An Investigation on the Non Thermal Pasteurisation Using Pulsed Electric Fields

Sally Alkhafaji

A Thesis Submitted in Partial Fulfilment of the Requirements for the Degree of Doctor of Philosophy at the University of Auckland, Auckland, New Zealand

Department of Chemical and Materials Engineering
School of Engineering
The University of Auckland

December 2006

Abstract

Increasing consumer demand for new products with high nutritional qualities has spurred a search for new alternatives to food preservation. Pulsed electric field (PEF) is an emerging technology for non thermal food pasteurisation. Using this technology, enzymes, pathogenic and spoilage microorganisms can be inactivated without affecting the colour, flavour, and nutrients of the food. PEF treatment may be provided by applying pulsed electric field to a food product in a treatment zone between two electrodes at ambient , or slightly above ambient temperature. Exposure of microbial cells to the electric field induces a transmembrane potential in the cell membrane, which results in electroporation (the permeabilization of the membranes of cells and organelles) and/or electrofusion (the connection of two separate membranes into one) of the cells.

An innovative pulsed electric field (PEF) unit was designed and constructed in the University of Auckland using modern IGBT technology. The system consists of main equipments, the high voltage pulse generator and the treatment chambers. The main focus of this work was to design an innovative PEF treatment chamber that provide uniform distribution of electric field, minimum increase in liquid temperature, minimum fouling of electrodes and an energy efficient system.

Four multi pass treatment chambers were designed consisting of two stainless steel mesh electrodes in each chamber, with the treated fluid flowing through the openings of the mesh electrodes. The two electrodes are

electrically isolated from each other by an insulator element designed to form a small orifice where most of the electric field is concentrated. Dielectric breakdown inside the chambers was prevented by removing the electrodes far from the narrow gap. The effect of the chambers different geometries on the PEF process in terms of electric parameters and microbial inactivation were investigated.

Electric field intensity in the range of (17-43 kV/cm) was applied with square bipolar pulses of 1.7 μs duration. The effect of PEF treatment on the inactivation of gram-negative Escherichia coli ATCC 25922 suspended in simulated milk ultra-filtrate (SMUF) of 100%, 66.67% and 50% concentration was investigated. Treatments with the same electrical power input but higher electric field strengths provided larger degree of killing. The inactivation rate of E coli was significantly increased with increasing the electric field strength, treatment time and processing temperature.

Morphological changes on E coli as a result of PEF treatment were studied under transmission electron microscopy (TEM). Significant morphological changes on E coli after PEF treatment were observed. The TEM studies suggested that the microbial inactivation was a consequence of electroporation and electrofusion mechanisms.

Kinetic analysis of microbial inactivation due to PEF and thermal treatment of E coli suspended in SUMF were also studied. Comparison between measured (experimental) and predicted (theoretical) variation of E coli concentration with time following the PEF treatment was discussed, taking into consideration the recirculation mode of the PEF treatment. The treated

liquid was circulated more than once through the treatment chamber to provide higher microbial inactivation. Arrhenius constants and activation energies of E coli inactivation using combined PEF and thermal treatment were calculated and generalized correlation for the inactivation rate constant as a function of electric field intensity and treatment temperature was developed.

Acknowledgements

I would like to express my sincere thanks to my academic supervisor Professor Mohammed Farid for his continuous advice and guide ness. And also I would like to thank my industrial supervisor Dr. Andrew Fletcher for his professional support and assistance.

I like to show my appreciation to Fonterra Research Institute and to the Foundation for Research Science and Technology for funding this project, by offering me the bright future scholarship.

I would also like to thank my colleges and the staff in Chemical and Materials engineering department for creating the best working environment. Thanks mum and dad for your encouragement and support all over the years, and thank you Sam for your tolerance and support and finally,

A very big and special thank to my sons, Abdullah, Mohammed and Ameer for their patience.

