Pulsed electric field treatment for microbial decontamination of heat-sensitive foods

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1. Abstract

Pulsed electric field is a promising preservation technique for heat sensitive liquid food. In this study the effect of PEF treatment on the reduction of *L. monocytogenes* was determined in phosphate buffer and milk. A three to four log unit reduction of *L. monocytogenes* in phosphate buffer was achieved by either combining PEF treatment with 55°C heat treatment or by applying four repeated PEF treatments with chilling in between the treatments. In milk the reduction was 5 log CFU/ml. The PEF treatment had an effect on the *rpoB* expression indicating that the bacteria´s protein synthesis can be disturbed by PEF treatment. A difference was obtained between low and high energy input.

2. Introduction

Treatment by electric pulses has useful applications in biotechnology (1), medicine (2), biofouling control and sludge disintegration (3, 4) and as a preservation technique for liquid food (5, 6). Pulsed Electric Field (PEF) is a non-thermal decontamination technique and is today recognised as a promising alternative method to heat treatment of food. One major advantage with PEF treatment over heat preservation is that flavour compounds and nutrients in foods are better preserved (7) and therefore the possibility to develop safe food with fresh-like properties may be accomplished. Examples of food that are of high interest for PEF preservation treatment are juices, dressings, sauces, egg and smoothies.

During PEF processing, the bacteria are exposed to an electric field greater than 20 kV/cm under short time (μs) resulting in membrane disruption and leakage of intracellular components. Dependent on PEF treatment the exposed bacteria are killed or just injured; in the later case the induced pores in the cell membrane will reseal. During this time for recovery the bacteria are sensitive and stressed; a condition of the bacteria that can be used in combination with other preservation techniques in order to achieve bacterial inactivation.

The sensitivity towards PEF varies between different types of bacteria and also with the size of a bacterial cell. Gram positive, small cells such as *Listeria monocytogenes* are very difficult to inactivate by PEF (5, 8). This pathogenic bacterium is an important target when optimizing PEF conditions for in particular ready-to-eat foods that are consumed without prior heat treatment.
The inactivation of microorganisms is influenced by several factors such as electrical field strength, number of pulses, pulse length and resulting energy input. The higher the energy input, the higher temperature increase during the treatment. When treating heat sensitive products, it is essential to balance the energy input by choosing appropriate combinations of electrical parameters leading to sufficient bacterial inactivation.

In the study, the effect of PEF treatment on the reduction of *L. monocytogenes* was determined for conditions that may be used for heat-sensitive foods such as milk. The aim was to find operating conditions where this highly PEF-resistant bacterium could be reduced without increasing the temperature during the treatment above 55°C. Furthermore, the activity of the *rpoB* was analysed before and after PEF treatment in order to better understand how the bacterium cell respond to PEF treatment. The *rpoB* gene is a housekeeping gene that is vital for the survival of the cell. The gene codes for the β-subunit on the RNA polymerase that is involved in the transcription of the protein synthesis (9).

3. Materials and Methods

3.1 Pulsed Electric Field

The PEF treatment chamber used in the study is developed at SIK. The design of the chamber provides that a homogenous electric field is applied over a defined volume.
Four different PEF treatments were used throughout the study; 27kV/cm, 6μs, 5 pulses; 35kV/cm, 6μs, 3 pulses; 35kV/cm, 6μs, 5 pulses and 32kV/cm, 10μs, 5 pulses. The inlet temperature was 5°C and depending on the PEF treatment the temperature increase during the treatments varied (Figure 1). The temperature increase during the treatments was 15, 15, 32 and 45°C, respectively. The sample temperature was maximum 55°C.

