Pulsed electric field treatment to increase intracellular trehalose in *L. plantarum* WCFS1

Evelien Vaessen, Heidy den Besten, Niels van Mossevelde, Tiffany Patra, Remko Boom, Maarten Schutyser
Food Process Engineering and Food Microbiology
Wageningen University and Research
Contact: evelien.vaessen@wur.nl

Background

Robustness of probiotics after spray drying is relatively low due to thermal and dehydration stresses. Intracellular accumulation of trehalose could enhance the robustness of bacteria during processing. One way of increasing intracellular trehalose could be the use of pulsed electric field (PEF) treatment to create pores in the membrane, through which trehalose can diffuse into the cells.

Objective

The aim of this work was to increase the intracellular trehalose concentration in *L. plantarum* WCFS1 upon PEF treatment while maintaining a high survival. (Fig. 1)

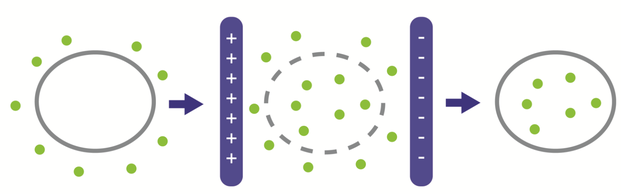

Figure 1. Schematic representation of trehalose diffusing into a cell upon PEF treatment.

Approach

Pulsed electric field treatment

PEF treatment was performed with *L. plantarum* WCFS1 in a solution containing 290 mM trehalose and with a conductivity of 0.15 S/m. The treatment chamber was a small scale cuvette (Fig. 2).

PEF process parameters
- Electric field strength: 2.5, 5, 7.5, 10 and 12.5 kV/cm
- 2 pulses of 100 µs with an interval of 5 s

Figure 2. Small scale PEF cuvette

Survival after PEF was assessed by plate counting and trehalose content of the samples was measured via HPLC analysis.

Membrane permeability

Propidium iodide (PI) and SYTO 9 staining were used to discriminate between bacteria that had a permeable or intact cell membrane. PI (red stain) can only enter cells with a damaged membrane, and SYTO 9 (green stain) enters all cells. Reversibility of pore formation in the membrane was studied by addition of the stains at different moments (Fig. 3). Results were based on fluorescent microscopy images (Fig. 4).

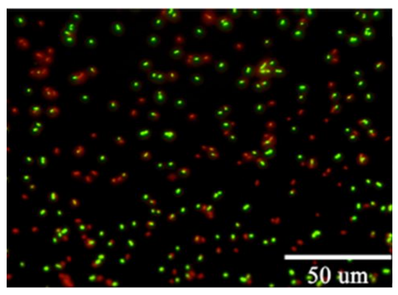

Figure 4. Fluorescent microscope image of *L. plantarum* WCFS1 stained with PI and SYTO 9

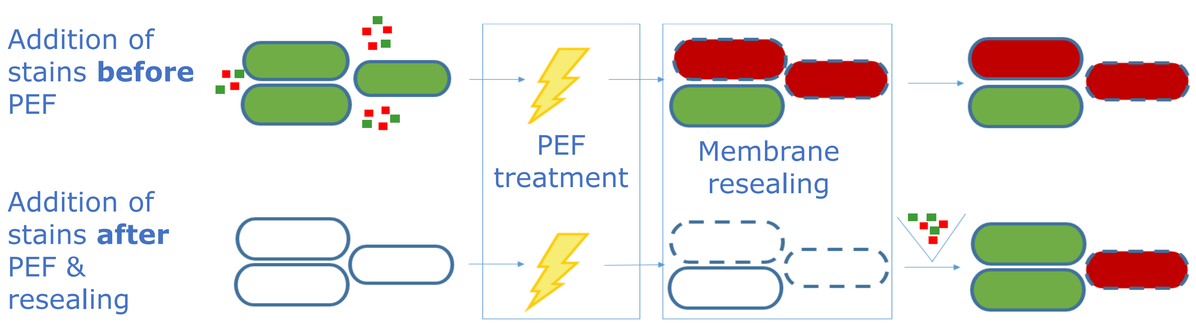

Figure 3. Study of the membrane permeability by addition of PI (red stain) and SYTO 9 (green stain) at different moments

Highlights

- PEF is used for increasing intracellular trehalose in *L. plantarum* WCFS1.
- Both intracellular trehalose and culture viability are high at 7.5 kV/cm.
- The percentage of reversible permeablized cells is approximately 12% at 7.5, 10 and 12.5 kV/cm.