Table of Contents

Part one: Literature Review

Part Two: Experimental Investigations

List of Figures

List of Tables

List of Symbols

A	*Electrode area,* (cm^2)
a	*Microbial cell radius,* (cm)
b_E	*Regression coefficient*
C_0	*Capacitive of the energy storage capacitor,* $(Colomb/V)$
C_P	*Specific heat,* $(J\ kg^{-1}k^{-1})$
d	*Gap between two electrodes,* (cm)
d	*Diameter,* (cm)
E	*Electric field strength,* (kV/cm)
E_C	*Critical electric field strength,* (kV/cm)
q	*Fluid flow rate,* $(ml/\sec)$
f	*Pulse repetition rate, (Hz)*
$f^{`}$	*Shape Factor, dimensionless*
f^*	*Form factor, dimensionless*
I	*Current,* (A)
k	*Inactivation rate constant* (sec^{-1})
$k_C,\ k_{CO}$	*Constant factors*
k_1, k_2	*Constant factors*

L	*Length,* (cm)
s	*Survival fraction of microorganisms*
n	*Number of pulses applied*
P_e	*Electric compressive force,* $(\mu F.V)$
Q	*Power,* $(J/\sec)$
$Q`$	*Energy density,* (J/cm^3)
R	*Effective resistance of food in the treatment chamber,* (Ω)
ΔT	*Change in food temperature*
t	*Treatment time,* $(\sec)$
V	*Voltage,* (kV)
V_0	*Initial charge voltage over the energy storage capacitor,* (kV)
V_r	*Volume of PEF chamber,* (cm^3)
Z	*Impedance,* (Ω)

Greek Symbols

ε_o	*Permittivity of free space,* $8.84\times10^{-8}(\mu F/cm)$
ε_r	*Relative permittivity of the food material, dimensionless*

σ	*Conductivity of the food, (Siemens/m)*
ρ	*Resistivity of the food,* (Ωcm)
τ	*Pulse width,* (μs)
φ	*Electrical potential, (V)*
ρ_f	*Density of fluid inside the treatment chamber,* (g/cm^3)
δ	*Membrane Thickness,* (cm)
ρ^*	*Density of food (g/cm^3)*
υ	*Treatment volume,* (cm^3)

1. Chapter One: Overview of the Pulsed Electric Field Technology

This chapter presents the background of the non thermal pasteurisation technology using pulsed electric fields (PEF), the basic aspects of the technology, the generation of pulsed electric fields, energy requirements, electric and dielectric properties of some fluid foods and the objective of this research and the thesis outline.

1.1 Background

The preservation of fluid food is initially based on the work of Louis Pasteur. In 1864 he developed a method for preventing extraordinary fermentation in wine by destroying the microorganisms responsible by heat treatment. Until recently, thermal processing had been the most commonly method used in the food industry to increase shelf-life and maintain food safety by inactivating spoilage and pathogenic microorganisms. However, because studies have shown that color, flavor, and nutrients may be degraded by heat, there is now a growing demand for alternative methods of food preservation (Barbosa-Ca`novas, Gorgano-Nieta, Pothakamury & Swanson, 1999).

One of the promising non thermal processes is the pulsed electric fields (PEF) process. The process has the ability to inactivate microorganisms at

ambient or near-ambient temperatures, thereby avoiding the deleterious effects that heat has on the flavour, colour and nutrient value of foods (Barbosa-Ca`novas, Gorgano-Nieta, Pothakamury & Swanson, 1999).

The inactivation of microorganisms by PEF and its effects on quality and shelf life of foods has been the topic of large number of investigations (Yang, Li & Zhang (2004), Álvarez, Raso, , Sala & Condón (2003), Angersbach, Heinz & Knor (2000), Aronsson, Lindgren, Johansson & Ronner (2001a), Aronsson & Rönner (2001), Bendicho, Espachs, Arantegu, and Martin (2002),. Calderon-Miranda, Barbosa-Ca`novas, & Swanson (1999), Castro, Barbosa-Ca`novas, & Swanson (1993), Dejong & Vanheesch (1998) and many others).

