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# Pulsed electric field effects on inactivation of microorganisms in acid whey

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ABSTRACT

Prospects of pulsed electric field technology application on acid whey concentrate pretreatment were analyzed. Stationary and flow pre-treatment systems were combined with different treatment parameters: electric field strength ($E$ = 39 kV/cm, 95 kV/cm, 92 kV/cm), pulse duration ($\tau$ = 60 ns, 90 ns, 1000 ns) and pulse number ($pn$ = up to 100 pulses). Isolates of *Saccharomyces* sp. and *Lactobacillus* sp. were predominant in concentrate. Significant non-thermal inactivation effect was achieved after PEF treatment. Exposure to short pulses selectively inactivated yeast cells, as a result PEF technology can be applied for low-energy acid whey processing.

## 1. Introduction

Acid whey, also known as sour whey is a byproduct of manufacturing process of acidic dairy products such as cottage cheese or strained yoghurt. Nowadays consumers pay a major attention to food quality as well as health benefits therefore healthier options, e.g., cultured buttermilk and yoghurt are often selected for healthy diet. As a result, food manufacturers are interested in possibilities to reprocess production byproducts such as sweet and acid whey (Chen et al., 2016; Helen Shiphrah et al., 2013; Panesar et al., 2010; Pescuma et al., 2008). Acid whey contains significant amounts of lactose, galactose, calcium phosphate and lactic acid. These components can be further reused in manufacturing of functional foods as well as in improving their color, texture or flavor (Alsaed et al., 2013; Lievore et al., 2015; Pescuma et al., 2010; Yang and Silva, 1995). Moreover, whey contains unique bioactive compounds, e.g., α-lactalbumin, β-lactoglobulin, glycomacropeptide, lactoferrin, lactoperoxidase, lysozyme, growth factors, cytokines, serum albumin, immunoglobulins which can be used as supplements in their active form (Park and Haenlein, 2013). It was previously shown that low-fat dairy products can be beneficial in certain human disease management, e.g., hypertension, type 2 diabetes, cardiovascular diseases, reduce oxidative and inflammatory stresses, facilitate obesity control (Aihara et al., 2005; Baer et al., 2011; McGregor and Poppitt, 2013; Mills et al., 2011). Some proteins are suggested to be active components regulating intestinal enzymes and hormones that play a role in satiety (Luhovyy et al., 2007) and digestion, other proteins are possible mediators for prevention of certain cancer types, e.g., colon cancer (Parodi, 2007).

In addition to nutritious compounds, whey also contains the microorganisms. Some of them are beneficial for the human gut microbiota, yet others can be harmful and cause food poisoning. Microorganisms may also decrease product shelf life. Seeking an effective assimilation of whey beneficial properties, it is important to apply specific concentration and pasteurization methods. Whey concentrates are usually contaminated, therefore they should be pasteurized and processed immediately. During pasteurization food products are treated with heat to eliminate pathogens and extend shelf life. On the downside, pasteurization affects physicochemical properties of food including changes in appearance, flavor and quality of proteins (Qi et al., 2015; Rynne et al., 2004), fats, minerals, vitamins. One of mentioned chemical changes is formation of advanced glycation products (Uribarri et al., 2010) which are related to diabetes and several chronic diseases (Clarke et al., 2016). Since thermal pasteurization of acid whey leads to secondary straining, i.e., formation of insoluble protein aggregates, non-thermal pasteurization methods are on demand.

Some methods such as centrifugation and microfiltration, high-pressure treatment, ultra-sonication, ultraviolet light, ionizing radiation were suggested and, in some specific cases, are even applied in production of dairy foods. Yet whey is often considered as a byproduct and no specific treatment is applied. Pulsed electric field (PEF) is an emerging technology in dairy product industry (McAuley et al., 2016). PEF technology has been previously applied for pasteurization of various liquid food products (Buckow et al., 2013; Milani et al., 2015; Min

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et al., 2003). It has also been applied in combination with other pasteurization methods and/or substances for improved efficiency (Caminiti et al., 2011; Pataro et al., 2014a; Saldaña et al., 2014). When a cell in a suspension or a tissue is exposed to electric field, electric potential difference across insulating membrane changes and it may lead to membrane integrity loss. Efficacy of PEF treatment usually depends on the following parameters: electric field strength (E), pulse length (τ), pulse number (pn) and pulse shape (Schoenbach et al., 2015; Pakhomov et al., 2015; Weaver and Chizmadzhev, 1996). PEF based pasteurization technologies inactivate the microorganisms by irreversibly permeabilizing the membranes.

