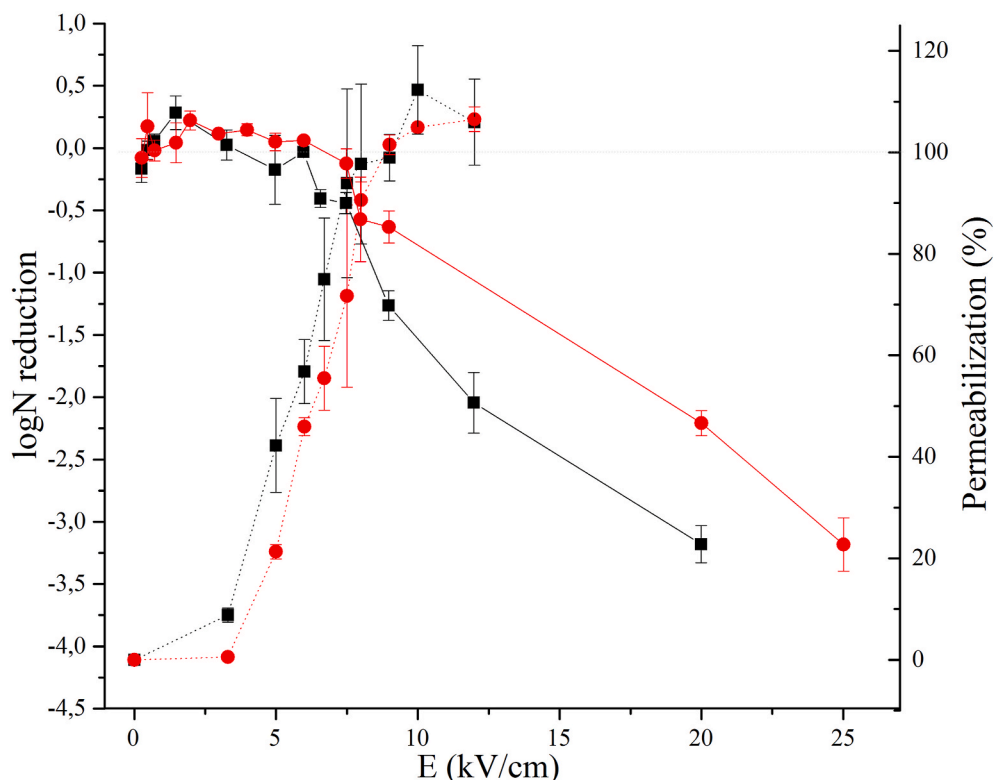


Fig. 3. Permeabilization and inactivation curves for *L. rhamnosus* and *L. paracasei* in batch PEF treatment mode. Symbols: solid line – viability curves, dotted line – permeabilization curves; black lines, square – *L. rhamnosus*, red lines, circle – *L. paracasei*. Experimental conditions: batch treatment in cuvettes, 8 pulses, pulse duration 100 μs, 1 Hz frequency. Please note that the scale for logN reduction is logarithmic, while that for permeabilization is linear. Experiments were repeated three times on different days to prove repeatability. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

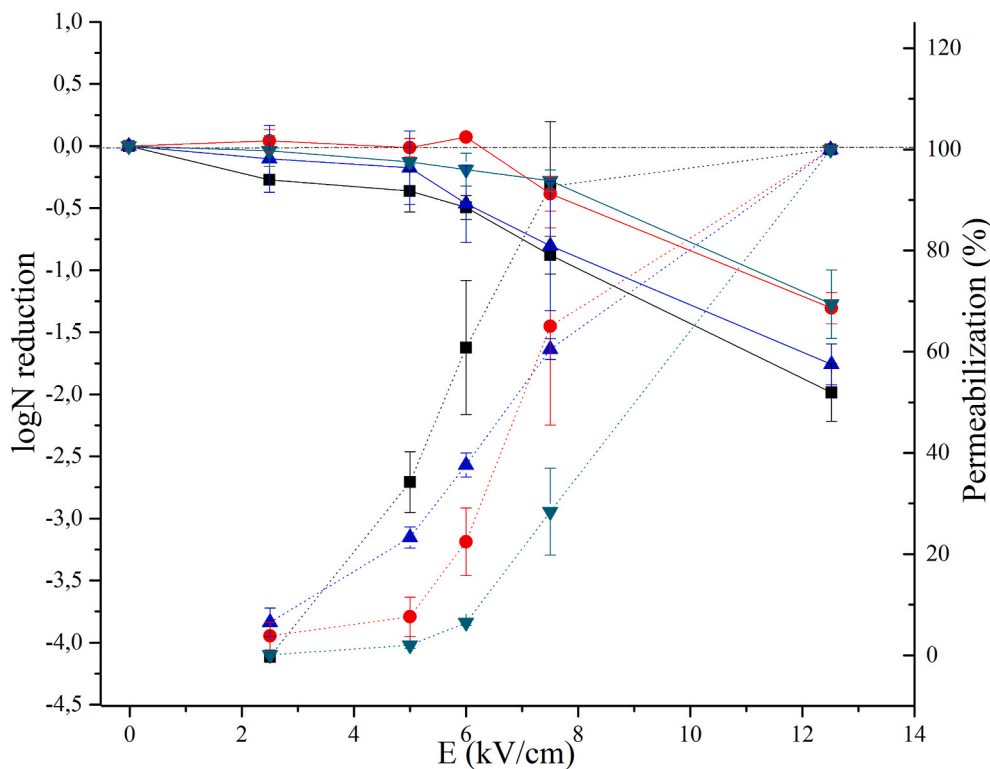


Fig. 4. Inactivation and permeabilization curves for *L. rhamnosus*, *L. paracasei*, *E. coli* and *L. innocua* in flow-through chamber PEF treatment mode. Experimental conditions: flow-through chamber, 8 pulses, pulse duration 100 μ s, 1 Hz frequency, 3.8 ml/min flow rate, total sample volume 5 ml. Symbols: dotted line – permeabilization curves; solid line – viability curves; black lines, square – *L. rhamnosus*, red lines, circle – *L. paracasei*, blue line, up-pointing triangle – *E. coli*, green line, down-pointing triangle – *L. innocua*. Please note that the scale for logN reduction is logarithmic, while that for permeabilization is linear. Experiments were repeated three times on different days to prove repeatability. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

determined as follows:

$$C_{\text{extracted}} = C_{\text{PEF}} - C_{\text{control}} \quad (4)$$

where C_{PEF} represents the protein concentration in a sample exposed to electric pulses and C_{control} represents the protein concentration in a sample not exposed to electric pulses.

2.7. Susceptibility of *L. rhamnosus* and *L. paracasei* to different antibiotics

The disc diffusion test procedure for susceptibility to different antibiotics described by Bauer et al. (1966) was slightly modified (Djukić-Vuković et al., 2015). Briefly, antibiotic test discs (Torlak, Serbia) of eight antibiotics, including erythromycin (15 μ g), tetracycline (30 μ g), chloramphenicol (30 μ g), penicillin G (10 IU), cephalixin (30 μ g), gentamicin (15 μ g), kanamycin (30 μ g) and streptomycin (10 μ g), were placed on MRS agar plates inoculated with 2% electroporated *L. rhamnosus* and *L. paracasei* (bacterial suspensions prepared as described in Section 2.1; batch treatment, 5 kV/cm, $8 \times 100 \mu$ s or $20 \times 100 \mu$ s, 1 Hz) or *L. rhamnosus* and *L. paracasei* culture without treatment, as controls. After an overnight incubation at 37 °C, the diameter of the inhibition zone was measured. The results were interpreted according to the proposed cut-off levels. Strains were considered resistant if the inhibition zone diameters were equal to or less than 22 mm for the tested antibiotics.

2.8. Statistical analysis

Experiments were repeated two or three times on different days to prove repeatability. The results were evaluated using an unpaired *t*-test analysis (OriginLab 8.0, USA) and were considered significantly different at $p < 0.05$. Error bars represents the standard deviation of the mean value from two or three experiments.

3. Results and discussion

Many *Lactobacillus* spp. (*acidophilus*, *gasseri*, *johnsonii*) and species separated into new genera, such as *Lacticaseibacillus* spp. (*L. casei*, *L. rhamnosus*, *L. paracasei*), *Ligilactobacillus* spp. (*L. salivarius*), *Lactiplantibacillus* spp. (*L. plantarum*), *Limosilactobacillus* spp. (*L. fermentum*) (Zheng et al., 2020) and *Bifidobacterium* (*adolescentis*, *animalis*, *bifidum*, *breve* and *longum*), are accepted as probiotics if their daily intake is at least 1×10^9 CFU per day (Health Canada, 2009). Probiotic biomass with lower viability or inactivated probiotics with beneficial effects on health could still be administered as paraprobiotics. Although mostly live bacteria are used for the treatment of gut diseases (Sanders et al., 2019) or in functional food, paraprobiotics can be more convenient in some cases. Paraprobiotics are safe for immunocompromised consumers and can be added after the sterilization of food without the risk of recontamination. Additionally, both viable LAB and LAB-derived postbiotics can adsorb mycotoxins or other contaminants and improve the safety of food in that way (Moradi et al., 2020; Sevim et al., 2019).