Micro organisms, when subjected to pulsed electric fields generated from a high voltage electric power source, may grow at an inhibited rate or be rendered reproductively inactive or killed. The application of electric fields to cellular suspensions produces a large increase in the electrical conductivity and permeability of the microbial membrane (Sale & Hamilton, 1967 and 1968, Kinosita & Tsong, 1977, Zimmermann, Vienken & Pilwat, 1980 and Hulsheger, Potel & Niemann, 1983). Depending on the potential of the electric field applied, breakdown or lysis of the membrane, with the consequent inactivation of the microorganism, can be achieved (Barbosa-Ca`novas & Zhang, 2001).

Other non thermal pasteurisation methods of food include high hydrostatic pressure, oscillating magnetic fields and intense light pulses. High pressure processing (HPP) is used for the inactivation of microorganisms and some enzymes and for extending the self life of the food. However, some of the

applications of HPP technology include modification of the texture and sensory properties of food. One of the technical difficulties in using HPP is the fabrication of equipment. Oscillating magnetic fields can be applied to food sealed in plastic bag in order to inactivate microorganisms, which reduces the possibility of contamination during packaging. Pulsed light technology is applicable for surface pasteurisation of different food including meat, vegetables, fruits, bakery and solid milk products. Each of the non thermal technologies has specific application in terms of the type of food that can be processed, high pressure and oscillating magnetic fields can be applied to liquid and solid foods, while the light pulses are useful for surface pasteurisation and the pulsed electric fields are more suitable for liquid foods (Barbosa-Ca`novas & Pothakamury, 1998).

1.2 Basic Aspects of Pulsed Electric Fields

PEF treatment of liquid foods is based on the application of a high intensity pulse electric field to the food product, which flows between two electrodes that confine the treatment gap of the PEF chamber. Liquid food materials are usually considered electrical conductors because they contain large concentrations of ions as electrical charge carriers. To generate a high-intensity PEF within a food, a large flux of current must flow in a very short period of time, and because the time between pulses is much longer than the pulse width, the generation of pulses involves slow charging and fast discharging of an electrical energy storage device such as a capacitor (Barbosa-Ca`novas, Gongora-Nieto, Pothakamury & Swanson, 1999).

The key variables involved in a PEF process are electric field strength (E), pulse duration or pulse width (τ), treatment time *(t)*, pulse repetition rate *(f),* wave form of the pulse and treatment temperature. Average electric field strength E is defined as the electric potential difference V for two given points in space divided by the distance d between them $E = V / d$.

The PEF treatment time is defined as $t = n\tau$, where n number of pulses, τ pulse width. In general, an increase in any of these variables results in an increase in microbial inactivation (Barbosa-Ca`novas, Gongora-Nieto, Pothakamury & Swanson, 1999).

The critical treatment time, t_c beyond which the microbial survival rate decreases linearly with respect to treatment time in a log-log plot, ranges from 2 to $80\mu s$ (Hulsheger, Potel & Niemann, 1983). However, this depends largely on the electric field intensity, conductivity of the treated liquid and treatment temperature.

For a given configuration of electrodes the electrical field can be found at every point by solving Laplace's equation ($\nabla^2\varphi = 0$) for a given boundary conditions. At the microbial membranes, which are electrically insulating, the electrical field produces an accumulation of charges with opposite polarity on either side of the bi-layer structure. Pore formation occurs when a certain threshold value of the electric field strength E is exceeded (Heinz, Alvarex, Angersbach & Dietrich, 2001).

Electric field pulses may be applied in the form of exponential decaying (Fig. 1.1), square wave (Fig. 1.2), and bipolar (Fig. 1.3). Exponential decay pulse has a long tail with a low electric field, during which excess heat is generated into the food without bactericidal effect (Wouters & Smelt, 1997).

Square wave pulse maintains a peak voltage for a longer time than exponential decay pulse. Although both waveforms inactivate microorganisms, square wave pulses save energy and require less cooling effort (Wouters & Smelt, 1997).

