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Quantifying the Efficiency and Efficacy of Pulsed Electric Field Apparatus for Liquid Food Preservation

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Abstract - Sufficient shelf life of food product is the one of major factor in the food processing industry mainly include liquid food. Pasteurization is more preferably thermal method to kill pathogenic bacteria and increase life of food product. Thermal application involved in Pasteurization might degrade food quality. Amongst non-thermal methods, Pulsed Electric Field (PEF) apparatus has received wide attention recently compare to other method. This method involves of passing liquid food sample through a single pulsed electric field by providing 12-35 kV/cm electricity. This causes the inactivation of the microbial population in the samples based on electromechanical stability of the microbial membrane with regard to the electric field. The two main factors in this regard are the voltage of electric field and time of exposure of the sample to the field. When the food samples are exposed to the electric field, transient or permanent pores develop in the cell membranes of the microorganisms, either by enlargement of preexisting pores or by development of new pores. These pores may be temporary or permanent and these pores cause cell lysis and loss of cell contents, ultimately leading to the cell death. The experiment aim is to quantify the efficiency and efficacy of a prototype PEF machine to sterilize the liquid foods. This machine has added the advantage of being able to process foods without tampering with their nutritional quality.

Keywords-Cell lysis, Microbial Membrane, Non-thermal methods, Pulsed Electric Field (PEF), Pasteurization, Shelf life.

I. INTRODUCTION

The primary demand for consumers is that the food they obtain from the market is sterile and significantly free from fungal spores and microbes. This can be achieved by a variety of methods such as freezing, irradiation, pasteurization, storage in a modified gaseous environment and so on. However, the major concern with many of these methods is the effect the processing method has on the food quality. The Pulsed electric field electroporation is a technique that has been testified to be highly effective in the disinfection of liquid food, in order to enhance their shelf life. In addition, this technique interferes minimally with the food quality. [2], [3], [6]. Recent progresses in non-thermal food sterilization technologies have shown that pulsed electric field (PEF) of high intensities is a highly viable alternative for sterilization of liquid foods such as fruit juices, milk, liquid egg and many others. Prior studies that have been carried out in this [16], [8],[9], [22], demonstrate the key fact that the PEF technology is an effective method for inactivation of yeasts, molds, and bacterium. The PEF technology for inactivation of fungal spores and microbes in liquid food is dependent upon the application of a high voltage electric field over the liquid food. This application of the high voltage electricity ensures causes the development of transient or permanent pores in the cell membranes of the yeast, mold or bacterium, leading to the dielectric breakdown of the microorganism. The extent of pore formation is dependent upon the lethality of the electric pulse applied to the liquid and also the duration for which the liquid is exposed to the radiation.

Experimental results from prior studies have also revealed that the PEF Treatment effectively inactivates liquid food without causing any drastic change to the nutritional attributes of the liquid [13], [22]. Earlier works have, in addition, demonstrated that the Electric Pulse does not cause any change to the color or flavor of the liquid food.

The PEF based protocol consumes approximately 1/1000th less power than these aforementioned methods. Technically speaking the PEF method uses high voltage rather than current. Therefore, the temperature of the system rarely rises throughout the experimental procedure. The average temperature high of the system is around 5-degree C, so obviously the concerns regarding temperature-induced denaturation of the liquid food can be put to rest.

In this study, a prototype PEF machine developed in the lab was used to sterilize three different liquid foods (namely milk and two fruit juices), to quantify the efficiency and efficacy of the machine in enhancing the shelf life of these foods.

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II. MATERIALS REQUIRED

A. Apparatus

- Pulsed Electric Field Apparatus
- Batteries
- Battery Eliminator
- Cathode Ray Oscilloscope
- Multimeter

B. Media Requirements

- Czapek Dox Agar
- Mannitol Salt Agar
- Rose Bengal Agar
- MacConkey Agar

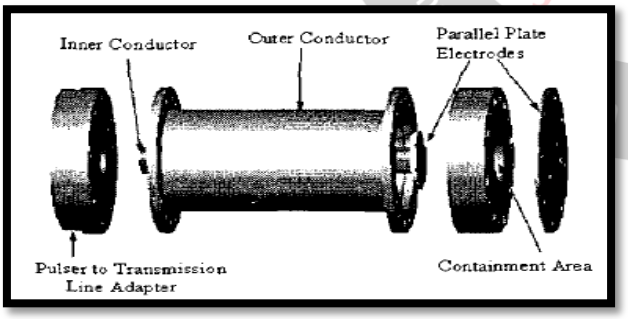
C. Glassware

- Petri plates
- Conical Flasks
- Beakers

III. METHODOLOGY

Processing of liquid food using Pulsed Electric Field (PEF) involves an application of high voltage pulses to the liquids placed in between two stationary electrodes in the treatment chamber (*Refer Fig 1*). The liquid foods are essentially considered as the conductors since they comprise a high concentration of ions, which act as carriers of electric charge. Therefore, a large electric flux should flow in a short time span to generate a high-intensity electric pulse.