When fruits and vegetables are harvested, they begin to lose quality mainly due to microbiological spoilage. Today, many food preservation methods aim to extend the shelf-life of food and ensure its safety. The ideal preservation method should inactivate spoilage and pathogenic microorganisms and not change food's organoleptic and nutritional properties (i.e., affecting food vitamins, flavour, colour or texture) (Raso & Barbosa-Cánovas, 2003). Although thermal treatments are most often used in the food industry, for some foods, the application of rather high heat is needed, which can considerably affect food properties. Therefore, nonthermal preservation methods are being sought to preserve nutritional and organoleptic food properties (Morales-de la Peña et al., 2019). One of the promising nonthermal methods in the food industry is PEF, where the food temperature during treatment is lower than that during traditional thermal treatments. PEF can inactivate various microorganisms in different foods (Gómez et al., 2019) without significant loss of food flavour, colour, and nutrients. Furthermore, the treatment time is substantially shorter (a few seconds) than that of traditional thermal treatments; thus, PEF is gaining interest as a gentle treatment

Table 4

Linear correlation of logN reduction and permeabilization with specific energy input or current in studied bacterial suspensions during applied continuous mode PEF treatments.

logN reduction (y)	Specific energy input (x) [kJ/L]		Current (x) [A]	
	Pearson's correlation coefficient	Linear fitting	Pearson's correlation coefficient	Linear fitting
<i>L. rhamnosus</i>	$r = -0.99529$, $p = 0.00039^a$	$y = -0.2029 - 0.0901x$, $r^2 = 0.976$	$r = -0.99077$, $p = 0.001^a$	$y = -0.0294 - 0.2632x$, $r^2 = 0.946$
<i>L. paracasei</i>	$r = -0.97978$, $p = 0.00344^a$	$y = 0.26885 - 0.03557x$, $r^2 = 0.827$	$r = -0.9607$, $p = 0.009^a$	$y = -0.52793 - 0.14558x$, $r^2 = 0.609$
<i>E. coli</i>	$r = -0.9888$, $p = 0.00142^a$	$y = -0.04634 - 0.08369x$, $r^2 = 0.991$	$r = -0.9984$, $p = 0.002^a$	$y = -0.58041 - 0.45623x$, $r^2 = 0.998$
<i>L. innocua</i>	$r = -0.99917$, $p = 0.00003^a$	$y = -0.00171 - 0.01689x$, $r^2 = 0.988$	$r = -0.98869$, $p = 0.001^a$	$y = -0.19837 - 0.0703x$, $r^2 = 0.907$
Permeabilization (y)	Specific energy input (x) [kJ/L]		Current (x) [A]	
	Pearson's correlation coefficient	Linear fitting	Pearson's correlation coefficient	Linear fitting
<i>L. rhamnosus</i>	$r = 0.99597$, $p = 0.004^a$	$y = -11.2028 + 16.5775x$, $r^2 = 0.996$	$r = 0.99634$, $p = 0.005^a$	$y = -38.1602 + 45.4665x$, $r^2 = 0.946$
<i>L. paracasei</i>	$r = 0.93942$, $p = 0.061$	$y = -0.49784 + 3.06274x$, $r^2 = 0.601$	$r = 0.879$, $p = 0.121$	$y = -5.57256 + 6.98358x$, $r^2 = 0.465$
<i>E. coli</i>	$r = 0.99576$, $p = 0.004^a$	$y = 0.96716 + 8.42891x$, $r^2 = 0.987$	$r = 0.99468$, $p = 0.005^a$	$y = -21.3655 + 27.0649x$, $r^2 = 0.983$
<i>L. innocua</i>	$r = 0.92749$, $p = 0.072$	$y = -1.3835 + 0.666188x$, $r^2 = 0.812$	$r = 0.87214$, $p = 0.1278$	

^a Significant, $p < 0.05$.

for food preservation. Therefore, understanding treatment parameters that can induce significant permeabilization, cause the release of metabolites or inactivate probiotic LAB is crucial for the treatment and formulation of functional food.

3.1. The effects of batch PEF treatment on the permeabilization and viability of *L. rhamnosus* and *L. paracasei*

The effects of pulse duration and electric field strength were studied in batch treatment mode with the aim of obtaining reversible electroporation of the studied LAB to achieve membrane permeability of bacteria while preserving their viability. The results are presented in Fig. 2. (A) and 2. (B), while the correlation of logN reduction and specific energy input and current is provided in Table 3.

The loss of viability was less pronounced for shorter pulses for both studied bacteria, while *L. rhamnosus* showed higher sensitivity to PEF treatment than *L. paracasei*. If longer pulses were applied, a significant loss in viability was observed for *L. rhamnosus* even at lower field strengths (5 kV/cm). With an increase in field strength or application of longer pulses, the viability of both bacteria was more affected (Table 1). Other authors (Raso et al., 2016; Wouters et al., 2001) also observed an increase in microbial inactivation with higher total specific energy input. A significant correlation of logN reduction and specific energy was obtained in the case of *L. rhamnosus* for shorter and longer pulses

(Fig. 2B., Table 3.). *L. paracasei* was less susceptible to PEF treatment. Lower specific energies did not affect its growth under the studied conditions, and the correlation of logN reduction and specific energy was not significant.

The behaviour of bacteria exposed to PEF treatment is certainly species- and even strain-dependent (Coustets et al., 2015; Gurtler et al., 2010; Heinz et al., 2001; Rieder et al., 2008), but the reasons for the difference in susceptibility are not well understood. *L. rhamnosus* and *L. paracasei* are members of the same genus, and they have similar probiotic characteristic profiles (Djukić-Vuković et al., 2015; Mladenović et al., 2019). Both bacteria are rod-shaped, but on average, *L. rhamnosus* cells (2.3–4.2 $\mu\text{m} \times 0.4$ –0.6 μm) are larger than *L. paracasei* cells (1.2–2.5 $\mu\text{m} \times 0.7$ –1.0 μm). We observed easier permeabilization of *L. rhamnosus*, which is consistent with theoretical predictions in which a larger induced transmembrane voltage is expected in larger cells. This particularly stands for elongated and rod-shaped cells in flow-through chambers for PEF treatment (El-Hag et al., 2011; Valic et al., 2003). Easier permeabilization is achieved if cells are positioned with their longer axis in the direction of the electric field (Kotnik & Miklavcic, 2000; Valic et al., 2003). Very subtle differences in cell dimensions, such as those between *Lactiplantibacillus plantarum* (0.9–1.2 $\mu\text{m} \times 3$ –8 μm), *Levilactobacillus brevis* (0.7–1.0 $\mu\text{m} \times 2$ –4 μm) and *Listeria monocytogenes* (0.4–0.5 $\mu\text{m} \times 0.5$ –2 μm), lead to easier inactivation of larger *L. plantarum*, according to Heinz et al. (2001).

For more in-depth studies of permeabilization and its effects on selected LAB, a pulse duration of 100 μs was selected to preserve the viability of probiotic bacteria. The permeabilization and logN reduction curves for *L. rhamnosus* and *L. paracasei* induced by batch PEF treatment are presented in Fig. 3.

The application of an electric field strength of up to 8 kV/cm resulted in permeabilization of approximately 90% and mainly preserved the viability of both bacteria. Larger *L. rhamnosus* was permeabilized at a lower field strength than *L. paracasei*, which is consistent with theoretical predictions. With a further increase in field strength above 8 kV/cm, the viability of *L. rhamnosus* was also more affected than the viability of *L. paracasei*. The peak temperature recorded during the PEF treatment at maximal applied electric field strength (25 kV/cm, 8 pulses, 100 μs pulse duration, used for positive control in PEF treatment) was 38.6 °C. Therefore, thermal inactivation is not plausible under the studied conditions. The viability of *L. rhamnosus* and *L. paracasei* can be significantly preserved if field strengths of 3–8 kV/cm are applied. Under these conditions, the membranes of both bacteria are permeabilized.

3.2. The effects of PEF treatment in continuous mode on the permeabilization and viability of *L. rhamnosus* and *L. paracasei*

The same electric field strengths as in batch PEF treatment (Section 3.3.) were applied for PEF treatment in continuous mode, which is most often used on an industrial scale. Bacterial suspensions of the studied LAB and undesired *E. coli* and *L. innocua*, model pathogen microorganisms, were subjected to continuous mode PEF treatment, and the obtained results are presented in Fig. 4.

For *L. paracasei*, a slightly higher field strength was necessary in continuous mode PEF treatment (Fig. 4.) than in the batch system (Fig. 3.) to achieve the same permeabilization and logN reduction. Although the difference between batch and continuous treatment was also in the electrodes used, stainless steel in continuous and aluminium electrodes in the batch system, no statistically significant difference was noticed when batch treatments with aluminium or stainless-steel electrodes were compared under the same conditions (data not shown).

In the continuous PEF treatment, *L. rhamnosus* and *E. coli* had similar inactivation kinetics, while *L. paracasei* and *L. innocua* had similar inactivation rates, although some differences in permeabilization were observed. Please note that different ranges of electric fields are shown in Figs. 3 and 4. Elongated cells are expected to be oriented with their longer axis perpendicular to the direction of the electric field in laminar