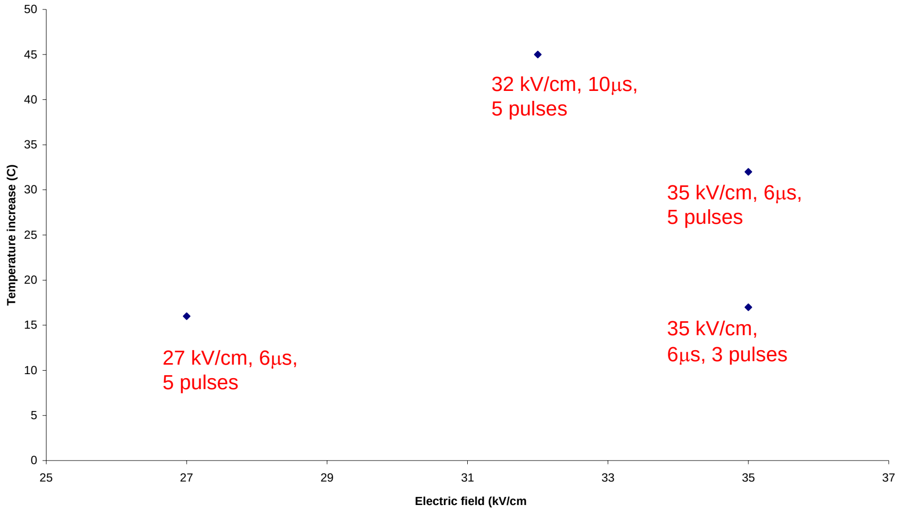

Figure 1. The temperature increase during the PEF treatment in phosphate buffer. Each dot represents different levels of electric field, pulse width and number of pulses applied. The inlet temperature was 5°C and the temperature increase during the treatments was 16, 17, 32 and 45°C respectively.

PEF treatments were performed as (i) single treatment, (ii) treatment in repeated cycles, (iii) single treatment in combination with a subsequent heat treatment at 55°C, and (iv) single treatment in combination with 0.1 % potassium sorbate. All PEF treatments were performed in a phosphate buffer with a conductivity of 5 mS/cm. A comparison of the results performed in phosphate buffer was made by repeating some experiment in low fat milk (0.1%) with the same conductivity.

3.2 Bacteria and microbial analysis

Two strains of *L. monocytogenes* was used; Scott A and UNIBO 56 Ly. The bacterial count (CFU) was determined by plate count on either TSA (phosphate buffer) or Palcam (milk) agar at 30°C for 48 hours. The time to growth (TTG) for *L. monocytogenes* post a PEF treatment was defined as 0.5 log CFU/ml higher than before PEF treatment.

3.3 *rpoB* expression

The activity of *rpoB* in *L. monocytogenes* Scott A was determined using quantitative reverse transcription PCR. Total RNA was extracted before PEF treatment and 6, 15 and 20 minutes after the PEF treatment. Total RNA was extracted using RNAprotect Bacteria

Reagent (QIAGEN, Venlo, Netherlands) and RNAqueous kit ((Ambion, Austin, Tex). After DNase treatment the RNA was translated into cDNA using High Capacity cDNA Archive Kit (Applied Biosystems, Foster City, CA) which includes random primers. DNA amplification was performed using AmpliTaq Gold DNA polymerase in realtime PCR 7500 (Applied Biosystems). Relative quantification was determined using *16S rRNA* as a reference gene and all calculation were performed according to Pfaffl et al. 2001 (10).

4. Results and discussion

4.1 Single and repeated PEF treatments

Reduction of *L. monocytogenes* Scott A in phosphate buffer during up to 4 cycles of PEF treatment is shown in Figure 2 for treatments with 32kV/cm, 10µs, 5 pulses; 35kV/cm, 6µs, 3 pulses; 35kV/cm, 6µs, 5 pulses and 27kV/cm, 6µs, 5 pulses. The reduction of *L. monocytogenes* was highest at the highest energy input applied (32kV/cm, 10µs, 5 pulses), irrespective the number of repeated PEF treatments. The inactivation increased for each PEF cycle applied. After four PEF treatments of 32kV/cm, 10µs, 5 pulses, a 3 log reduction of *L. monocytogenes* Scott A was achieved, which is to compare with a single PEF treatment that gave 1 log reduction (Figure 2). A similar reduction was obtained for *L. monocytogenes* UNIBO 56 Ly as for strain Scott A, i.e. no strain variation was detected.