In this study, we aim to elucidate PEF effects on i) acid whey solution, ii) inactivation efficiency, iii) effects on different microorganisms in acid whey concentrate and iv) energy-related aspects of the treatment.

## 2. Materials and methods

### 2.1. Whey composition

Acid whey was collected and concentrated at a dairy company (Pieno žvaigždės, Lithuania) during cottage cheese production process. Milk was incubated with a mixture of mesophilic cultures Ceska-star G-700 (CSK, Netherlands) until whey separated from cottage cheese. Whey composition measurements were performed by the manufacturer according to international standards: ISO 9622:2013 (IDF 141:2013). The composition (except the content of viable microorganisms) remained constant prior and after the PEF treatment (Table 1.) and this is in accordance with data from previous milk pasteurization research (Al-Hilphy, 2012). Freshly prepared whey concentrate contained bacterial ($> 10^6$ cells per mL) and yeast ($< 10^4$ cells per mL) cells. During storage at 4 °C, yeast cell count reached approximately $10^5$ cells per mL. The PEF treatment was performed at room temperature.

Conductivity was measured with a conductivity meter AD3000 AC/TDS (ADWA, Hungary). Log reduction was evaluated by a plate counting method on a solid MRS media (10 g/L peptone from casein (Merck KGaA, Germany), 10 g/L meat extract (Merck KGaA, Germany), 4 g/L yeast extract (Merck KGaA, Germany), 20 g/L glucose (Merck KGaA, Germany), 2 g/L disodium hydrophosphate (SIGMA-ALDRICH, USA), 1 g/L Tween 80 (Merck KGaA, Germany), 2 g/L diammonium hydrocitrate (SIGMA-ALDRICH, USA), 5 g/L sodium acetate (SIGMA-ALDRICH, USA), 0.2 g/L magnesium sulphate (SIGMA-ALDRICH, USA), 0.04 g/L manganese sulphate (Merck KGaA, Germany), 12 g/L agar (Merck KGaA, Germany).

### 2.2. Evaluation of viability

Efficacy of microbial inactivation was evaluated by log reduction (LR), defined by equation:

$$LR = -\log\left(\frac{N}{N_0}\right). \quad (1)$$

N and $N_0$ are numbers of colony forming units in treated and untreated suspensions respectively. Experimental data of log reduction was analyzed by mathematical model (Mafart et al., 2002):

$$LR = \left(\frac{pn}{pn_{red1}}\right)^{\rho}. \quad (2)$$

**Table 1**
Chemical composition of acid whey.

| Fat, % | Protein, % | Dry solids, % | Conductivity, mS/cm | pH |
|---|---|---|---|---|
| 0.026 ± 0.005 | 2.26 ± 0.02 | 17.78 ± 0.05 | ~8.33 | 4.54 ± 0.01 |

**Table 2**
List of primers.

| Primer | Primer sequence (Sanchez and Sanz, 2011; White et al., 1990) |
|---|---|
| 27F | 5′-GAGAGTTTGATCCTGGCTCAG-3′ |
| 1495R | 5′-CTACGGCTACCTTGTTACGA-3′ |
| ITS1 | 5′-TCCGTAGGTGAACCTGCGG-3′ |
| ITS4 | 5′-TCCTCCGCTTATTGATATGC-3′ |

Eq. (2) includes data from our experiments (LR, pn) and computed generated parameters ($pn_{red1}$, ρ). $Pn_{red1}$ describes pulse number required viable cell count reduction by one log and ρ is the parameter of curve shape. Convex curves are described by $\rho > 1$, concave curves are described by $\rho < 1$, $\rho = 1$ represents linear change. Data lines approximated with this model are survival curves.