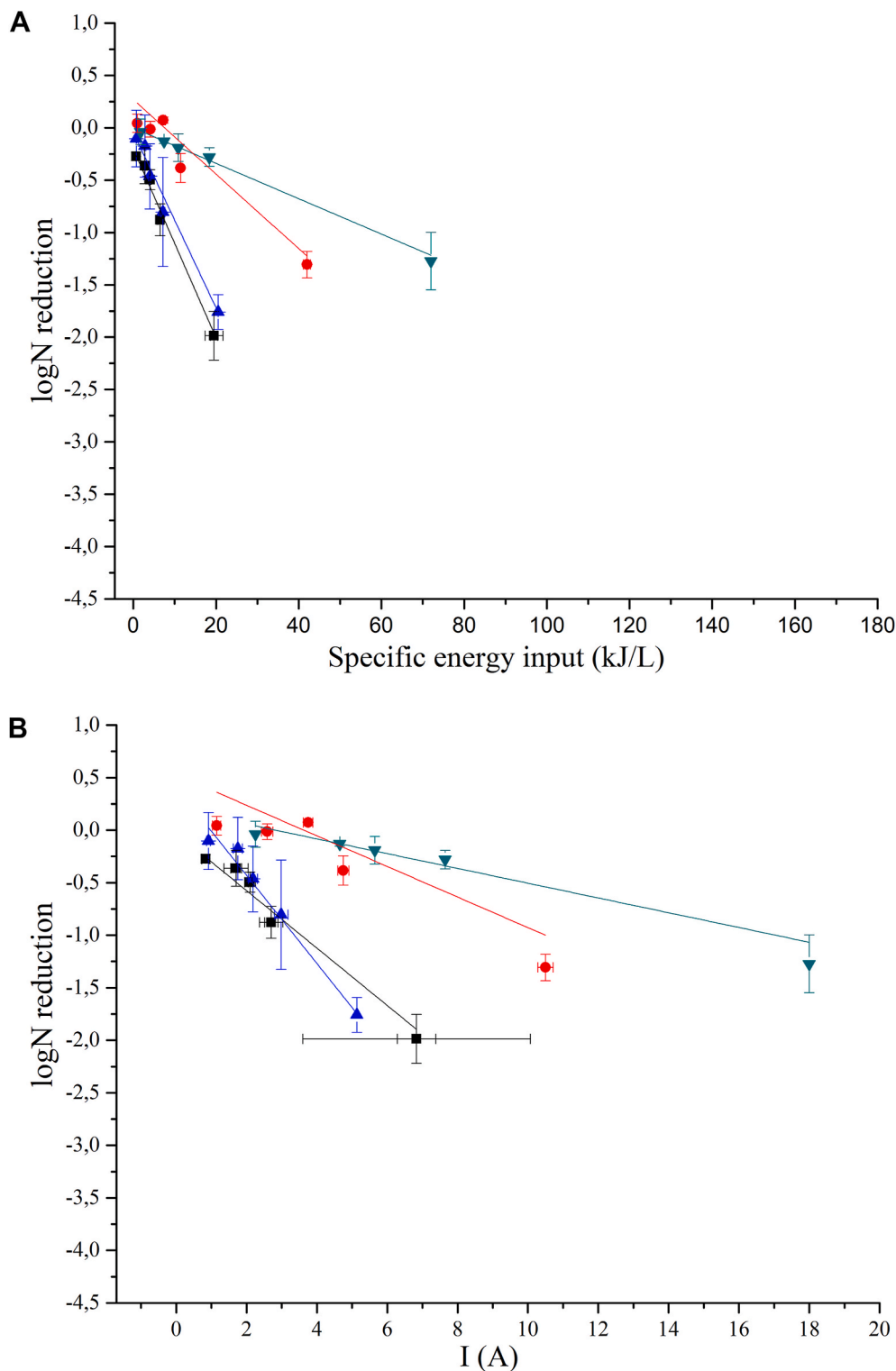


Fig. 5. Correlation of logN reduction and applied specific energy input (A) or current (B) for four tested bacteria subjected to continuous mode PEF treatment. Experimental conditions: flow-through chamber, 8 pulses, pulse duration 100 μ s, 1 Hz frequency, 3.8 ml/min flow rate, total sample volume 5 ml. Symbols: black lines, square – *L. rhamnosus*, red lines, circle – *L. paracasei*, blue line, up-pointing triangle – *E. coli*, green line, down-pointing triangle – *L. innocua*. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

flow; therefore, slightly higher field strength is needed to achieve the same permeabilization as in batch PEF treatment where the cells are randomly distributed. This effect was noticed for *L. paracasei*, while for *L. rhamnosus*, there was no difference between permeabilization in the batch and flow systems. When cells are aggregated, such as *L. rhamnosus*, due to exopolysaccharide production, the effect of cell orientation during flow treatment can be less pronounced at low flow rates, which could explain the absence of a difference between the batch and flow PEF treatment for *L. rhamnosus*.

To analyse the observed effects, logN reduction and permeabilization were correlated with the specific energy input or current (Table 2., Table 2S, 3S, 4S) for continuous mode treatment and are presented in Fig. 5. (A) and 5. (B). The results of the linear fit are provided in Table 4.

A similar amount of specific energy is needed for logN reduction of *L. rhamnosus* and *E. coli*, and their behaviour was similar over the whole range of applied energies. *L. paracasei* and *L. innocua* showed two- and three-fold higher resilience to applied energy and current, respectively, during PEF treatment than *L. rhamnosus* or *E. coli*. In addition,

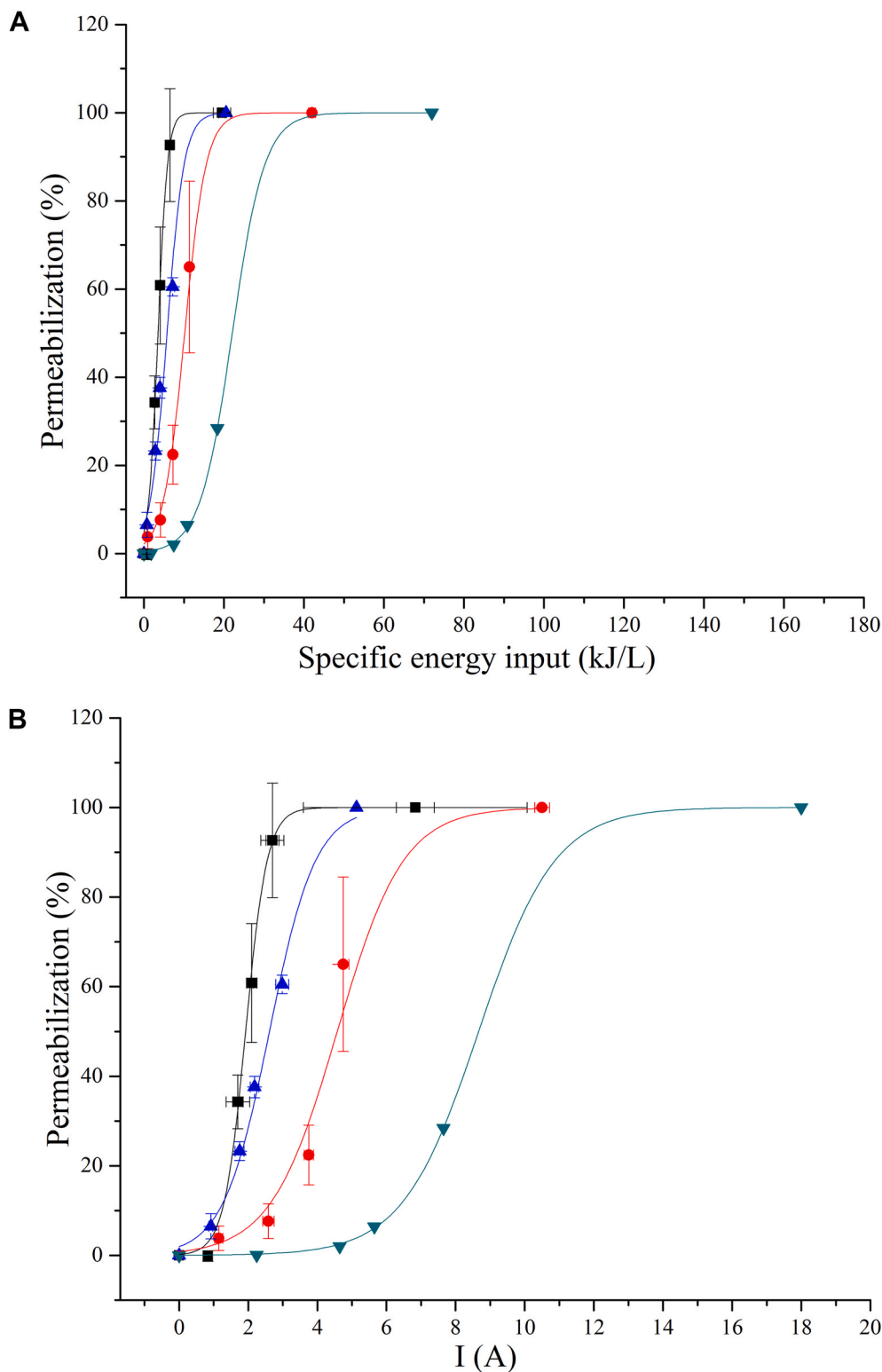


Fig. 6. Sigmoidal fitting for the correlation of permeabilization and specific energy (A) or current (B) in the studied bacterial suspensions during applied continuous mode PEF treatment. Experimental conditions: flow-through chamber, 8 pulses, pulse duration 100 μ s, 1 Hz frequency, 3.8 ml/min flow rate, total sample volume 5 ml. Symbols: black lines, square – *L. rhamnosus*, red lines, circle – *L. paracasei*, blue line, up-pointing triangle – *E. coli*, green line, down-pointing triangle – *L. innocua*. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

differences in the slopes of linear fitting of logN reduction and specific energy input show a range of energies that are tolerable for different bacteria, with *L. innocua* and *L. paracasei* being less susceptible to inactivation by PEF (Fig. 5A). *L. paracasei* can act as a surrogate microorganism for the model food-borne pathogen *E. coli*, since *L. paracasei* has shown higher resistance to PEF treatment than *E. coli*.

From the permeabilization dataset, a linear correlation was only significant for *L. rhamnosus* and *E. coli*, which were permeabilized at low

specific energies (Table 4). To fit better observed results, sigmoid fitting of permeabilization with specific energy (A) and current (B) is presented in Fig. 6. The equations for sigmoid fitting are given in Table 5.

In bacteria where the electroporation threshold is reached at low specific energies (*L. rhamnosus* and *E. coli*), a linear correlation explains the good permeabilization as a function of specific energy. In bacteria that need a higher specific energy input to initiate electroporation, sigmoidal fitting better explains the correlation of

Table 5

Sigmoidal fitting for correlation of permeabilization and specific energy or current in studied bacterial suspensions during applied continuous mode PEF treatments.