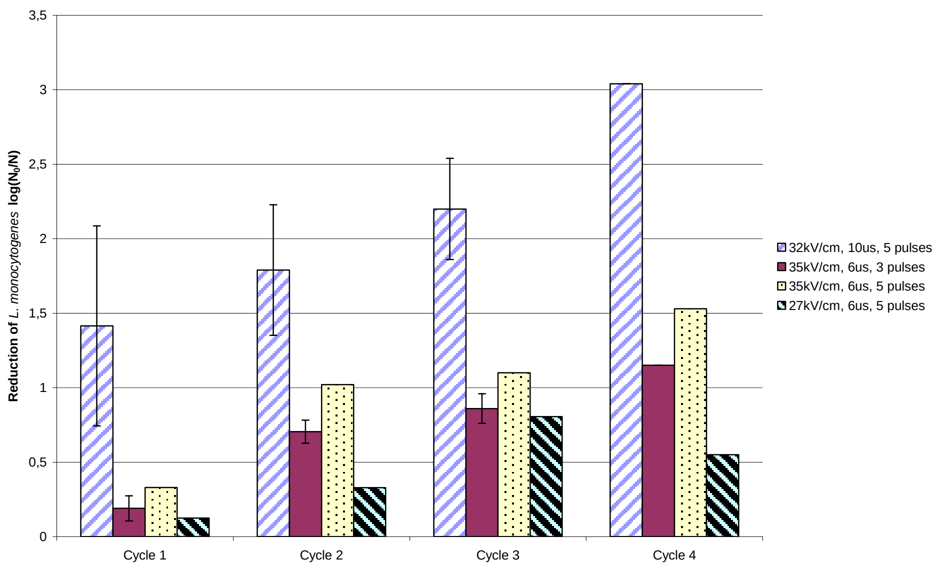

Figure 2. Reduction of L. monocytogenes Scott A after different cycles of PEF treatments in phosphate buffer.

For heat sensitive products a satisfactory reduction of *L. monocytogenes* may be achieved by applying repeated PEF treatments with chilling between the treatments and maintaining the product temperature below 50°C.

4.2 PEF and mild heat treatment

A single PEF treatment at 32kV/cm, 10µs, 5 pulses was combined with a recovery period of 10 minutes at 25°C, and a subsequent heat treatment of 15 minutes at 55°C. This resulted in almost a 4 log reduction of *L. monocytogenes* Scott A (Figure 3). This is to compare with an almost 1.5 log reduction when applying only PEF treatment at 32kV/cm, 10µs, 5 pulses or less than one log reduction when applying heat treatment at 55°C for 15 minutes alone. Similar results were obtained with PEF treatment at 35kV/cm, 6µs, 5 pulses in combination with 55°C heat treatment. Here the effect of treatment at 55°C is even larger than when applying only PEF treatment or only heat treatment (Figure 3).

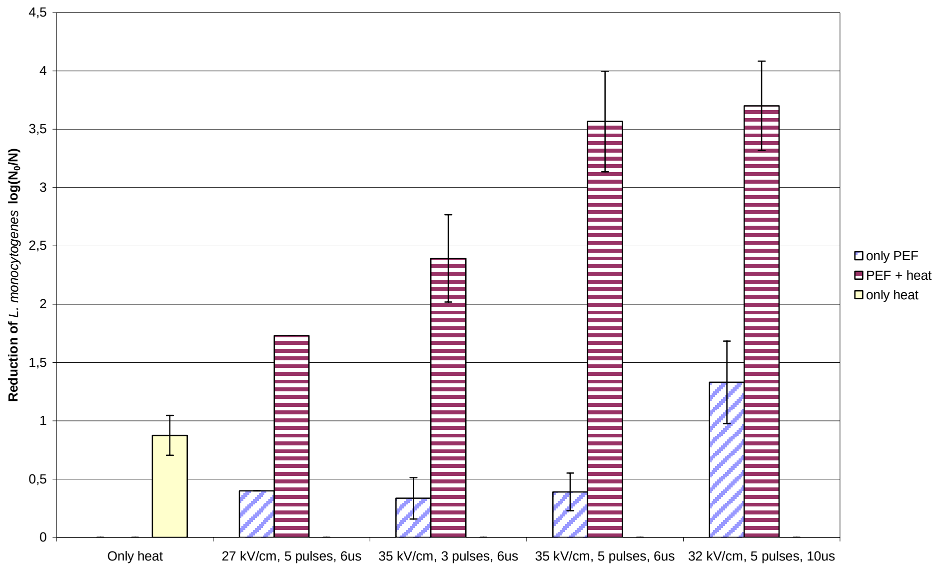

Figure 3. The effect of 55°C heat treatment for 15 minutes alone, PEF treatment alone, and the combination of PEF and 55°C heat treatment on the reduction of L. monocytogenes in phosphate buffer. The bacteria were maintained at 25°C for 10 minutes between the PEF treatment and the subsequent heat treatment.