Results

Increasing electric field strength led to a decrease in survival after PEF treatment (Fig. 5A). Furthermore, field strengths of 7.5 kV/cm and higher led to an increase in intracellular trehalose compared to the control (Fig. 5B). At 7.5 kV/cm both intracellular trehalose and survival were high.

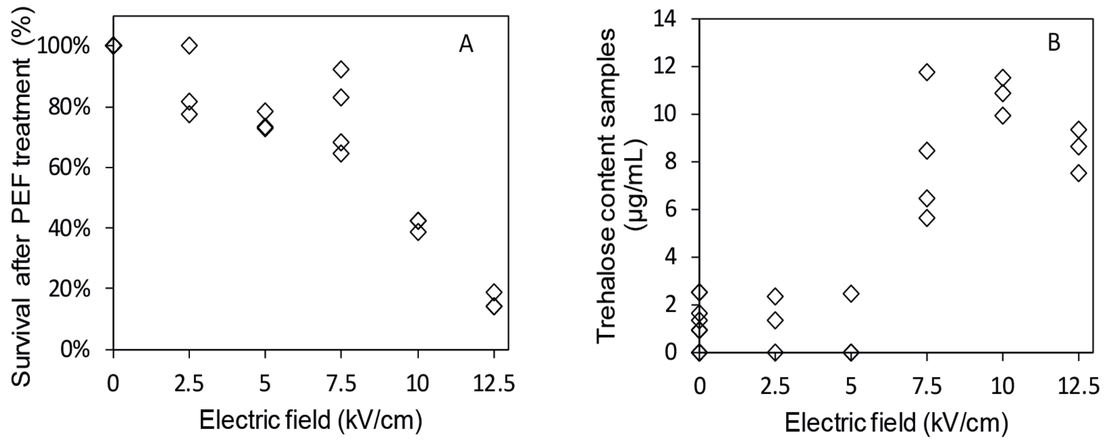

Figure 5. Survival (A) and trehalose content of the samples (B) after PEF treatment. Each data point represents one biological replicate. Data points at 0 kV/cm represent control samples (without PEF).

Study of the membrane permeability (Fig. 6) showed that:

- The irreversible permeated fraction increased upon increasing field strength.
- The reversible permeated fraction was for all three field strengths approximately 12%.
- A large fraction of the population remained unaffected at 7.5 kV/cm.
- When assuming that the intracellular trehalose (Fig. 5) is in the reversible electroporated part of the population, the intracellular trehalose concentration in these cells is approximately 100 mM.

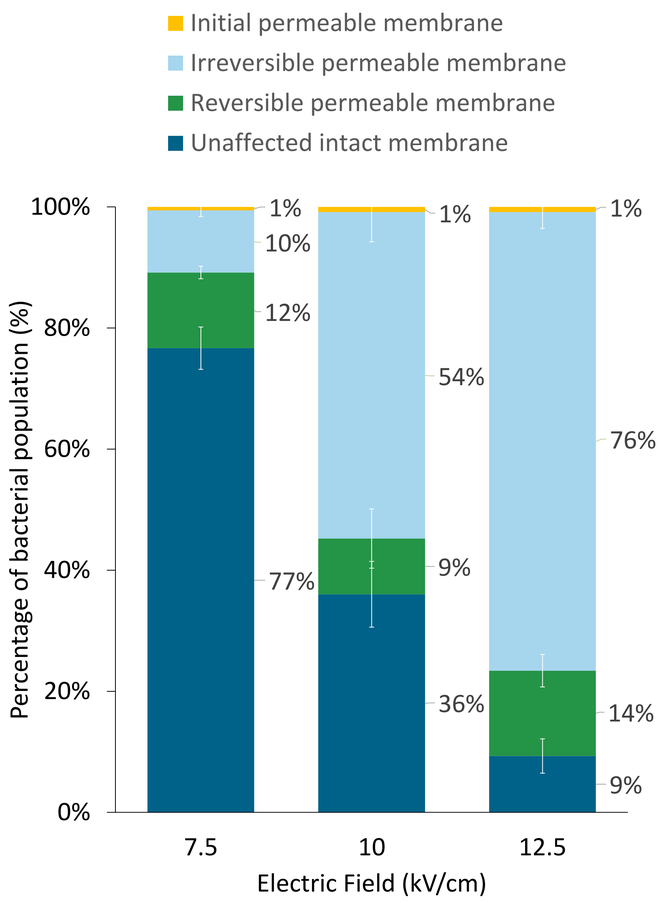

Figure 6. Membrane permeability for PI upon PEF treatment at various electric field strengths. Results were obtained by addition of PI and SYTO 9 at different time points. Error bars represent standard deviations of biological triplicates.