The magnitude of electric fields fashioned is usually in the range of 4000 volts/cm to 20, 000 volts/cm and their application can be in the form of exponentially decaying, square wave, bipolar or even oscillatory pulses. The peak voltage is maintained for an extended time in case of square wave pulse and these are more fatal and energy effective than the exponential decaying pulse as well as demanding less cooling energy[21]



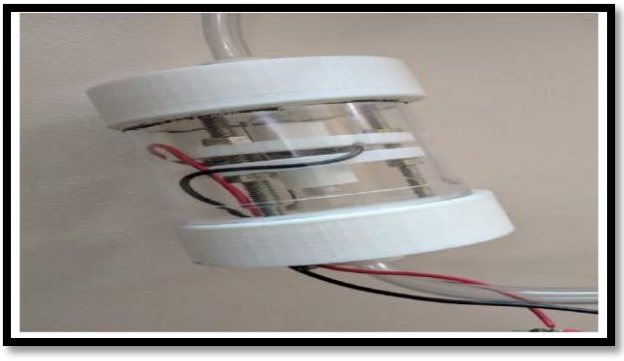


Figure 1: PEF Chamber

PEF application for liquid food sterilization can be functional in both Batch Flow as well as Continuous Flow Operations. In order to provide a model that could be used in future for industrial processes, a continuous circulation system was developed, which enables circulation of the contaminated liquid from the reservoir containing the feed, through the PEF Chamber and finally obtaining sterilized liquid in the recipient reservoir.

A. PEF System Components

Processing of Liquid Food using Pulsed Electric Field involves the application of High Voltage Pulses to the Liquids placed in between two stationary electrodes in the treatment chamber. The components of a PEF system can be enumerated as follows (*Refer Fig 2 a,b*):

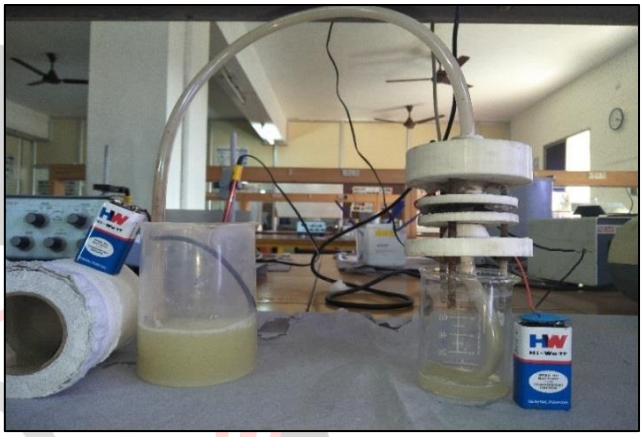


Figure 1: PEF Chamber

B. Electric Field

The electric field is demarcated as the electric force per unit charge. It is the potential difference engendered amid two plates when electricity is allowed to flow in between them. The electric field is radially outward from a positive charge and radially in toward a negative point charge. Hence, Electric field can be defined by the following equation:

$$E = F/q$$

Where E is the electric field, F is the electric force, and q is the charge.

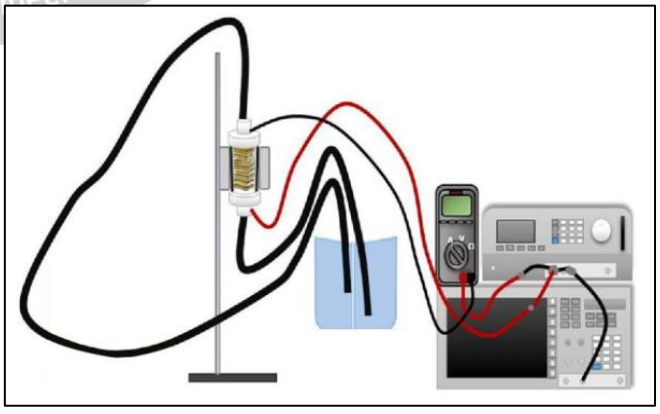


Figure 1a: PEF Set-up

C. Capacitor

A capacitor is a reflexive electronic module that stores energy in the form of an electrostatic field. Inside a simple parallel plate capacitor, the termini connected to two metal

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plates separated by a non-conducting ingredient, or dielectric.

Application of a DC voltage between the two electrodes reasons the shift of electrons to one side, which reasons one of the electrodes to have high negative charge. The other electrode thus has a deficiency of electrons and therefore has a high positive charge. PEF electrode is considered as a capacitor since there is an absenteeism of any direct contact between the cathode and anode when DC voltage is applied between the electrodes.