Bipolar pulses are more lethal than monopolar pulses; the alternating stress produced by the bipolar pulses results in structural fatigue of the membrane and enhances its susceptibility to electrical breakdown, even when the amplitude of the cell membrane motion is not large enough to result directly in a mechanical breakdown (Chang, 1989). Bipolar pulses also offer the advantages of minimum energy utilization, reduced deposition of solids on the electrode surface, and decreased food electrolysis. Using monopolar pulses, charged particles inside the liquid food migrate to electrodes with opposite polarity, which will cause an electric field distortion (Qin, Zhang, Barbosa-Ca`novas, Swanson & Pedrow, 1994).

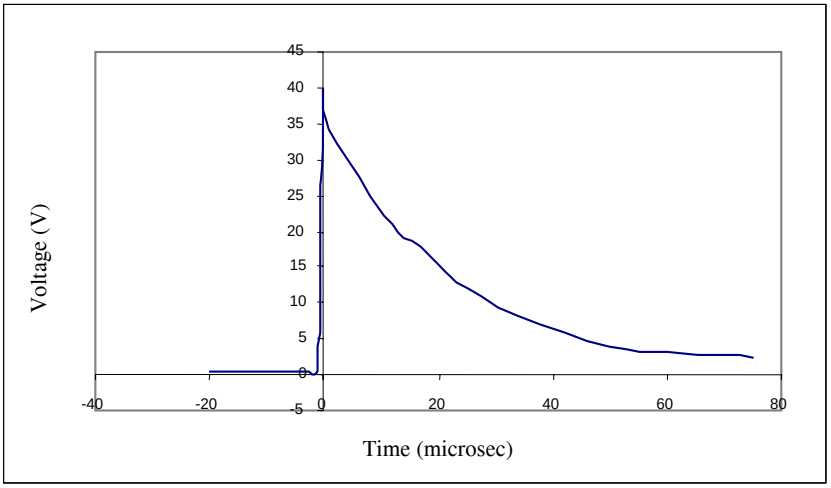

Fig. 1.1: A voltage trace of an exponential decaying pulse wave (Barbosa-Ca`novas & Gongora-Nieto, Pothakamury & Swanson, 1999).

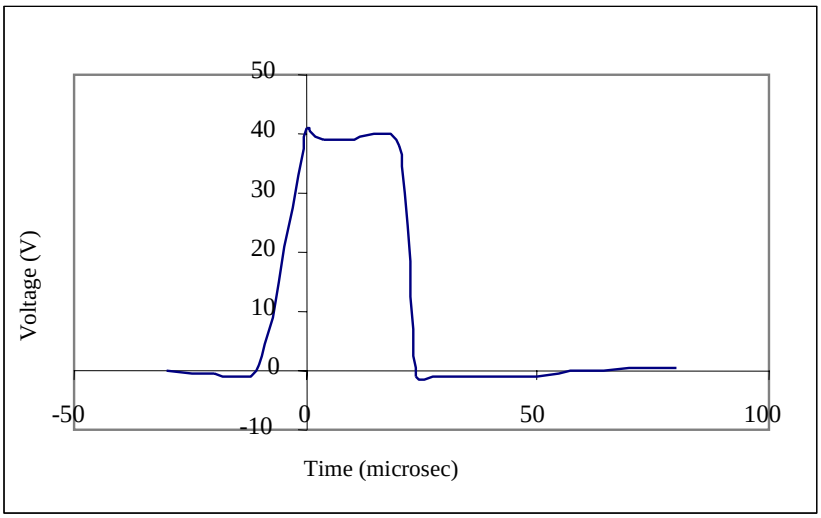

Fig. 1.2: A voltage trace across a treatment chamber of a square pulse wave (Barbosa-Ca`novas & Gongora-Nieto, Pothakamury & Swanson, 1999).