### 2.3. Microorganism identification

To identify species of isolated microorganisms, 16S rRNA gene sequence from bacterial isolate (Sanchez and Sanz, 2011) and highly variable Internal Transcribed Spacer (ITS) regions of fungal ribosomal DNA from yeast isolate (White et al., 1990) were amplified and analyzed. The amplification reactions were performed by a colony PCR method. Two pairs of primers were selected (Table 2): 27F and 1495R for 16S rRNA gene amplification, ITS1 and ITS4 for amplification of ITS region sequences. All primers were synthesized by Metabion (Germany).

Microorganism isolation was performed on chloramphenicol glucose agar (Sharlau Chemie S.A., Spain) and plate count skim milk agar (Merck KGaA, Germany) growth media. Single dominant bacterial and yeast isolates were then separated and cultured overnight at 30 °C on MRS agar and YPD agar plates (20 g/L peptone from casein (Merck KGaA, Germany), 10 g/L yeast extract (Merck KGaA, Germany), 20 g/L glucose (Merck KGaA, Germany), 12 g/L agar (Merck KGaA, Germany) respectively in INCULine (VWR, USA) incubator. Single colonies from overnight cultures of each isolate were re-suspended in 50 μL of ultrapure water and mixed. Cells were lysed at 95 °C for 10 min. 2 μL of each lysate was added to prepared PCR reaction mixtures. 50 μL PCR reaction mixture contained 2 μL of template DNA, 1.25 U of DreamTaq DNA Polymerase (Thermo Scientific, Lithuania), 5 μL of 10× Dream Taq buffer, 1 μM of forward and 1 μM of reverse primer, 4 mM of $MgCl_2$, 0.4 mM of each of four dNTPs. Amplification was performed under the following conditions: 2 min of initial denaturation at 98 °C; 35 cycles of denaturation (30 s at 96 °C), annealing (30 s at 51 °C for samples with 27F and 1495R primers, 54 °C for samples with ITS1 and ITS4 primers), extension (1 min at 72 °C) and final extension at 72 °C for 10 min. Amplification products were analyzed on 0.9% (wt/vol) agarose gel, purified using a GeneJET Gel Extraction and a DNA Cleanup Micro Kit (Thermo Scientific, Lithuania). Purified fragments were sequenced by BaseClear B.V. (The Netherlands).

The consensus sequences were obtained using BioEdit software package version 7.0.5 (North Carolina State University, Raleigh, USA) and compared to those in the NCBI database (http://www.ncbi.nlm.nih.gov/BLAST/). Yeast isolate was ascribed to the species showing the highest matched sequence identity. In case of bacterial isolate, sequences showing highest identity were chosen to generate phylogenetic tree and determine evolutionary relationships. Sequences were aligned using ClustalW method. The phylogenetic tree was generated using neighbor-joining method from phylogenetic distances calculated by the Jukes-Cantor method with MEGA v. 7 software (Pennsylvania State University, USA). Bootstrap values were obtained after 1000 replicates.

### 2.4. PEF generation systems

Two different PEF generation systems were used for acid whey treatment. Square-wave monopolar pulses (duration of 60 and 90 ns) were formed by a generator based on an optically triggered spark-gap switch. In these experiments, the coaxial line (75 Ω) was charged up to 23 kV resulting in generation of electric field E = 95 kV/cm across a cuvette with resistance of approximately 50 Ω. 60 and 90 ns pulses generated 0.11 J and 0.16 J of energy respectively. A schematic diagram of the experimental setup and working parameters were previously described by Balevicius et al., 2013. A cuvette with cylinder shaped stainless-steel electrodes (diameter D = 5.3 mm), spaced at a distance of d = 0.75 mm, was used in stationary system (SS). Another experimental set-up involved a seven-stage Marx generator operating in free-running mode up to 10 Hz. In these experiments, the Marx generator was charged to 1.8 kV (threshold voltage for used spark-gaps) and discharged into the cuvette with liquid samples. A unipolar exponential damped pulse of about 1 μs duration (full width at half maximum) was achieved. For flow system (FS), a politetrafluorethylene (PTFE) cell with stainless steel electrodes was used. Acid whey was delivered to the treatment chamber by MasterFlex L/S (Cole-Parmer, USA) peristaltic pump at a flow rate of 2 mL/min. The active area of the electrodes was 130 $mm^2$. The distance between the plates of the electrodes was 2 mm. 0.26 mL of cell suspension was placed in a cuvette. Number of pulses per volume was adjusted by changing pulse frequency and flow speed. Pulse frequency was changed by adjusting a power supply voltage. In a stationary cuvette experiments, the electrical energy of about 1.8 J was produced with each pulse. Electric field of about 92 kV/cm was generated across a cuvette. In flow system experiments, the energy was about 2.6 J per pulse when electric field was approximately E = 39 kV/cm. To determine PEF effects on whey only, microorganisms were separated by centrifugation for 20 min at 20,000*g* (Jouan KR25i, Thermo Fisher Scientific, USA). Fig. 1 shows a rough experimental scheme and a summary of treatment parameters.