D. Introduction to Cell Membrane

The cell membrane of bacteria is composed of two coatings of phospholipids.

The phospholipid bilayer is recurrently arranged and proteins entrenched within the phospholipid bilayer normalize the movement of materials in and out of the microbial cell [17]. On administration of short (Microseconds tomilliseconds) yet high-intensity electric field pulses to the bacterial cell population, it primes to building up of charges on themembrane surface, triggering an electrical potential difference to develop across the plasma membrane [12], with a standard transmembrane potential of about 70 mV [7]. Upon exceeding the critical value, the electric field induces dielectric cessation of the plasma membrane, often causing loss of osmosis and homeostatic stability inside the cell. These changes in the chemical constitution of the cell are unalterable and eventually act as the antecedent for cell death [11].

IV. PROTOCOL

To carry out the experimental procedure the apparatus was set up as shown above. Nutrient Agar media were measured and prepared, followed by autoclaving to make then sterile. The Nutrient agar plates were prepared as per requirement and were allowed to solidify inside a laminar air flow cabinet. Then fresh liquid samples were obtained from VIT University Campus (namely milk and two fruit juices). The Samples were serially diluted to x10 -3 and x10 -5 and plated (using 0.1 ml sample for each plate) in the duplicates of sterile Petri Plates. The Petri plates were then incubated in an incubator.

The Liquid samples were then passed through the PEF Apparatus and the processed liquid was collected and immediately transferred to a laminar chamber. The Liquid samples were then again plated onto duplicate Petri plates and then left for incubation in the incubator.

The plates were allowed to incubate for 24hours and then the CFU were counted for the processed and unprocessed samples plates. The results obtained served to indicate the efficiency of the PEF apparatus for Liquid food sterilization. The efficiency is calculated by counting colonies of untreated and treated sample.

Fig 3: Untreated sample colonies growth with a) YEP Agar media (fungi)b) MacConkey Agar (Gram negative bacteria) c) Mannitol Salt Agar media (Gram positive bacteria)

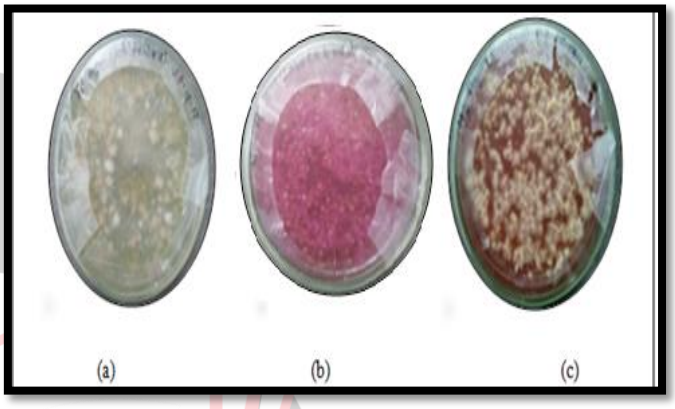

Fig 4: Treated sample colonies growth with a) YEP Agar media (fungi)b) MacConkey Agar (Gram negative bacteria) c) Mannitol Salt Agar media (Gram positive bacteria)

A. % Reduction Count (RC):

RC= [(untreated media colonies)−(treated media colonies) / untreated media colonies] ×100%

	Sample I: Citrus limetta Juice	Sample II: Saccharumbarberi	Sample III: Milk
Gram Positive Bacteria:			
Control	**0**	**0**	**0**
Untreated	**>250**	**>300**	**>250**
PEF Treated	**46**	**34**	**41**
% Reduction Count	**81.6%**	**88.6%**	**83.6%**
Gram Negative Bacteria:			
Control	**0**	**0**	**0**
Untreated	**>250**	**>300**	**>250**
PEF Treated	**52**	**36**	**44**
% Reduction Count	**79.2%**	**88%**	**82.4**
Yeast:			
Control	**0**	**0**	**0**
Untreated	**>250**	**>300**	**>250**
PEF Treated	**87**	**93**	**67**
% Reduction Count	**65%**	**69%**	**73%**

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V. EXPERIMENTAL RESULTS and INTERPRETATION

After plating the colony forming units were counted and statistical analysis was performed. The reduction for Gram Negative Bacteria and 69% for Yeast. For the Sample three plate count data reveals a percent reduction count of 83.6% reduction for Gram Positive Bacteria, 82.4% reduction for Gram Negative Bacteria and 73% for Yeast. Thus, it can be inferred from the Statistical analysis that the mean reduction count for Gram Positive Bacterial is around 84.6%, for Gram Negative Bacteria it is around 83.2%, whereas for Yeast is a somewhat less at around 69.

The overall Sterilization of the machine can therefore be stated to be approximately 78.93%. Thus, the PEF based method of sterilization is a novel and effective approach for enhancing the shelf life of liquid foods.

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