Fast flow-through non-thermal pasteurization using constant radiofrequency electric fields

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Abstract
Pulsed Electric Field technologies have captured the attention of researchers on food pasteurization because of their non-thermal inactivation mechanism, which results in fresh-like products. Nevertheless, high voltage pulsing required by these technologies implies complex and costly generators. Here, as an alternative, it is proposed a method, partially inherited from research on cell electroporation for gene transfection, in which the liquid to be treated flows at high speed through a miniature chamber where the electric field is permanently applied. In particular, it is proposed that the constantly applied electric field consists of an AC signal (> 100 kHz) so that electrochemical by-products are minimized. The method, while being compatible with batch processing, will allow use of lower voltages and will avoid the pulsation requirement.

Background
Thermal methods are the basis of most pasteurization technologies intended for food preservation. High temperatures, however, not only inactivate harmful microorganisms but also damage constituents of the medium under treatment, which may result in detrimental effects on nutrients, color, flavor and texture.

In the last decades, Pulsed Electric Field (PEF) technologies have captured the attention of researchers because:
- Growing demand for fresh-like foods.
- PEF technologies have the potential to minimize energy consumption.
- PEF technologies could be relevant in the context of renewable resources (for efficient oil extraction from microalgae for biofuel production).

Nevertheless, PEF methods have some disadvantages that hinder their industrial adoption. Among those disadvantages:
- High maintenance costs due to electrode erosion.
- High cost of electrical pulse generators.

The proposal
Electroporation is employed in microbiology laboratories for gene transfection. Recently, in order to transflect large quantities of cells, it has been proposed to electroporate the cells with high speed flowing through a miniature chamber where the electric field is permanently applied (Nat Protoc. 2011. 6(9):1102-20).

This concept is usually referred to as flow-through electroporation and it is:
1. Do not require ultra high voltages. In a small chamber the electric fields (i.e. voltage gradients) can be made very large with lower voltages.
2. Do not require voltage pulsation as pulsation intrinsically occurs.

Here we show that this concept is applicable to non-thermal pasteurization despite that much higher fields are required for killing cells than for transfection. In addition, we propose to employ constant radiofrequency voltages (>100 kHz) so that electrochemical reactions at the electrodes are minimized.

Numerical study
A model was designed for simulating a single conduit of the proposed three-layer structure. The results show that, in an scenario where feasible fluid speed is forced and high electric fields capable of killing bacteria are produced, mild temperature increase is induced.

The model consisted of slightly conductive water flowing through an insulator tube in between two metallic tubes. Dimensions and other parameters were selected as to be consistent with the in vitro study later described (total length = 40 mm; insulator diameter = 0.45 mm, inner diameter = 0.5 mm). It was simulated in COMSOL Multiphysics 4.2a, which is a numerical software platform able to concurrently model diverse physics. In this case, three physics were modeled: DC electrical conduction, turbulent flow and heat transfer. The applied DC voltage was 570 V which corresponds to the RMS value of the applied bipolar square pulses in the in vitro study. Water input speed was imposed to be 5 m/s. Axial symmetry was used for minimizing computation time.

In vitro study
A proof-of-concept system was built for demonstrating effectiveness of the fast flow-through non-thermal technique proposed here for killing E. coli bacteria.

The system consisted of a high voltage generator, a treatment chamber and a syringe pump. The generator delivered bipolar square signals at 100 kHz with a peak to peak amplitude of 1230 V. The treatment chamber was made from a stainless steel hypodermic needle ( gauge 21) the needle was cut into two sections and a dielectric epoxy seal with a length of 0.45 mm was created. The custom made syringe pump was set so that the water velocity through the chamber was 5 m/s.

Treated samples consisted of mineral water intentionally contaminated with Escherichia coli (strain ATCC # 25922); bacterial culture in LB broth was 1:20 diluted in the water. Electrical conductivity of the samples was approximately 0.15 S/m and concentration of bacteria was about 3×10^5 CFU/ml. Counting was performed measuring viable plate counts after 48 hours incubation on MacConkey's agar.

In order to emulate concatenated chambers, experiments were performed in which the treated water samples were treated again up to three times more.

Cell counting results. The method is indeed capable of producing bacteria inactivation but, for industrial applications, higher fields (or more treatments) will be required because the obtained CFU reductions were low. Fluid temperature was measured after treatment when 1280 V were delivered and it resulted in an average temperature increase of 4.5 °C. By performing dummy treatments (0 V) the average temperature increase was about 0.5 °C. That is, of the 4.5 °C increase during treatment about 0.5 °C is due to the heating of the water.

Treated samples were checked for iron contents, in a range of test samples which have detection sensitivity of 0.1 ppm, with negative results.

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Proposed implementation of the flow-through electroporation concept for industrial pasteurization: the fluid to be treated would flow at high speed through orifices in a three-layer structure consisting of a thick metallic electrode, a thin insulator and a second thick metallic electrode. The electrodes would be energized by a moderate AC voltage so that each portion of fluid would experience a high electric field AC burst during passage through the insulator. Concatenation of treatment chambers is proposed for higher effectiveness.

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