## 3. Results and discussion

### *3.1. Indirect effects on the viability of microorganisms*

Research was started by analyzing indirect PEF effects on the viability of microorganisms, i.e., generation of toxic reactive oxygen species (Drogui et al., 2001) as well as electrolysis and electrode corrosion products (Pataro et al., 2014a, b; Saulis et al., 2015). To test PEF induced chemical effects, whey was centrifuged, and the supernatant was exposed to PEF (τ = 1 μs; E = 39 kV/cm; pn = 32). The pellet was resuspended immediately after PEF treatment and subsequently plated on solid media. It was shown that LR increase up to 0.287 ± 0.094 can be attributed to indirect effects. Some of LR was achieved by centrifugation (LR = 0.159 ± 0.076) that could have caused formation of aggregates as well as cell death due to shear forces and, therefore, lead to decrease in the number of colony-forming units. It was concluded that LR of approximately 0.128 was caused by PEF generated chemical toxicity.

It is known that thermal treatment may lead to permanent inactivation of microorganisms. To test thermal impact on LR values, acid whey was incubated in water bath for 2 min and plated on solid medium immediately afterwards. It was observed that the temperature of 40 °C can act as a signal for proliferation or induce aggregate dispersion, therefore, lowering LR values (−0.046 ± 0.044). The viability of microorganisms in a sample that was incubated at 50 °C was similar to the control sample. The highest increase in LR (0.196 ± 0.066) was observed after incubating the sample at 60 °C. The conclusion was drawn that the temperature does affect the viability of microorganisms, therefore, to distinguish PEF effects from those caused by thermal pasteurization, it is important to monitor the sample temperature.

### *3.2. Electroporation in flow chamber*

For further whey processing, a continuous flow system was used (E = 39 kV/cm, τ = 1 μs). The viability of microorganisms was shown to be affected by the velocity of a fluid flow (data not presented): microorganisms were less viable at higher velocity rates. Hence, the flow speed was set to a constant 2 mL/min value that did not influence the viability of microorganisms. Pulse number was adjusted by changing frequency of pulses (up to 32 pulses per volume). Higher LR values were observed with raise in pulse number and the results are shown in Fig. 2. The highest value of microbial LR was achieved after 32 pulses (LR = 1.14 ± 0.08). Whey exposure to electric field also caused the rise of its temperature and it was dependent on pulse number applied. The highest temperature ($T$ = 50.10 ± 4.05 °C) was measured after 32 pulses, however, it is too low for thermal pasteurization. Therefore, it can be presumed that LR in our experiments was achieved by direct as well as indirect effects of PEF treatment but not by thermal effect only. It was concluded that PEF can be effectively applied for non-thermal acid whey concentrate pasteurization in the flow system. Since LR values changed when the number of pulses was increased, it suggests that the results could also be improved by changing E and/or τ.