Permeabilization (y)	Specific energy input (x) [kJ/L]	Current (x) [A]
<i>L. rhamnosus</i>	$y = 98.4568/(1+e^{-(x-3.6457)/1.0043})$, $r^2 = 0.9507$, $P = 0.2762^a$	$y = 99.3325/(1+e^{-(x-1.8176)/0.3711})$, $r^2 = 0.9019$, $P = 0.4082^a$
<i>L. paracasei</i>	$y = 99.9724/(1+e^{-(x-10.4205)/2.5248})$, $r^2 = 0.9586$, $P = 0.0238$	$y = 100.1191/(1+e^{-(x-4.5930)/0.68})$, $r^2 = 0.9558$, $P = 0.0657^a$
<i>E. coli</i>	$y = 99.9134/(1+e^{-(x-5.9276)/2.6197})$, $r^2 = 0.9730$, $P = 0.1611^a$	$y = 104.3755/(1+e^{-(x-2.7458)/0.7904})$, $r^2 = 0.9730$, $P = 0.1746^a$
<i>L. innocua</i>	$y = 100.0053/(1+e^{-(x-22.1688)/4.0252})$, $r^2 = 0.9942$, $P = 0.0009$	$y = 100.0265/(1+e^{-(x-8.7443)/1.1211})$, $r^2 = 0.9939$, $P = 0.0005$

^a Significant, normality test, $P > 0.05$.

permeabilization with specific energy over the whole studied range of specific energies, as in the case of *L. innocua* (Fig. 6A, B). Once the electroporation threshold is reached, permeabilization linearly correlates with both specific energy and current until it reaches 100%. Variations in permeabilization and inactivation within the population of PEF-treated bacteria due to the distribution of cell size were suggested previously by Puc et al. (2003).

For numerous PEF applications, there is a need to translate results obtained in batch PEF treatment to continuous mode PEF treatment in a predictive manner. Significant linear correlations obtained in batch and continuous mode treatment for *L. rhamnosus* are presented in Fig. 7.

At lower specific energies and inactivation rates, there was no significant difference between the batch and continuous mode treatments. However, for higher inactivation above $-1.8 \log N$, continuous mode treatment is more efficient in terms of specific energy input. Qin et al.

(1998) also observed that continuous mode PEF treatment was more efficient in terms of microbial inactivation than the batch system.

The obtained correlations enable prediction of the inactivation rate, which can be expected in continuous mode PEF treatment from data acquired in a batch system, for scale up and transition from batch to continuous setup and *vice versa*, under the studied conditions. Furthermore, PEF treatment parameters were examined for extraction of specific compounds or enhancement of particular metabolic activity in LAB related to their probiotic characteristics.

3.3. Effect of sublethal PEF treatment on the probiotic characteristics of *L. rhamnosus* and *L. paracasei*

3.3.1. Lactic acid production

LA production is an important probiotic characteristic responsible for antimicrobial activity against pathogens (Djukić-Vuković et al., 2015; Rajilić-Stojanović & de Vos, 2014). We first tested the effects of sublethal PEF treatment conditions ($8 \times 100 \mu s$, 1 Hz, 5 kV/cm), and LA production was completely preserved (data not shown). Therefore, a longer pulse duration ($8 \times 1 \text{ ms}$, 1 Hz, 5 kV/cm) was examined to evaluate the effect of higher energy input but still without a high loss of viability (Table 1, Table 2S., Fig. 2(A), (B)). These results are presented in Fig. 8. LA production was 10% higher after 24 h of LAF with 1 ms pulse-treated *L. rhamnosus*, while with *L. paracasei*, no significant difference in LA production was observed. Additionally, sugar consumption was increased during LAF with electroporated *L. rhamnosus*. A similar result was reported for *Saccharomyces cerevisiae*, where low electric field strengths (below 6 kV/cm) caused an increase in sugar consumption (Mattar et al., 2014, 2015). PEF treatment of *L. plantarum* at field strengths of 14 kV/cm or less also enhanced the metabolic activity and acidification rate during the first 24 h of fermentation (Seratlić et al., 2013).

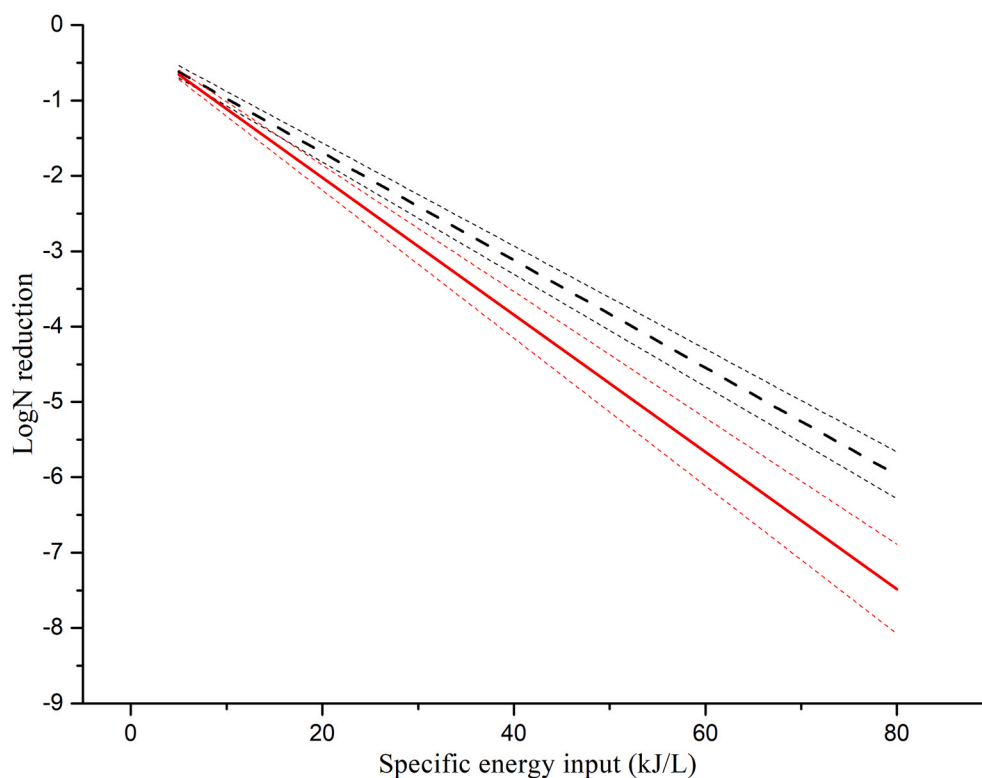


Fig. 7. Correlations of logN reduction and specific energy for batch and continuous mode PEF treatment of *L. rhamnosus*. Symbols: black, dashed line – linear fitting for batch treatment, red, solid line – linear fitting for continuous mode treatment, dotted lines – errors. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

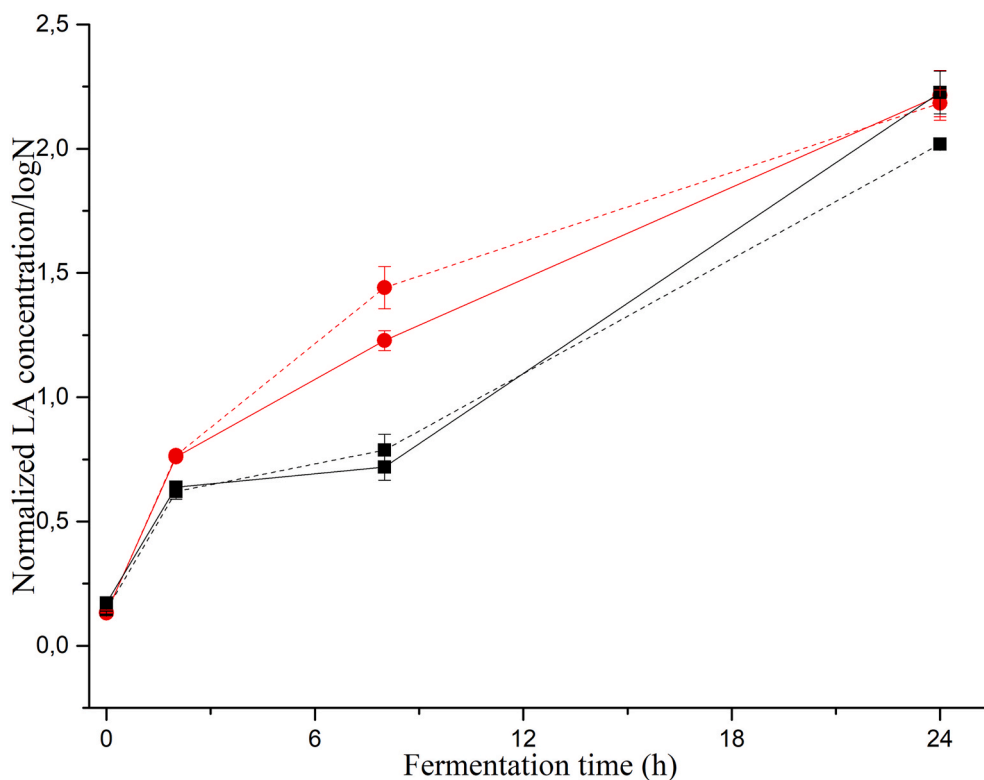


Fig. 8. Normalized LA production per number of viable *L. rhamnosus* and *L. paracasei* cells after sublethal PEF treatment. Experimental conditions: flow-through chamber, 8 pulses, electric field 5 kV/cm, pulse duration 100 μ s, 1 Hz frequency, 3.8 ml/min flow rate, total sample volume 5 ml. Symbols: red lines, circle – *L. paracasei*, black line, square – *L. rhamnosus*, solid line – PEF-treated bacteria, dashed line – control bacteria. Experiments were repeated two times on different days to prove repeatability. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 6
Extraction of proteins by means of electroporation^a.

Bacteria	Number of pulses	Pulse duration [μ s]	E [kV/cm]	Protein concentration [μ g/ml]
batch				
<i>L. rhamnosus</i>	8	100	5	not detected
	8	100	7.5	not detected
	20	100	5	not detected
	2	1000	5	1.5 \pm 0.3
	8	1000	5	2.1 \pm 0.4
<i>L. paracasei</i>	8	100	5	not detected
	8	100	7.5	not detected
	20	100	5	not detected
	2	1000	5	not detected
	8	1000	5	not detected
continuous				
<i>L. rhamnosus</i>	8	100	5	not detected
	8	1000	5	1.9 \pm 0.5
<i>L. paracasei</i>	8	100	5	not detected
	8	1000	5	not detected

^a Experiments were repeated two times, on different days to prove repeatability.