4.3 PEF and potassium sorbate

The Time To Growth (TTG) of *L. monocytogenes* UNIBO 56Ly after PEF treatment was prolonged when 0.1% potassium sorbate was included in the phosphate buffer during PEF treatment (32kV/cm, 10µs, 5 pulses), as shown in Figure 4. TTG was about 200 hours at 10°C (Figure 4). This is to be compared with a TTG of about 80 hours for the two controls, i.e. *L. monocytogenes* treated by PEF in absence of potassium sorbate or when not PEF treated but in presence of potassium sorbate.

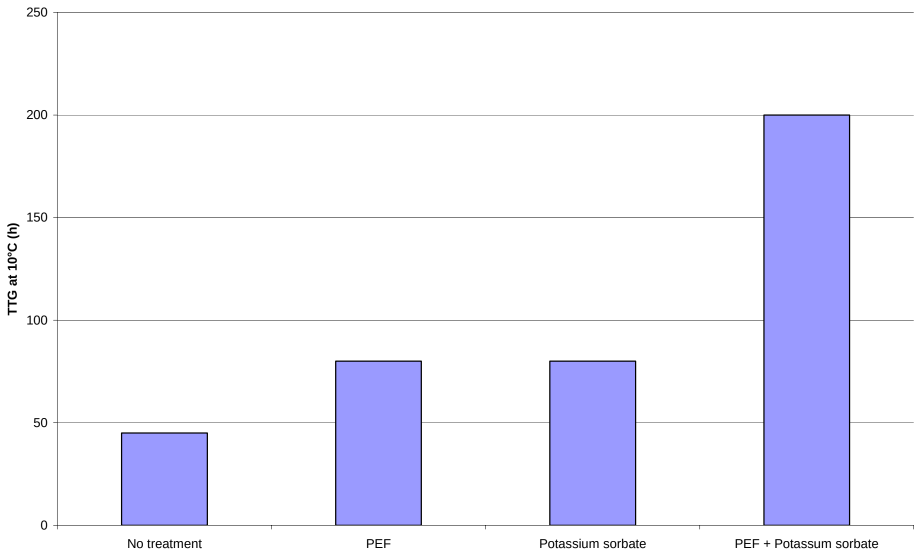

Figure 4. Time to growth of L. monocytogenes UNIBO 56 Ly at 10 °C after PEF treatment, addition of 0.1 % potassium sorbate and their combination. PEF treatment was performed at 32 kV/cm, 10 µs and 5 pulses. TTG corresponds to an increase of 0.5 log CFU/ml.

4.4 *rpoB* expression after PEF treatment

The PEF treatment that resulted in the highest log reduction of *L. monocytogenes* Scott A (32 kV, 10µs, 5 pulses) down regulated slightly the *rpoB* expression during 20 minutes after PEF (Figure 5). The opposite effect was however observed after the other two PEF treatments, 35 kV, 6µs, 3 pulses and 35 kV, 6µs, 5 pulses. After both these treatments the *rpoB* expression was up regulated, with the highest expression observed after 15 minutes. At this time the expression has increased 6-folded compared to untreated cells. Also after 20 minutes the *rpoB* expression was still high; between 4-6 times higher than the expression in untreated cells. This indicates a difference in the *rpoB* activity and subsequently a disturbance in the bacteria's protein synthesis depending on the PEF treatment.