### *3.3. Microorganism isolation*

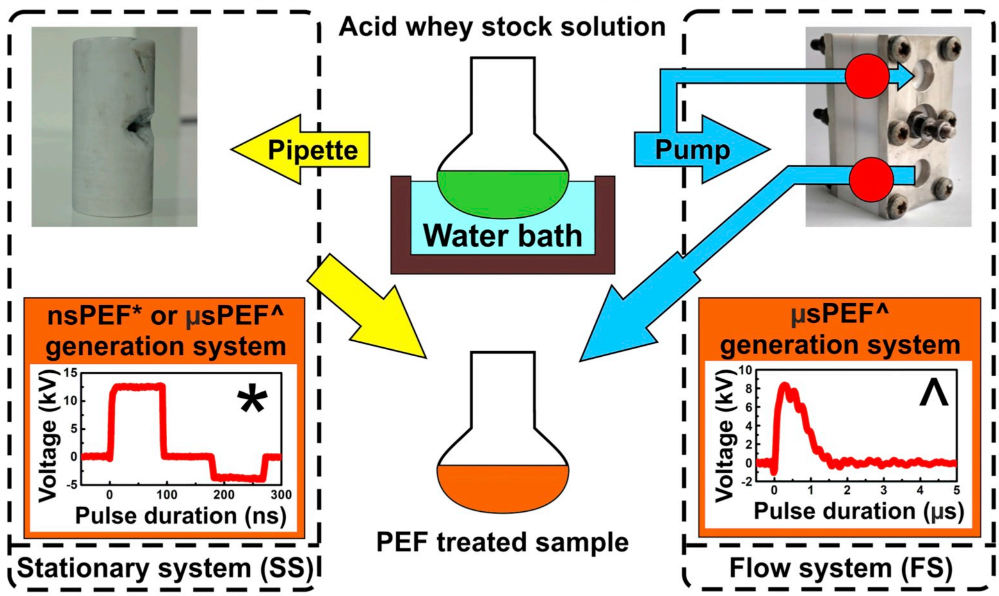
To evaluate PEF effects on specific microorganisms, isolation was performed. Two dominant microorganisms were chosen for identification by 16S rRNA gene and ITS region sequencing. The analysis of yeast ITS region sequences resulted in 99% match with the sequences of *Saccharomyces cerevisiae* strains published in NCBI database. The BLAST analysis showed significant bacterial isolate identity (> 98%) to *Lactobacillus* genus bacteria. Phylogenetic analysis indicated that a species most closely related to bacterial isolate is *L. rhamnosus* (Fig. 3). In general, bacteria from *Lactobacillus* genus are recognised as safe and are designated the GRAS status (Salminen et al., 1998). *L. rhamnosus* has significant positive impact on the intestinal microbiota and epithelial barrier functions (Johnson-Henry et al., 2008), it also relieves diarrhoea (Guandalini et al., 2000), displaces the pathogenic bacteria (Vesterlund et al., 2006), stimulates hosts' immune system (Saxelin et al., 2005) and plays a role in maintaining homeostasis of intestinal epithelial cells (Yan and Polk, 2002). Furthermore, *L. rhamnosus* strains act as probiotics for prevention of oral cavity disorders (Ahola et al., 2002) and respiratory infections (Hojsak et al., 2010).

PEF effects on each microorganism were analyzed separately. For simplicity purposes, PEF parameters are shown as $LR_{microorganism\_pulselength(ns)\_fieldstrength\_pulsenumber}$.

### *3.4. Selective pasteurization*

PEF effects on acid whey microorganisms were further analyzed in a static treatment chamber. The suspension was poured into a cuvette and exposed to various numbers of electric field pulses (pn = 1, 5, 10, 30, 50, 70, 100) of different pulse length (τ = 60 ns, 90 ns, 1000 ns) and field strength (E = 95 kV/cm, 92 kV/cm). When shorter pulses < 1000 ns were applied, minimal increase of bacterial LR values was observed ($LR_{B\_60\_95\_100}$ = 0.08 ± 0.01 and $LR_{B\_90\_95\_100}$ = 0.08 ± 0.01; Fig. 4). However, higher sensitivity of yeast cells was observed ($LR_{Y\_60\_95\_100}$ = 0.99 ± 0.08, $LR_{Y\_90\_95\_100}$ = 1.34 ± 0.36) and it suggests that PEF can be used for selective pasteurization. At pulse lengths of 60 ns and 90 ns yeast cells were at least ten times more sensitive to PEF treatment than bacterial cells. After whey exposure to longer pulses LR values were higher, but the difference of PEF effects on yeast and bacterial cells was less significant ($LR_{Y\_1000\_92\_50}$ = 1.79 ± 0.12; $LR_{B\_1000\_92\_50}$ = 1.47 ± 0.05).

