

# PEF and subsequent mild heat treatment increase the reduction of *L. monocytogenes* and affect the *rpoB* expression



Maria Lövenklev, Pernilla Arinder and Elisabeth Borch

SIK – The Swedish Institute for Food and Biotechnology, Sweden

maria.lovenklev@sik.se

## Objective

- To combine PEF with mild heat treatment to increase the reduction of *L. monocytogenes* in buffer system.
- To use qRT-PCR to understand changes in gene expression that are related to cell injury and recovery after PEF treatment.

## Background

In PEF treatment, the bacteria are exposed to electric field under short time leading to membrane disruption and leakage of cellular components. Dependent on PEF treatment the bacteria are killed or just injured; in the later case the induced pores will reseal. During the recovery time the bacteria are sensitive and stressed, which can be used in combination with a second hurdle in order to achieve a relevant bacterial inactivation. When bacteria are exposed to treatments like PEF, changes in gene expression occur that are related to stress and recovery of the cells.

*L. monocytogenes* is a highly PEF resistant bacteria. In this study different PEF treatments have been used in combination with a mild heat treatment to reduce *L. monocytogenes* Scott A. A developed qRT-PCR method for quantifying the *rpoB* gene expression before and up to 45 minutes after PEF was used.

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## Conclusion

- The combination of PEF and subsequent mild heat treatment lead to a reduction of *L. monocytogenes*.
- This approach shows a potential for heat sensitive products or ingredients.
- The relative *rpoB* expression varied up and down during a time frame of 45 minutes after PEF treatment.
- The study of gene expression may give an increased understanding on the bacterial response to PEF.

## Material & Methods

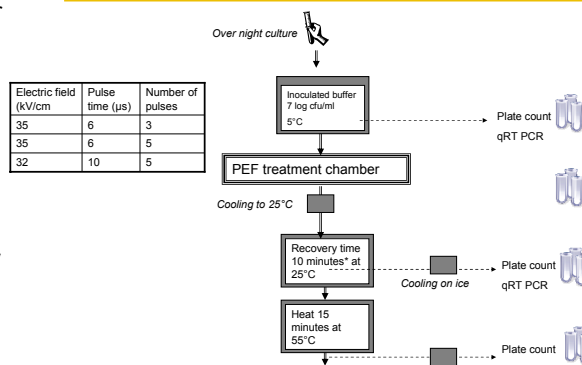


Fig. 1. Experimental setup.

\* In the qRT-PCR study samples were withdrawn at 6, 10, 15, 20 and 45 min after PEF.

For gene expression analysis, total RNA was extracted using RNAprotect Bacteria Reagent and RNAqueous kit. The RNA was then translated to cDNA using random primers in a reverse transcription step. DNA amplification was performed using AmpliTaq Gold DNA Polymerase in real time PCR 7500 (Applied Biosystems). Relative quantification was determined using *16S rRNA* as a reference gene.

## Results

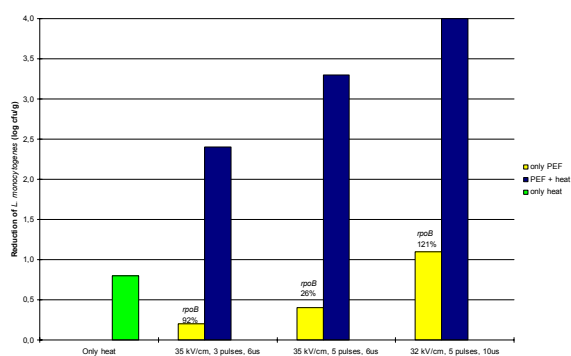


Fig. 2. Reduction of *L. monocytogenes* Scott A after different PEF treatment and after combination of PEF and mild heat treatment at 55°C for 15 minutes. PEF induced changes in *rpoB* expression are expressed as % relative untreated cells (100%) after 10 minutes.

The combination of PEF treatment and a subsequent mild heat treatment lead to a 4 log reduction of *L. monocytogenes*. This is to be compared with PEF (0,3-1,1 log reduction) and heat (0,8 log reduction) alone. The temperature in the sample was never above 55°C. The combination of PEF and subsequent mild heat has the potential to be applied on heat sensitive products or ingredients.

The relative *rpoB* expression showed a complex pattern of up and down regulation during a time frame of 45 minutes after PEF treatment. For PEF treatment almost having no effect on CFU, an initial down regulation was followed by a slow increase in gene activity. For the PEF treatment reducing *L. monocytogenes* with 1 log CFU, an initial up regulation took place which was followed by a decrease in gene activity. The study of gene expression may when coupled to reduction in CFU and possibly re-growth give an increased understanding on the bacterial response to PEF.

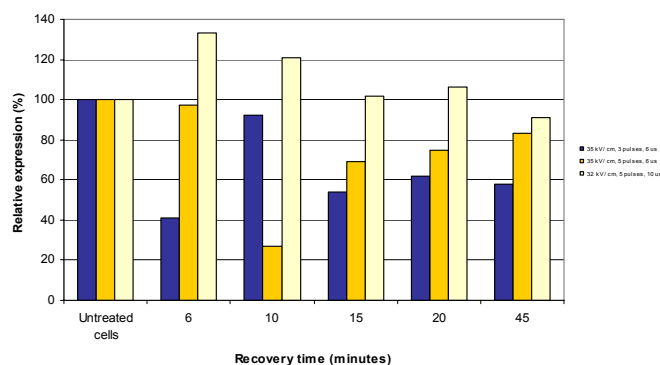


Fig. 3. *rpoB* expression before and up to 45 minutes after PEF.