3.3.2. Extraction of proteins

Enhancement of the performance of bacteria or yeasts due to electroporation is often attributed to the increase in transport of different molecules through membranes (Seratlić et al., 2013). However, it can also lead to leakage of intracellular contents, with proteins being most often studied (Coustets et al., 2015; Haberl Meglic et al., 2015; Haberl-Meglic et al., 2016), and this can provide new perspectives for PEF in the production of paraprobiotics and postbiotics. Haberl-Meglic et al. (2015) reported that electroporation enables protein extraction in *E. coli*.

Therefore, we determined the content of proteins released after different numbers of 100 μ s and 1 ms pulses while preserving the viability of bacteria (field strength 5 kV/cm, Fig. 2.) and the obtained results are presented in Table 6. The electroextraction of proteins in

filtered extracts of *L. rhamnosus* 10 min after PEF was significantly above the control for longer pulse durations. Pulse duration was also important for better extraction from *E. coli* (Haberl-Meglic et al., 2016).

The release of intracellular proteins could be particularly important for functional food with probiotics, enabling the treated product to have enhanced characteristics (Molae Parvare et al., 2021). Glucosidase, an enzyme responsible for the biotransformation of heterosides into their active aglycons (Ewe et al., 2012), is mostly intracellular in lactobacilli (Carevic et al., 2017; Michlmayr & Kneifel, 2014). The release of enzymes by reversible electroporation could be an important application of PEFs in functional food.

3.3.3. Susceptibility to antibiotics

Increased mass transfer between bacteria and their surroundings as a consequence of electroporation can also affect the susceptibility of the studied bacteria to antibiotics or other inhibitory molecules. Susceptibility to antibiotics is studied in probiotics to prevent the introduction of transferable resistance with probiotic-rich food into the host microbiome. When PEF treatment of 8 \times 100 μ s at 5 kV/cm was performed, there were no differences in the susceptibility to antibiotics between treated bacteria and controls (data not shown). With a higher number of pulses (20 \times 100 μ s, 5 kV/cm), some changes in the susceptibility are obtained and presented in Fig. 9. (A) and 9. (B).

For *L. rhamnosus*, there were no significant changes in the susceptibility to antibiotics after PEF application, except for chloramphenicol (Fig. 9. (A)), although it was more sensitive to PEF (Figs. 2., 3., Fig. 4.). After PEF application, *L. paracasei* was less susceptible to some antibiotics (Fig. 9. (B)) and generally showed less susceptibility to PEF treatment (Figs. 2., 3., Fig. 4.). One could hypothesize that bacteria will be more susceptible to antibiotics after electroporation due to permeabilization, as was observed for organic acids or antimicrobial compounds (Arroyo & Lyng, 2017; Martens et al., 2020; Terebiznik et al., 2016). Therefore, we examined the effect of permeabilization/resealing time to compare it for both studied bacteria 12.5 min and 30 min after electroporation. The results showed the same antibiotic susceptibility

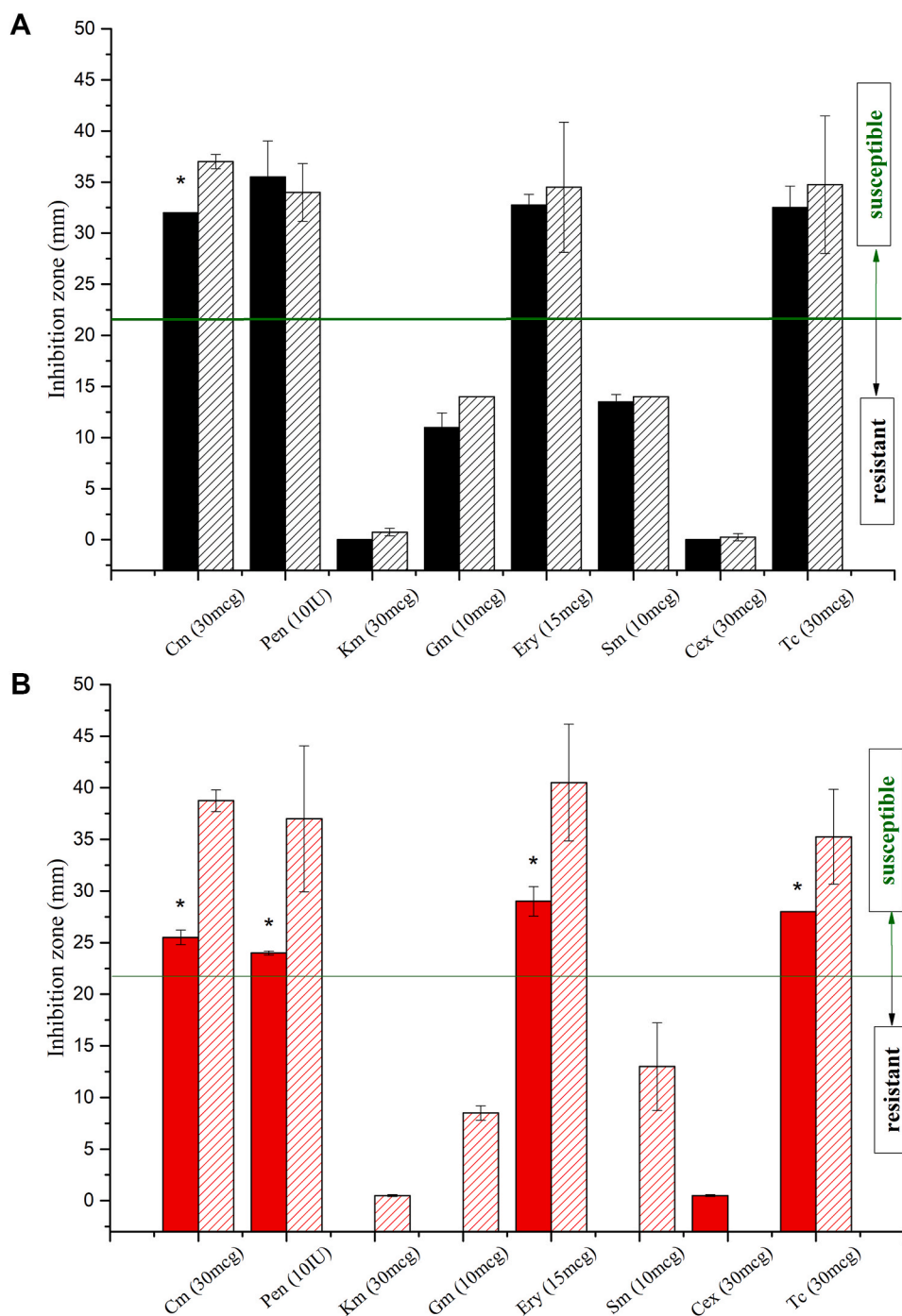


Fig. 9. Susceptibility of *L. rhamnosus* (A) and *L. paracasei* (B) to antibiotics. Symbols (A): black, solid bars – PEF treated samples, patterned bars – control sample, Symbols (B): red, solid bars – PEF-treated samples, patterned bars – control sample; Chloramphenicol (Cm), Penicillin G (Pen), Kanamycin (Km), Gentamicin (Gm), Erythromycin (Ery), Streptomycin (Sm), Tetracycline (Tc), Cephalexin (Cex). Asterisk (*) is used to designate statistically significant differences between treated and control samples. Experimental conditions: $20 \times 100 \mu\text{s}$, 5 kV/cm, 1 Hz (batch PEF treatment). Experiments were repeated two times on different days to prove repeatability. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

profile, regardless of time within the studied intervals. The permeability was $14.45 \pm 0.34\%$ (12.5 min) and $13.81 \pm 1.06\%$ (30 min) for *L. paracasei*. For *L. rhamnosus*, the permeability was $34.75 \pm 3.24\%$ (12.5 min) and $37.36 \pm 1.69\%$ (30 min).

Therefore, the observed decreased susceptibility to antibiotics is probably related to other changes in the cell caused by the application of PEF, not simply by membrane permeabilization and the potential influx of antibiotics into the cell. Regardless of the mechanism of action, all bactericidal antibiotics induce the production of highly reactive oxygen species (ROS) related to cell death (Dwyer et al., 2010; Kohanski et al., 2010). In contrast, PEF treatment causes stress to the cell mediated by the generation of ROS (Teissie, 2017). Upregulation of many genes was reported in PEF-treated bacteria, from TCA cycle and methylcitrate cycle

proteins related to β -oxidation of lipids and peroxidation in the membrane to genes related to protection from abiotic stress in general (UspB), oxidative stress (AcnB) and changes in membrane stability (OmpF, MacA) (Liu et al., 2019; Pakhomova et al., 2012; Tanino et al., 2012). Elevated amounts of ROS and increased expression of proteins responsible for the recovery of bacteria after sublethal PEF treatment (Chueca et al., 2015) could interact with conventional pathways of antibiotic action and decrease the efficiency of antibiotics. The pre-treatment of bacteria with 1–5 mM H_2O_2 before exposure to antibiotics caused the expression of natural oxidative stress-protecting enzymes and resulted in a 1 log decrease in susceptibility to antibiotics (Yang et al., 2014). However, other mechanisms unrelated to oxidative stress could also induce lower susceptibility to antibiotics after PEF treatment. For

example, PEF-treated *E. coli* showed expression of macrolide-specific efflux protein (MacA), which was not detected in the control untreated sample (Liu et al., 2019). This implies that PEF treatment could activate resistance genes for selected antibiotics by mechanisms associated with membrane transport, permeability and fluidity and not just through the oxidative stress response. This could be the case for other bacteria, including LAB, since *L. paracasei* in this study also showed lower susceptibility to macrolide antibiotic erythromycin after PEF treatment.

Further studies are needed to gain more insights into the mechanisms involved in changes in susceptibility to antibiotics due to PEF treatment, but it is evident that the interaction of probiotic LAB and PEF goes beyond the increase in membrane permeability and transmembrane transport. PEFs have the potential to be exploited in processing in novel ways, other than inactivation or electrotransformation, especially in the growing field of functional food production and biotransformation by bacteria.