Shorter pulses may cause selective effects by targeting mitochondria in yeast, yet the difference in size of the cells can be an alternative explanation of our results (Schoenbach et al., 2004). It was previously shown




| PEF parameters | SS + nsPEF | SS + µsPEF | FS + µsPEF |
|---|---|---|---|
| Electric field | 95 kV/cm | 92 kV/cm | 39 kV/cm |
| Flow rate | – | – | 2 mL/minute |
| Residence time | – | – | ~8 s |
| Pulse frequency | 1 Hz | 1 Hz | 1-4 Hz |
| Pulse width | 60 ns, 90 ns | 1 µs | 1 µs |
| Pulse number | 1-100 | 1-50 | 8-32 |
| Treatment time | up to 9 µs | up to 50 µs | 12-48 µs |
| Energy input per pulse | 0.11 J, 0.16 J | 1.8 J | 2.6 J |

**Fig. 1.** Scheme of PEF treatment setup and parameters. Residence time – is the time which specimen spends in PEF treatment area in flow system; treatment time – is the duration of an actual exposure to PEF of the specimen; * – pulse shape formed by nsPEF generation system; ^ – pulse shape formed by µsPEF generation system; ● – termocouples.

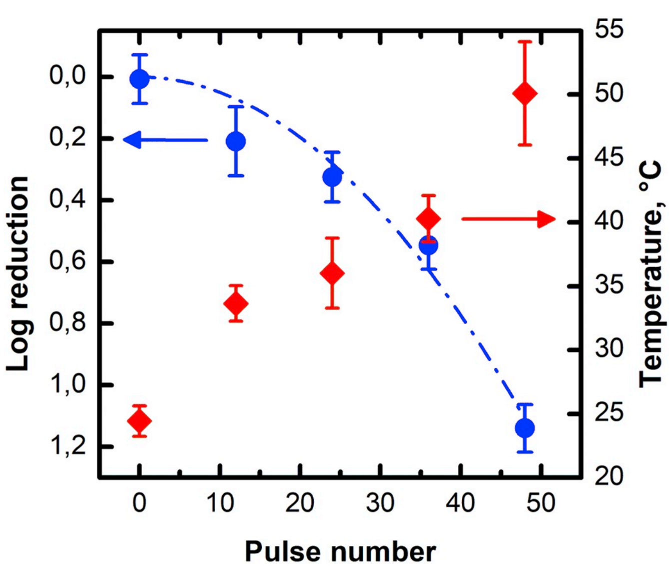



**Fig. 2.** Log reduction of total colony forming unit number (blue circles) and temperature (red diamonds) change dependence on pulse number in flow system (FS). Dash-dotted line represents survival curve by data fitting using Eq. (2) (Adj, R-square = 0,96,576). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

that pulse duration of 90 ns can induce apoptosis in yeast cells – number of colony forming units (CFU) of *S. cerevisiae* cells decreased ($CFU_{Y\_90\_96\_1}$ = 62 ± 6%, $CFU_{Y\_90\_96\_5}$ = 59 ± 5%) (Simonis et al., 2017). If we present whey $LR_Y$ results as CFU, where 100% represents number of yeast colony forming units in untreated samples, we get similar decrease values ($CFU_{Y\_90\_95\_1}$ = 63 ± 29%, $CFU_{Y\_90\_95\_5}$ = 66 ± 16%). However, cell treatment medium was different in both studies in terms of acidity and osmolarity. It suggests that acidity and osmolarity were secondary factors affecting yeast cell viability during/after PEF treatment (Saldaña et al., 2009). PEF effects on yeast and bacteria in same media were studied previously (Puértolas et al., 2009). Puértolas et al. demonstrate similar results: *Lactobacillus* strains were more resistant to PEF treatment than *S. bayanus*, but no significant selectivity was detected. Puértolas' team applied electric field strengths ranging from 16 to 31 kV/cm, number of pulses ranged from 0 to 100, specific energies per pulse ranged from 1.02 to 3.77 kJ/kg, pulse frequency was 1 Hz. Treatment of microorganisms in wine media was more energy efficient probably due to lower conductivity (~4 times lower than whey). In both of our studies (flow and stationary) increase in LR (for all whey microorganisms) was dependent on pn, τ and E, resulting in greater values with higher pn, longer τ and stronger E.

