Fundamental and Applied Aspects of Pulsed Electric Fields for Microbial Inactivation

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Abstract—Pulsed electric field (PEF) is a technology that causes electroporation of the cell membranes by application of intermittent electric fields of high intensity for short periods of time (µs). The local defects or pores created by the application of an external electric field may lead to the loss of the membrane integrity and uncontrolled molecular transport across microbial membranes. These events may abolish the microbial capacity to maintain the microbial homeostasis causing microbial inactivation at temperatures below those used in thermal processing. Based in this phenomenon PEF provides an excellent alternative to conventional thermal pasteurization of heat sensitive foods.

Keywords—microbial inactivation, preservation, Pulsed electric fields.

I. INTRODUCTION

Currently, the food industry’s primary intervention to supply safe foods with an extended shelf-life is thermal treatment. However, heating may cause unacceptable deterioration of foods with a heat labile chemical or physical structure. Indeed over the last decades considerable research efforts have been focus towards the development of non-thermal processing technologies such as high hydrostatic pressure, ultra-violet light, pulse light, ultrasound, high pressure homogenization or pulsed electric fields. The concept of nonthermal technologies referrers to a group of technologies whose effects in foods are similar to effects cause by heating, but the processing temperatures are lower to those used in thermal processing.

PEF technology is considered a promising no thermal alternative that has received considerable attention for microbial inactivation in foods. The treatment consists on the application of pulses of high voltage and short duration to a liquid placed between two electrodes. This voltage results in an electric field which intensity depends on the voltage delivered and the gap between the electrodes. PEF cause some type of structural rearrangement of the cell membranes that consists on the formation of local defects or pores (electroporation) that lead to an increment of the cell membrane permeability to ions and macromolecules. Depending on the intensity of the treatment applied (external electric field, treatment time) and the cell characteristics (size, shape, orientation in the electric field) the viability of the electroporated cell can be preserved by recovering the membrane integrity, or the electroporation can be permanent. Electroporation causes inactivation of vegetative forms of microorganisms at temperatures below those used in thermal processing [1].

II. BASICS PRINCIPLES OF MICROBIAL INACTIVATION BY PULSED ELECTRIC FIELDS

Although the mechanism underlying microbial inactivation by PEF has not been fully elucidated it is thought that is based on the electroporation of the microbial cytoplasmatic membrane. The local defects or pores created in the cytoplasmatic membrane by the application of an external electric field lead to the lost of its semipermeable barrier function and uncontrolled molecular transport across the membrane can occurs. These events may abolish the homeostatic capacity of the microorganisms and will eventually lead to microbial death [1]. However, the cytoplasmatic membrane is not the only barrier that separates the cytoplasm from the environment in microorganisms including bacteria and yeast. Cytoplasmatic membrane of Gram-positive bacteria is surrounded by a thick cell wall made of peptidoglycans and tectonic acids. On the other hand the cell wall in Gram negative bacteria is thinner but it is surrounded by an outer membrane that differs from typical biological membrane because the main molecular constituents are lipopolysacharides. Similarly to gram positive bacteria yeast cells are surrounded by a cell wall. Although it has been observed differences in the effect caused by PEF in gram positive and gram negative bacteria, how influence the envelopes surrounding the cytoplasmatic membrane on electroporation is an aspect still unknown.

Evidence of cytoplasmatic membrane electroporation caused by PEF has been observed by different authors using different techniques such as leakage of intracellular material, measurement of osmotic response or fluorescent dye exclusion assays.

Several authors have observed leakage of intracellular compounds from different microorganisms treated by PEF treatments were non-lethal indicating that the temporary loss of permeability control is not necessarily lethal [3].
Using the exclusion of propidium iodine technique to measure the permeabilization of the microbial membrane it has been observed that the membrane permeabilization was involved in microbial inactivation by PEF but relationship between electroporation and microbial inactivation depended on the treatment medium pH and the characteristics of microbial envelopes [4]. At pH 7 and 4, the loss of viability for Gram-positive bacteria investigated such as *Listeria monocytogenes* and *L. plantarum* was correlated with an irreversible loss of membrane integrity. However, for the Gram-negative bacteria (*E. coli* and *Salmonella senftenberg*), inactivation was correlated with the proportion of reversible and irreversible electroporated cells indicating that reversible changes in the cytoplasmic membrane of Gram-negative bacteria also may cause microbial death.

The capability of the microorganism of recovering the damage caused by PEF in the cytoplasmic membrane has been correlated with the occurrence of sublethal injury after the PEF treatment. Sublethally injured population fail to survive and multiply in harsh environments. Early studies indicated that microbial inactivation by PEF was an all-or-nothing effect because after the treatment alive or dead cells were detected but not sublethally injured ones [5]. However, currently it is well established that PEF causes sublethal injury depending on the microorganisms and pH of the treatment medium [6]. Generally, greater number of sublethally injured cells were detected in a population of Gram-negative bacteria when treated by PEF at pH 4 that at pH 7 but in Gram-positive bacteria occurrence of sublethal injury was greater at pH 7 than at pH 4.

The fact that the presence of sodium chloride in the recovery medium prevents the growth of sublethal injured cells after PEF treatment and the demonstration that this damaged cells required the synthesis of lipids for injury repair supports the involvement of the cytoplasmic membrane on the microbial inactivation by PEF [7].

### III. FACTORS AFFECTING MICROBIAL INACTIVATION BY PULSED ELECTRIC FIELDS

Microbial inactivation by PEF has been widely investigated. These studies have demonstrated that many factors influence microbial resistance to PEF. The definition of the processing conditions required to inactivate spoiling and pathogenic microorganism requires the understanding of the influence of these factors. Critical factors affecting microbial inactivation can essentially be classified into three groups: processing parameters, treatment medium characteristics, and microbial characteristics.

#### A. Processing Parameters

Electric field strength, treatment time, total specific energy and temperature are the most important processing parameters that affect inactivation of microorganisms by PEF.

A specific transmembrane voltage threshold (from 0.5 V to 1 V) exists for the manifestation of the electroporation phenomenon [8]. Transmembrane potential generated in a cell by application of an external electric field depends among other factor on the intensity of the electric field strength and on the cell size. The external electric field strength required to reach the transmembrane voltage threshold is called critical electric field strength (E₀). Due to the size of microbial cells (1-10 µm) is smaller than the eukaryote plant cells (40-200 µm) the critical electric field intensity to induce electroporation of microbial cells (>10 kV/cm) is much higher than for induce electroporation in eukaryote cells of plant or animal tissues (<5 kV/cm). Over the critical electric field strength, microbial sensitivity to PEF enhances by increasing the electric field strength and the treatment time (Figure 1).

![Fig. 1 Typical survival curves corresponding to microbial inactivation by pulsed electric field treatments of different intensity (E₂ < E₁ < E₀)](image)

Treatment time is defined as a function of the duration of pulse width and the number of pulses applied. The survival curves (log10 of survivors along the time) at constant electric field strength are characterized by a fast inactivation in the first moments of the treatment and then the number of survivors slowly decreases as the treatment time becomes longer (Figure 1). Different studies have been conducted in order to found the most adequate mathematical equations to describe this kinetic of inactivation.