4. Conclusions

The inactivation and permeabilization kinetics of *L. rhamnosus* and *L. paracasei* by batch and continuous mode PEF treatment showed the higher susceptibility of *L. rhamnosus* to PEF treatment in both studied treatment systems. A slightly larger size probably enables easier permeabilization of *L. rhamnosus*.

In parallel, the permeabilization and inactivation kinetics of *E. coli* and *L. innocua* as model foodborne pathogens were studied, and they followed those of *L. rhamnosus* and *L. paracasei*, respectively. Therefore, *L. paracasei* can be used as a surrogate microorganism in processes for validation of PEF treatment potentially challenged by *E. coli*.

Similar linear correlations between a reduction in viability and specific energy input were established for all studied bacteria. The results obtained in batch treatment mode can be used to predict logN reduction in continuous mode under the defined conditions. In terms of specific energy input, continuous treatment was more efficient for achieving the same inactivation of *L. rhamnosus*. These results could be useful in the selection of promising candidates for the application of PEFs for the treatment of inoculated functional food for biotransformation, the production of paraprobiotics or postbiotics, etc.

The effects of the determined sublethal PEF treatment conditions on probiotic characteristics were also studied. The application of sublethal PEF treatment of 5 kV/cm with 8×1 ms pulses increased LA production and sugar consumption by *L. rhamnosus* in LAF. Significant extraction of proteins was obtained by application of longer pulses. PEF treatment did not change the susceptibility of *L. rhamnosus* to specific antibiotics, while for *L. paracasei*, a decrease in the susceptibility to antibiotics was observed. This is probably a result of the PEF-induced stress and recovery mechanisms initiated by PEF treatment. The obtained results are valuable for PEF treatment of functional food with probiotics to improve its safety or functionality. This study sets the foundation for novel PEF applications in the production of postbiotics or paraprobiotics by tuning PEF treatment parameters. Further studies are needed, but biotransformation in functional food with PEF-assisted electroextraction of enzymes or other LAB metabolites could be a very promising option in the future.

CRedit authorship contribution statement

Aleksandra Djukić-Vuković: Conceptualization, Design, Methodology, Formal analysis, Interpretation, Writing – original draft, revisions and editing. **Saša Haberl Meglič:** Methodology, Formal analysis, Interpretation, Writing, Reviewing. **Karel Flisar:** Methodology, Formal analysis, Interpretation. **Ljiljana Mojović:** Supervision, Writing, Reviewing. **Damijan Miklavčič:** Conceptualization, Design, Methodology, Supervision, Writing, revisions and editing, Project administration.

Declaration of competing interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.lwt.2021.112304>.

References

- Alimolaei, M., Golchin, M., & Daneshvar, H. (2016). Oral immunization of mice against *Clostridium perfringens* epsilon toxin with a *Lactobacillus casei* vector vaccine expressing epsilon toxoid. *Infection, Genetics and Evolution*, 40, 282–287. <https://doi.org/10.1016/j.meegid.2016.03.020>
- de Almada, C. N., Almada, C. N., Martinez, R. C. R., & Sant’Ana, A. S. (2016). Paraprobiotics: Evidences on their ability to modify biological responses, inactivation methods and perspectives on their application in foods. In *Trends in food science and technology* (Vol. 58, pp. 96–114). Elsevier Ltd. <https://doi.org/10.1016/j.tifs.2016.09.011>
- Andresen, V., Gschossman, J., & Layer, P. (2020). Heat-inactivated *Bifidobacterium bifidum* MIMBb75 (SYN-HI-001) in the treatment of irritable bowel syndrome: A multicentre, randomised, double-blind, placebo-controlled clinical trial. *The Lancet Gastroenterology and Hepatology*, 5(7), 658–666. [https://doi.org/10.1016/S2468-1253\(20\)30056-X](https://doi.org/10.1016/S2468-1253(20)30056-X)
- Arroyo, C., & Lyng, J. G. (2017). Pulsed electric fields in hurdle approaches for microbial inactivation. In *Handbook of electroporation* (Vol. 4, pp. 2591–2620). Springer International Publishing. https://doi.org/10.1007/978-3-319-32886-7_190
- Barba, F. J., Parniakov, O., Pereira, S. A., Wiktor, A., Grimi, N., Boussetta, N., Saraiva, J. A., Raso, J., Martin-Belloso, O., Witrowa-Rajchert, D., Lebovka, N., & Vorobiev, E. (2015). Current applications and new opportunities for the use of pulsed electric fields in food science and industry. *Food research international*. <https://doi.org/10.1016/j.foodres.2015.09.015>
- Barros, C. P., Pires, R. P. S., Guimaraes, J. T., Abud, Y. K. D., Almada, C. N., Pimentel, T. C., Sant’Anna, C., De-Melo, L. D. B., Duarte, M. C. K. H., Silva, M. C., Sant’Ana, A. S., Freitas, M. Q., & Cruz, A. G. (2021). Ohmic heating as a method of obtaining paraprobiotics: Impacts on cell structure and viability by flow cytometry. *Food Research International*, 140. <https://doi.org/10.1016/j.foodres.2020.110061>
- Batista Napotnik, T., & Miklavčič, D. (2018). *Vitro electroporation detection methods - an overview* (Vol. 120, pp. 166–182). Amsterdam, Netherlands: Bioelectrochemistry. <https://doi.org/10.1016/j.bioelechem.2017.12.005>
- Bauer, A. W., Kirby, W. M., Sherris, J. C., & Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology*, 45(4), 493–496. <http://www.ncbi.nlm.nih.gov/pubmed/5325707>
- Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72(1–2), 248–254. [https://doi.org/10.1016/0003-2697\(76\)90527-3](https://doi.org/10.1016/0003-2697(76)90527-3)
- Carevic, M., Vukasinovic-Sekulic, M., Banjanac, K., Milivojevic, A., Corovic, M., & Bezbradica, D. (2017). Characterization of β -galactosidase from *Lactobacillus acidophilus*: Stability and kinetic study. *Advanced Technologies*, 6(1), 5–13. <https://doi.org/10.5937/savteh1701005c>
- Chueca, B., Pagán, R., & García-Gonzalo, D. (2015). Transcriptomic analysis of *Escherichia coli* MG1655 cells exposed to pulsed electric fields. *Innovative Food Science & Emerging Technologies*, 29, 78–86. <https://doi.org/10.1016/j.ifset.2014.09.003>
- Coustets, M., Ganeva, V., Galutzov, B., & Teissie, J. (2015). Millisecond duration pulses for flow-through electro-induced protein extraction from *E. coli* and associated eradication. *Bioelectrochemistry*, 103, 82–91. <https://doi.org/10.1016/j.bioelechem.2014.08.008>
- Cuevas-González, P. F., Liceaga, A. M., & Aguilar-Toalá, J. E. (2020). Postbiotics and paraprobiotics: From concepts to applications. In *Food research international* (Vol. 136, p. 109502). Elsevier Ltd. <https://doi.org/10.1016/j.foodres.2020.109502>
- Djukić-Vuković, A. P., Mojović, L. V., Jokić, B. M., Nikolić, S. B., & Pejin, J. D. (2013). Lactic acid production on liquid distillery stillage by *Lactobacillus rhamnosus* immobilized onto zeolite. *Bioresource Technology*, 135, 454–458. <https://doi.org/10.1016/j.biortech.2012.10.066>