### 3.5. Efficiency analysis

Efficiency of PEF treatment was evaluated by calculating energy

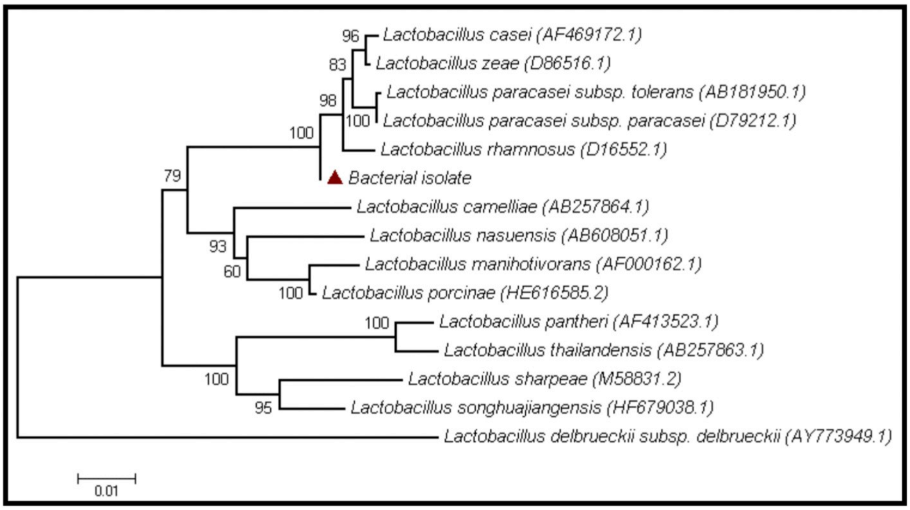



**Fig. 3.** Phylogenetic tree highlighting the position of bacterial isolate relative to other *Lactobacillus* species. The tree was constructed based on 16S rRNA gene sequences. GenBank accession numbers are presented in parentheses. The scale bar indicates an estimated 0.01 nucleotide change per nucleotide position. Horizontal distances correspond to genetic distances; vertical distances are arbitrary. Numbers at the nodes indicate the bootstrap values on neighbor-joining analysis.

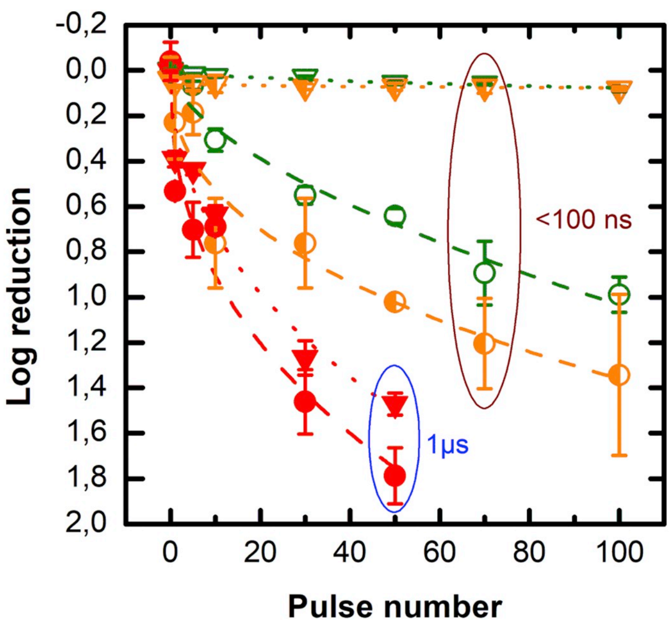



**Fig. 4.** Selective inactivation of acid whey concentrates in stationary system. Circles – yeast cells; triangles – bacteria; hollow symbols, green color – E = 95 kV/cm, $\tau$ = 60 ns; half-filled symbols, orange color – E = 95 kV/cm, $\tau$ = 90 ns; filled symbols, red color – E = 92 kV/cm, $\tau$ = 1 μs; dashed and dotted curves were obtained by fitting with Eq. (2) for yeast and bacteria cells respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

**Table 3**
Log reduction analysis through model assessed data. SS – stationary system, FS – flow system, Y – yeast cells, B – bacteria, T – yeast and bacteria.