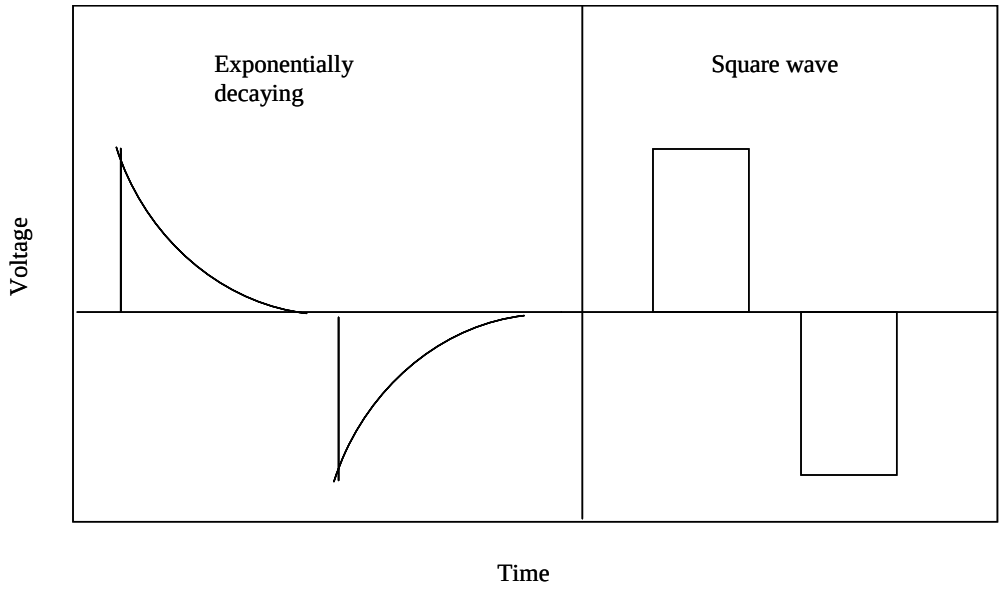

Fig. 1.3: An ideal voltage trace of bipolar pulse wave shapes.

The chemical changes in the processed food can be minimised by controlling the process conditions. A proper selection of pulse duration can minimise the potential for electrochemical change. All electrochemical changes occur near the field region (Fig. 1.4). When an electric pulse is applied to liquid food, most of the early energy in the pulse is consumed by the orientation of water molecules as shown in (Fig. 1.5), the oriented layers of water dipoles are formed at each electrode. These layers result in a delay in the appearance of the full potential of the electrical pulse in the near field region at the electrodes (Fig. 1.6), where all electrochemical reactions occur. The delay in the field potential near the field region at the electrodes, means that insufficient electromotive force is available to initiate electron exchange reactions for some time during the pulse

(Barbosa-Ca`novas & Zhang, 2001). Thus, by selecting appropriate pulse duration, it is possible to prevent the occurrence of specific reactions and the potential for electrochemical change to be minimized (Barbosa-Ca`novas & Zhang, 2001).

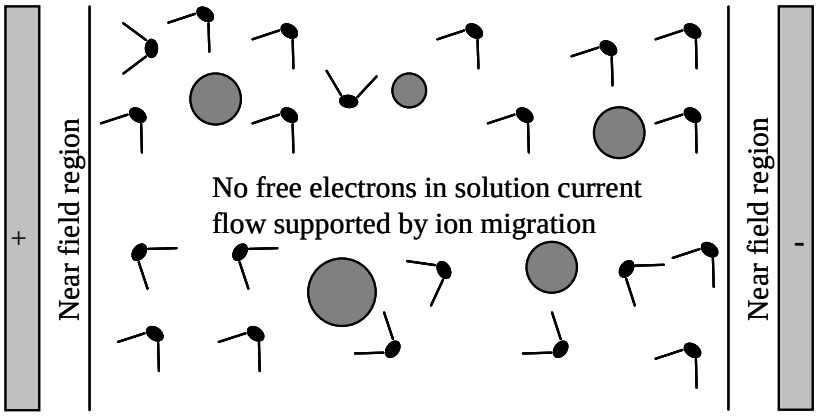

Fig. 1.4: Ion migration in a parallel plate chamber (Barbosa-Ca`novas & Zhang, 2001).