- Djukić-Vuković, A. P., Mojević, L. V., Semenčenko, V. V., Radosavljević, M. M., Pejin, J. D., & Kocić-Tanackov, S. D. (2015). Effective valorisation of distillery stillage by integrated production of lactic acid and high quality feed. *Food Research International*, 73, 75–80. <https://doi.org/10.1016/j.foodres.2014.07.048>
- Dwyer, D. J., Kohanski, M. A., & Collins, J. J. (2010). Role of reactive oxygen species in antibiotic action and resistance. *Current Opinion in Microbiology*, 12(5), 482–489. <https://doi.org/10.1016/j.mib.2009.06.018> (Role).
- El-Hag, A., Jayaram, S., Rodríguez-González, O., & Griffiths, M. W. (2011). The influence of size and shape of microorganism on pulsed electric field inactivation. *IEEE Transactions on NanoBioscience*, 10(3), 133–138. <https://doi.org/10.1109/TNB.2011.2163078>
- Ewe, J. A., Wan-Abdullah, W. N., Alias, A. K., & Liang, M. T. (2012). Enhanced growth of lactobacilli and bioconversion of isoflavones in biotin-supplemented soy milk by electroporation. *International Journal of Food Sciences & Nutrition*, 63(5), 580–596. <https://doi.org/10.3109/09637486.2011.641940>
- Feng, T., & Microbes, J. W.-G. (2020). undefined. (2020). Oxidative stress tolerance and antioxidant capacity of lactic acid bacteria as probiotic: A systematic review. *Taylor & Francis*, 12(1). <https://doi.org/10.1080/19490976.2020.1801944>
- Flisar, K., Meglic, S. H., Morelj, J., Golob, J., & Miklavcic, D. (2014). Testing a prototype pulse generator for a continuous flow system and its use for E. coli inactivation and microalgae lipid extraction. *Bioelectrochemistry*, 100, 44–51. <https://doi.org/10.1016/j.bioelechem.2014.03.008>
- Galindo, F. G., Dejmek, P., Lundgren, K., Rasmusson, A. G., Vicente, A., & Moritz, T. (2009). Metabolomic evaluation of pulsed electric field-induced stress on potato tissue. *Planta*, 230(3), 469–479. <https://doi.org/10.1007/s00425-009-0950-2>
- Gómez, B., Munekata, P. E. S., Gavahian, M., Barba, F. J., Martí-Quijal, F. J., Bolumar, T., Campagnol, P. C. B., Tomasevic, I., & Lorenzo, J. M. (2019). Application of pulsed electric fields in meat and fish processing industries: An overview. In *Food research international* (Vol. 123, pp. 95–105). Elsevier. <https://doi.org/10.1016/j.foodres.2019.04.047>
- Gurtler, J. B., Rivera, R. B., Zhang, H. Q., & Geveke, D. J. (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, 1–8. <https://doi.org/10.1016/j.ijfoodmicro.2010.02.023>
- Haberl Meglic, S., Marolt, T., & Miklavcic, D. (2015). Protein extraction by means of electroporation from E. coli with preserved viability. *Journal of Membrane Biology*, 248(5), 893–901. <https://doi.org/10.1007/s00232-015-9824-7>
- Haberl-Meglic, S., Levčičnik, E., Luengo, E., Raso, J., & Miklavcic, D. (2016). The effect of temperature and bacterial growth phase on protein extraction by means of electroporation. *Bioelectrochemistry*, 112. <https://doi.org/10.1016/j.bioelechem.2016.08.002>
- Health Canada. (2009). Guidance document – the use of probiotic microorganisms in food. In *Food directorate health products and food branch, april, 1–8*. https://www.canada.ca/content/dam/hc-sc/migration/hc-sc/fn-an/alt_formats/hpfb-dgpsa/pdf/le_gislation/probiotics_guidance-orientation_probiotiques-eng.pdf
- Heinz, V., Alvarez, I., Angersbach, A., & Knorr, D. (2001). Preservation of liquid foods by high intensity pulsed electric fields—basic concepts for process design. *Trends in Food Science & Technology*, 12(3–4), 103–111. [https://doi.org/10.1016/S0924-2244\(01\)00064-4](https://doi.org/10.1016/S0924-2244(01)00064-4)
- Hill, C., Guarner, F., Reid, G., Gibson, G. R., Merenstein, D. J., Pot, B., Morelli, L., Canani, R. B., Flint, H. J., Salminen, S., Calder, P. C., & Sanders, M. E. (2014). The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nature Reviews Gastroenterology & Hepatology*, 11(8), 506–514. <https://doi.org/10.1038/nrgastro.2014.66>
- Kazemi, A., Soltani, S., Nasri, F., Clark, C. C. T., & Kolahdouz-Mohammadi, R. (2020). The effect of probiotics, paraprobiotics, synbiotics, fermented foods and other microbial forms on immunoglobulin production: A systematic review and meta-analysis of clinical trials. *International Journal of Food Sciences & Nutrition*, 1–19. <https://doi.org/10.1080/09637486.2020.1857710>
- Kohanski, M. A., Dwyer, D. J., & Collins, J. J. (2010). How antibiotics kill bacteria: From targets to networks. *Nature Reviews Microbiology*, 8(Issue 6), 423–435. <https://doi.org/10.1038/nrmicro2333>. NIH Public Access.
- König, H., & Fröhlich, J. (2017). Lactic acid bacteria. In *Biology of microorganisms on grapes, in must and in wine* (pp. 3–41). Springer International Publishing. https://doi.org/10.1007/978-3-319-60021-5_1
- Kotnik, T., Frey, W., Sack, M., Haberl Meglic, S., Peterka, M., & Miklavcic, D. (2015). Electroporation-based applications in biotechnology. In , 33. *Trends in biotechnology* (pp. 480–488). Elsevier Current Trends. <https://doi.org/10.1016/j.tibtech.2015.06.002>, 8.
- Kotnik, T., & Miklavcic, D. (2000). *Analytical description of transmembrane voltage induced by electric fields on spheroidal cells analytical description of transmembrane voltage induced by electric fields on spheroidal cells* (Vol. 79). Elsevier. August <https://www.sciencedirect.com/science/article/pii/S0006349500763259>
- Kotnik, T., Pucihar, G., & Miklavcic, D. (2019). Induced transmembrane voltage and its correlation with electroporation-mediated molecular transport. *Journal of Membrane Biology*, 236(1), 3–13. <https://doi.org/10.1007/s00232-010-9279-9>
- Kotnik, T., Rems, L., Tarek, M., & Miklavcic, D. (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, S., Lee, J., Jin, Y. I., Jeong, J. C., Chang, Y. H., Lee, Y., Jeong, Y., & Kim, M. (2017). Probiotic characteristics of Bacillus strains isolated from Korean traditional soy sauce. *LWT - Food Science and Technology*, 79, 518–524. <https://doi.org/10.1016/j.lwt.2016.08.040>
- Lin, R., Zhang, Y., Long, B., Li, Y., Wu, Y., Duan, S., Zhu, B., Wu, X., & Fan, H. (2017). Oral immunization with recombinant Lactobacillus acidophilus expressing espA-tir-M confers protection against enterohemorrhagic Escherichia coli O157:H7 challenge in mice. *Frontiers in Microbiology*, 8(MAR), 417. <https://doi.org/10.3389/fmicb.2017.00417>
- Liu, Z., Zhao, L., Zhang, Q., Huo, N., Shi, X., Li, L., Jia, L., Lu, Y., Peng, Y., & Song, Y. (2019). Proteomics-Based mechanistic investigation of Escherichia coli inactivation by pulsed electric field. *Frontiers in Microbiology*, 2644. <https://doi.org/10.3389/fmicb.2019.02644>
- Mahnčić-Kalamiza, S., Vorobiev, E., & Miklavcic, 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>
- Marco, M. L., Sanders, M. E., Gänzle, M., Arrieta, M. C., Cotter, P. D., De Vuyst, L., Hill, C., Holzapfel, W., Lebeer, S., Merenstein, D., Reid, G., Wolfe, B. E., & Hutkins, R. (2011). The international scientific association for probiotics and prebiotics (ISAPP) consensus statement on fermented foods. *Nature Reviews Gastroenterology & Hepatology*, 18(3), 196–208. <https://doi.org/10.1038/s41575-020-00390-5>
- Martens, S. L., Klein, S., Barnes, R. A., TrejoSanchez, P., Roth, C. C., & Ibey, B. L. (2020). 600-ns pulsed electric fields affect inactivation and antibiotic susceptibilities of Escherichia coli and Lactobacillus acidophilus. *AMB Express*, 10(1). <https://doi.org/10.1186/s13568-020-00991-y>
- Mattar, J. R., Turk, M. F., Nonus, M., Lebovka, N. I., El Zakhem, H., & Vorobiev, E. (2014). Stimulation of Saccharomyces cerevisiae cultures by pulsed electric fields. *Food and Bioprocess Technology*, 7(11), 3328–3335. <https://doi.org/10.1007/s11947-014-1336-4>
- Mattar, J. R., Turk, M. F., Nonus, M., Lebovka, N. I., El Zakhem, H., & Vorobiev, E. (2015). S. cerevisiae fermentation activity after moderate pulsed electric field pre-treatments. *Bioelectrochemistry*, 103, 92–97. <https://doi.org/10.1016/j.bioelechem.2014.08.016>
- Michlmayr, H., & Knäfel, W. (2014). β-Glucosidase activities of lactic acid bacteria: Mechanisms, impact on fermented food and human health. In , Vol. 352. *FEMS microbiology letters* (pp. 1–10). Oxford University Press. <https://doi.org/10.1111/1574-6968.12348>. Issue 1.
- Mladenović, D., Djukić-Vuković, A., Stanković, M., Milašinović-Šeremešić, M., Radosavljević, M., Pejin, J., & Mojević, L. (2019). Bioprocessing of agro-industrial residues into lactic acid and probiotic enriched livestock feed. *Journal of the Science of Food and Agriculture*. <https://doi.org/10.1002/jsfa.9759>
- Molaei Parvareh, M., Khorshidian, N., Fazeli, M. R., Mortazavian, A. M., Sarem Nezhad, S., & Mortazavi, S. A. (2021). Comparative effect of probiotic and paraprobiotic addition on physicochemical, chemometric and microstructural properties of yogurt. *LWT*, 144, 111177. <https://doi.org/10.1016/j.lwt.2021.111177>
- Moradi, M., Kousheh, S. A., Almasi, H., Alizadeh, A., Guimarães, J. T., Yilmaz, N., & Lotfi, A. (2020). Postbiotics produced by lactic acid bacteria: The next frontier in food safety. *Comprehensive Reviews in Food Science and Food Safety*, 19(6), 3390–3415. <https://doi.org/10.1111/1541-4337.12613>
- Morales-de la Peña, M., Welti-Chanes, J., & Martín-Belloso, O. (2019). Novel technologies to improve food safety and quality. In *Current opinion in food science* (Vol. 30, pp. 1–7). Elsevier. <https://doi.org/10.1016/j.cofs.2018.10.009>
- Najim, N., & Aryana, K. J. (2013). A mild pulsed electric field condition that improves acid tolerance, growth, and protease activity of Lactobacillus acidophilus LA-K and Lactobacillus delbrueckii subspecies bulgaricus LB-12. *Journal of Dairy Science*, 96(6), 3424–3434. <https://doi.org/10.3168/jds.2012-5842>
- Odrizola-Serrano, I., Aguiló-Aguayo, I., Soliva-Fortuny, R., & Martín-Belloso, O. (2013). Pulsed electric fields processing effects on quality and health-related constituents of plant-based foods. In , Vol. 29. *Trends in food science and technology* (pp. 98–107). <https://doi.org/10.1016/j.tifs.2011.10.003>. Issue 2.
- Ohba, T., Uemura, K., & Nabetani, H. (2016). Moderate pulsed electric field treatment enhances exopolysaccharide production by Lactococcus lactis subspecies cremoris. *Process Biochemistry*, 51(9), 1120–1128. <https://doi.org/10.1016/j.procbio.2016.05.027>
- Pakhomova, O. N., Khorokhorina, V. A., Bowman, A. M., Rодаite-Riševičienė, R., Saulis, G., Xiao, S., & Pakhomov, A. G. (2012). Oxidative effects of nanosecond pulsed electric field exposure in cells and cell-free media. *Archives of Biochemistry and Biophysics*, 527(1), 55–64. <https://doi.org/10.1016/j.abb.2012.08.004>
- Park, J. K., Ryu, J. C., Kim, W. K., & Kang, K. H. (2009). Effect of electric field on electrical conductivity of dielectric liquids mixed with polar additives: DC conductivity. *Journal of Physical Chemistry B*, 113(36), 12271–12276. <https://doi.org/10.1021/jp9015189>
- Patato, G., Senatore, B., Donsi, G., & Ferrari, G. (2011). Effect of electric and flow parameters on PEF treatment efficiency. *Journal of Food Engineering*, 105(1), 79–88. <https://doi.org/10.1016/j.jfoodeng.2011.02.007>
- Peng, K., Koubaa, M., Bals, O., & Vorobiev, E. (2020). Recent insights in the impact of emerging technologies on lactic acid bacteria: A review. In *Food research international* (Vol. 137). <https://doi.org/10.1016/j.foodres.2020.109544>
- Puc, M., Kotnik, T., Mir, L. M., & Miklavcic, D. (2003). Quantitative model of small molecules uptake after in vitro cell electropermeabilization. *Bioelectrochemistry*, 60(1–2), 1–10. [https://doi.org/10.1016/S1567-5394\(03\)00021-5](https://doi.org/10.1016/S1567-5394(03)00021-5)
- Qin, B.-L., Barbosa-Canovas, G. V. V., Swanson, B. G. G., Pedrow, P. D. D., Olsen, R. G. G., Qin, B.-L., Barbosa-Canovas, G. V. V., Swanson, B. G. G., Pedrow, P. D. D., & Olsen, R. G. G. (1998). Inactivating microorganisms using a pulsed electric field continuous treatment system. *IEEE Transactions on Industry Applications*, 34(1), 43–50. <https://doi.org/10.1109/28.658715>
- Rajilić-Stojanović, M., & de Vos, W. M. (2014). The first 1000 cultured species of the human gastrointestinal microbiota. *FEMS Microbiology Reviews*, 38(5), 996–1047. <https://doi.org/10.1111/1574-6976.12075>
- Raso, J., & Barbosa-Cánovas, G. V. (2003). Nonthermal preservation of foods using combined processing techniques. In , Vol. 43. *Critical reviews in food science and*