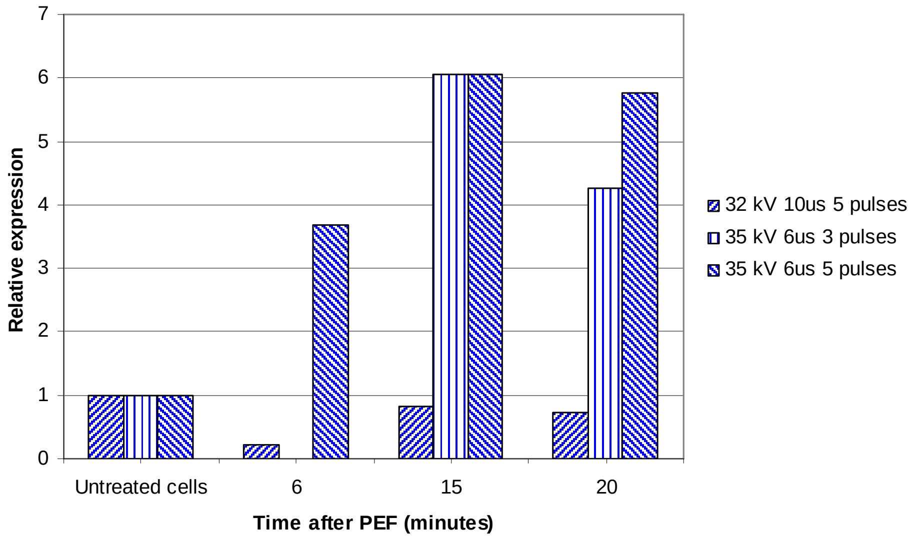

Figure 5. rpoB expression before and up to 20 minutes after PEF treatment. All samples after PEF are compared to untreated cells (relative expression = 1). No data was obtained after 6 minutes of 35 kV, 6 µs, 3 pulses.

4.5 Reduction in milk

The reduction of *L. monocytogenes* UNIBO 56Ly in low fat milk was evaluated after each cycle of repeated (n=4) PEF treatment (32kV/cm, 10µs, 5 pulses) and compared with the same treatment in phosphate buffer (Figure 6). The milk and the phosphate buffer had the same conductivity (5 mS/cm). The reduction of *L. monocytogenes* was always higher in low fat milk than in phosphate buffer. After 4 repeated PEF cycles a 5 log reduction of *L. monocytogenes* was observed. This is to be compared with a 3 log reduction after 4 PEF cycles in phosphate buffer (Figure 6).

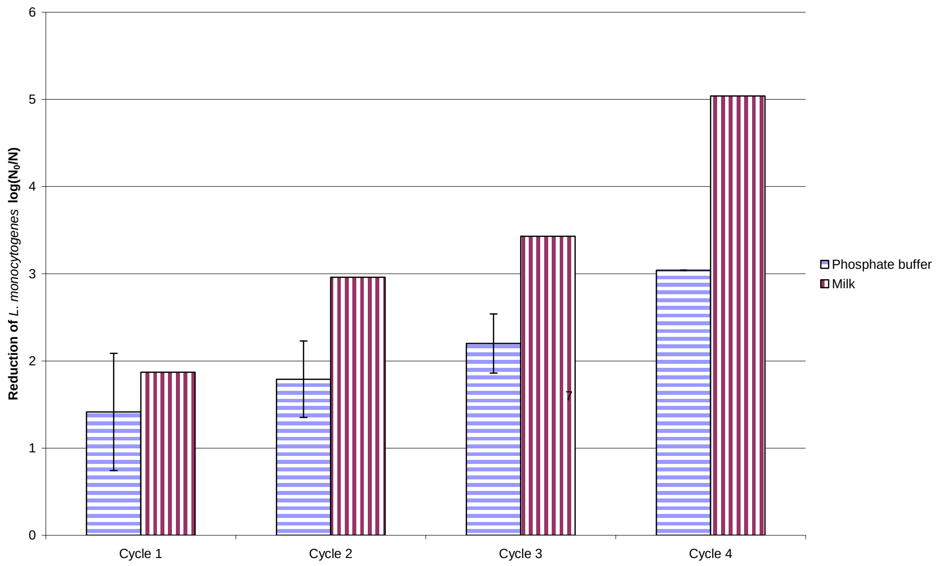

Figure 6. Reduction of L. monocytogenes UNIBO 56Ly in phosphate buffer and in low fat milk after different cycles of PEF treatment. In each PEF cycle 32 kV/cm, 10 μs and 5 pulses were applied. The conductivity was 5 mS/cm in the phosphate buffer and in the milk.

5. Acknowledgements

The project HighQ RTE (www.highqrte.eu) is funded by the EU´s 6th Framework Programme´s "Food Quality and Safety" activity.

6. References

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