| PEF system | SS | SS | SS | SS | SS | FS |
|---|---|---|---|---|---|---|
| Microorganisms | Y | Y | Y | B | T | T |
| E, kV/cm | 95 | 95 | 92 | 92 | 92 | 39 |
| Pulse width | 60 ns | 90 ns | 1 μs | 1 μs | 1 μs | 1 μs |
| Pnred1 | 94.37 | 47.34 | 12.75 | 20.77 | 19.69 | 30.36 |
| ρ | 0.61 | 0.41 | 0.41 | 0.46 | 0.45 | 1.98 |
| Energy per 1 LR, kJ/L | 189.52 | 137.63 | 417.27 | 679.75 | 644.4 | 303.6 |

required for log reduction (Table 3). Pnred1 and ρ values were calculated from eq. (2). In order to increase $LR_Y$ by one, when $\tau$ = 90 ns and E = 95 kV/cm, 137.63 kJ/L of energy was consumed. Same $LR_Y$ was achieved after exposure of whey to multiple pulses when $\tau$ = 1 μs and E = 92 kV/cm. Such treatment required 417.27 kJ/L of energy. It was shown that selective PEF treatment is an energy efficient process. In addition, overall microbial inactivation in flow system demonstrated significantly higher energy efficiency compared to stationary system (303.6 kJ/L in flow system and 644.4 kJ/L in stationary system when 1 μs pulses with 92 kV/cm electric field strength were used). In order avoid electrical discharges, extra liquid was added into stationary treatment chamber. Untreated volume could have caused lower efficiency.

Another parameter obtained from the model was ρ which describes the shape of the curve (Cebrián et al., 2016; Mafart et al., 2002). The only convex curve (ρ = 1.98) was observed in log reduction pattern of flow system. This could mean that not all cells were affected equally (due to shear forces, turbulence inside treatment chamber, uneven flow) in the system and specific number of pulses should be applied to compensate this phenomenon. In stationary system all curves were concave with ρ < 1 suggesting that most of the cells were affected during the treatment. Some cells remained untreated possibly due to uneven electric field distribution, cell size difference or involvement in aggregates. In theory, if we multiply applied pulses by factor ten, LR in stationary system will increase up to ~4 times. LR would reach plateau due to our particular setup where some of the cells remain outside the treatment chamber. On the other hand, in FS, tenfold raise in pulse number, theoretically would result in ~95-fold higher LR suggesting that in our setup some cells remained untreated, but they would be if exposed to higher number of pulses. It was concluded that electric field pulses of nanosecond duration can be used for selective and energy efficient pasteurization of the product, yet it is very important to optimize treatment parameters as well as treatment chamber.

## 4. Conclusions

We analyzed prospects of PEF application on acid whey concentrate in flow and stationary treatment chambers. We demonstrate that a significant microbial reduction can be achieved ($LR_{T\_1000\_92\_50}$ = 1.47 ± 0.05). As the current flows through the chamber during PEF application, the temperature of the sample increases and toxic compounds are potentially produced. In flow system, $LR_{T\_1000\_39\_60}$ = 1.14 ± 0.08 was achieved with $\Delta T$ = 27.41 ± 3.26 °C. Temperature raise was not high enough to significantly affect LR. This suggests that PEF could be applied as a non-thermal pasteurization technology and it could be used for treatment of heat sensitive products. To lower the temperature of the system and to achieve log reduction of higher values, multiple treatment chambers with different PEF parameters could be used in combination with cooling lines.

Efficiency of electropermeabilization is not significantly affected by pulse shape and primarily depends on time during which the amplitude of pulse exceeds a certain critical value (Kotnik et al., 2003). In our study, we showed that PEF pulses of different duration can result in selective LR of different cell types (in our case yeast and bacterial cells) making it applicable for food processing technologies where beneficial bacteria are required to be preserved. On the other hand, yeast cells usually play an undesirable role as spoilage microorganisms and preferably should be inactivated (Fleet and Mian, 1987). Yeast can survive in acidic and/or cool environments and are resistant to physicochemical stresses, therefore, they can grow in manufacturing lines of certain dairy products (e.g., yoghurt) (Jakobsen and Narvhus, 1996). PEF technology enables pre-treatment and non-thermal pasteurization of various food products including cottage cheese, yoghurt and acid whey preserving the beneficial strains of bacteria for further fermentation.

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