- nutrition (pp. 265–285). Taylor & Francis Group. <https://doi.org/10.1080/104608690390826527>. Issue 3.
- Raso, J., Frey, W., Ferrari, G., Pataro, G., Knorr, D., Teissie, J., & Miklavčič, D. (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 & Emerging Technologies*, 37, 312–321. <https://doi.org/10.1016/j.ifset.2016.08.003>
- Rems, L., & Miklavčič, D. (2016). Tutorial: Electroporation of cells in complex materials and tissue. *Journal of Applied Physics*, 119(20), 201101. <https://doi.org/10.1063/1.4949264>
- Rieder, A., Schwartz, T., Schön-Hözl, K., Marten, S. M., Süß, J., Gusbeth, C., Kohnen, W., Swoboda, W., Obst, U., & Frey, W. (2008). Molecular monitoring of inactivation efficiencies of bacteria during pulsed electric field treatment of clinical wastewater. *Journal of Applied Microbiology*, 105(6), 2035–2045. <https://doi.org/10.1111/j.1365-2672.2008.03972.x>
- Rochat, T., Gratadoux, J.-J., Gruss, A., Corthier, G., Maguin, E., Langella, P., & Guchte, M. (2006). Production of a heterologous nonheme catalase by *Lactobacillus casei*: An efficient tool for removal of H₂O₂ and protection of *Lactobacillus bulgaricus* from oxidative stress in milk. *Applied and Environmental Microbiology*, 72(8), 5143. <https://doi.org/10.1128/AEM.00482-06>
- Sack, M., & Mueller, G. (2016). Scaled design of PEF treatment reactors for electroporation-assisted extraction processes. *Innovative Food Science & Emerging Technologies*, 37, 400–406. <https://doi.org/10.1016/j.ifset.2016.09.005>
- Salmerón, I., Thomas, K., & Pandiella, S. S. (2015). Effect of potentially probiotic lactic acid bacteria on the physicochemical composition and acceptance of fermented cereal beverages. *Journal of Functional Foods*, 15, 106–115. <https://doi.org/10.1016/j.jff.2015.03.012>
- Sanders, M. E., Merenstein, D. J., Reid, G., Gibson, G. R., & Rastall, R. A. (2019). Probiotics and prebiotics in intestinal health and disease: From biology to the clinic. In *Vol. 16. Nature reviews gastroenterology and hepatology* (pp. 605–616). Nature Publishing Group. <https://doi.org/10.1038/s41575-019-0173-3>. Issue 10.
- Sepulveda, D. R., Góngora-Nieto, M. M., Guerrero, J. A., & Barbosa-Cánovas, G. V. (2005). Production of extended-shelf life milk by processing pasteurized milk with pulsed electric fields. *Journal of Food Engineering*, 67(1), 81–86. <https://doi.org/10.1016/j.jfoodeng.2004.05.056>
- Seratić, S., Bugarski, B., Radulović, Z., Dejmek, P., Wadsö, L., & Nedović, V. (2013). Electroporation enhances the metabolic activity of *Lactobacillus plantarum* 564. *Food Technology and Biotechnology*, 51(4), 446–452. <http://www.ftb.com.hr/archives/136-volue-51-issue-no-4/1160-electroporation-enhances-the-metabolic-activity-o-f-lactobacillus-plantarum-564>
- Sevim, S., Topal, G. G., Tengilimoglu-Metin, M. M., Sancak, B., & Kizil, M. (2019). Effects of inulin and lactic acid bacteria strains on aflatoxin M1 detoxification in yoghurt. *Food Control*, 100, 235–239. <https://doi.org/10.1016/j.foodcont.2019.01.028>
- Sharma, P., Tomar, S. K., Goswami, P., Sangwan, V., & Singh, R. (2014). Antibiotic resistance among commercially available probiotics. In *Food research international* (Vol. 57, pp. 176–195). Elsevier. <https://doi.org/10.1016/j.foodres.2014.01.025>
- Tanino, T., Sato, S., Oshige, M., & Ohshima, T. (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.jelstat.2012.01.003>
- Teissie, J. (2017). Involvement of reactive oxygen species in membrane electroporation. In *Handbook of electroporation* (Vol. 1, pp. 473–488). Springer International Publishing. https://doi.org/10.1007/978-3-319-32886-7_40
- Terebiznik, M. R., DE Huerdo, M. S., Pilosof, A. M. R., Cerrutti, P., & Jagus, R. J. (2016). Combined effect of nisin and pulsed electric fields on the inactivation of *Escherichia coli*. *Journal of Food Protection*, 63(6), 741–746. <https://doi.org/10.4315/0362-028x-63.6.741>
- Tsakalidou, E., & Papadimitriou, K. (2011). Stress responses of lactic acid bacteria. In E. Tsakalidou, & K. Papadimitriou (Eds.), *Stress responses of lactic acid bacteria*. Springer US. <https://doi.org/10.1007/978-0-387-92771-8>.
- Vaessen, E. M. J., den Besten, H. M. W., Leito, K. M. N., & Schutyser, M. A. I. (2020). Pulsed electric field pre-treatment for enhanced bacterial survival after drying: Effect of carrier matrix and strain variability. *Innovative Food Science & Emerging Technologies*, 66, 102515. <https://doi.org/10.1016/j.ifset.2020.102515>
- Vaessen, E. M. J., den Besten, H. M. W., Patra, T., van Mossevelde, N. T. M., Boom, R. M., & Schutyser, M. A. I. (2018). Pulsed electric field for increasing intracellular trehalose content in *Lactobacillus plantarum* WCFS1. *Innovative Food Science & Emerging Technologies*, 47, 256–261. <https://doi.org/10.1016/j.ifset.2018.03.007>
- Valic, B., Golzio, M., Pavlin, M., Schatz, A., Faurie, C., Gabriel, B., Teissie, J., Rols, M. P., & Miklavcic, D. (2003). Effect of electric field induced transmembrane potential on spheroidal cells: Theory and experiment. *European Biophysics Journal*, 32(6), 519–528. <https://doi.org/10.1007/s00249-003-0296-9>
- Virtanen, T., Pihlanto, A., Akkanen, S., & Korhonen, H. (2007). Development of antioxidant activity in milk whey during fermentation with lactic acid bacteria. *Journal of Applied Microbiology*, 102(1), 106–115. <https://doi.org/10.1111/J.1365-2672.2006.03072.X>
- WHO/FAO. (2002). *Guidelines for the evaluation of probiotics in food*. 1–11.
- Wouters, P., Alvarez, I., & Raso, J. (2001). Critical factors determining inactivation kinetics by pulsed electric field food processing. *Trends in Food Science & Technology*, 12(3–4), 112–121. <https://www.sciencedirect.com/science/article/pii/S092422440100067X>.
- Yadav, R., Kumar, V., Baweja, M., & Shukla, P. (2017). Gene editing and genetic engineering approaches for advanced probiotics: A review. *Critical Reviews in Food Science and Nutrition*, 1–12. <https://doi.org/10.1080/10408398.2016.1274877>
- Yang, J. H., Dwyer, D. J., Ting, A. Y., Ralifo, P. S., Khalil, A. S., Takahashi, N., Ye, J. D., Schwarz, E. G., Collins, J. J., Walker, G. C., MacDonald, I. C., Belenky, P. A., Martell, J. D., Pati, M., Chan, C. T. Y., Allison, K. R., Vercruyse, M., Lobritz, M. A., & Braff, D. (2014). Antibiotics induce redox-related physiological alterations as part of their lethality. *Proceedings of the National Academy of Sciences*, 111(20), E2100–E2109. <https://doi.org/10.1073/pnas.1401876111>
- Zheng, J., Wittouck, S., Salvetti, E., Franz, C. M. A. P., Harris, H. M. B., Mattarelli, P., O’toole, P. W., Pot, B., Vandamme, P., Walter, J., Watanabe, K., Wuyts, S., Felis, G. E., Gänzle, M. G., & Lebeer, S. (2020). A taxonomic note on the genus *Lactobacillus*: Description of 23 novel genera, emended description of the genus *Lactobacillus* Beijerinck 1901, and union of *Lactobacillaceae* and *Leuconostocaceae*. *International Journal of Systematic and Evolutionary Microbiology*, 70(4), 2782–2858. <https://doi.org/10.1099/ijsem.0.004107>