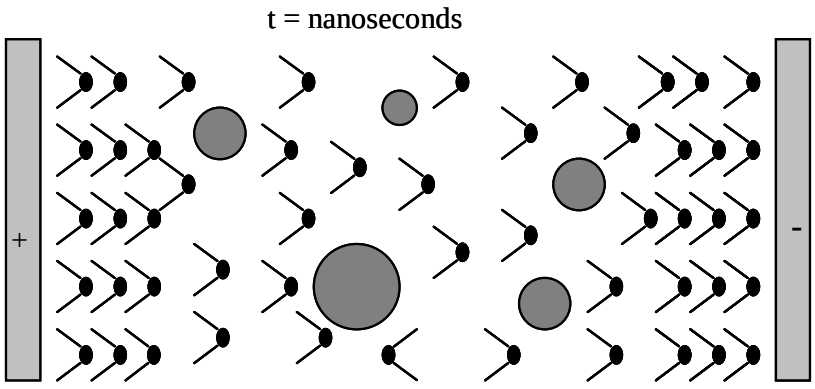

Fig. 1.5: Orientation and distribution of ions and electrodes due to capacitive effect (Barbosa-Ca`novas & Zhang, 2001).

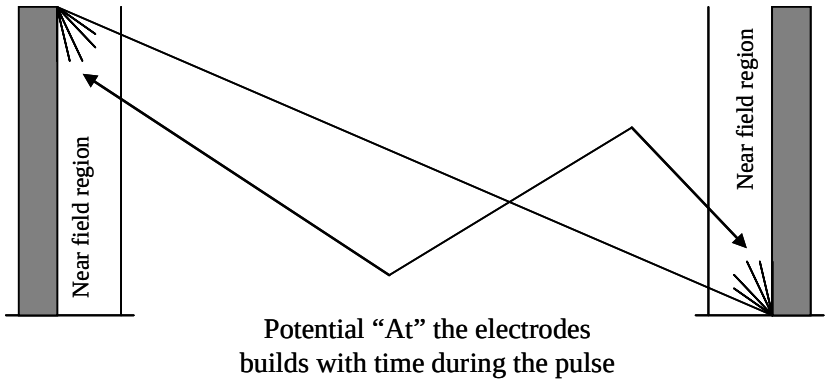

Fig. 1.6: Electrical-field in near field of the electrodes (Barbosa-Ca`novas & Zhang, 2001).

1.3 Electric and Dielectric Properties of Fluid Foods

The physical structure of food products is closely related to its dielectric strength. A homogeneous liquid with low electrical conductivity provides ideal conditions for PEF treatment (Qin, 1995). When food is subjected to electric field, polarisation of dipole molecules and movement of charged molecules takes place and induces a capacitive and a resistive currents. The circuit model for dielectric polarisation is a capacitor and the circuit model for charge carrying conduction is a resistor. The combined circuit can be explained as a parallel resistor-capacitor as shown in (Fig. 1.7) (Zhang, Barbosa-Ca`novas & Swanson, 1995).

Assuming the food material has homogeneous dielectric and electrical properties, the effective capacitance (C) and effective resistance (R) can be calculated as:

$$C = \frac{\varepsilon_O \varepsilon_r A}{d} \quad \text{.....(1.1)}$$

$$R = \frac{d}{\sigma A} = \frac{\rho d}{A} \quad \text{.....(1.2)}$$

Where, ε_r is the permittivity of free space, ε_o is the relative permittivity d is the gap between two electrodes, A is the electrode area, ρ is the resistivity of the food and σ is the conductivity of the food.

In general, dielectric constant increases with increasing water content and decreases with increasing temperature (Zhang, Barbosa-Ca`novas & Swanson, 1995).

When the applied field strength E becomes equal to the dielectric strength $E_в$ of the food, dielectric breakdown of the food inside the treatment chamber takes place as a bright luminous spark with explosive sound. The passage of a spark through the liquid is characterized by a large electrical current flow in a narrow channel, evolution of bubbles of gas, the formation of pits on the electrodes and an impulsive pressure through the liquid. Dielectric breakdown in pure liquids is attributed to several factors including the electron emission from the cathode surface, collision and ionisation of originally neutral molecules and localized energy input and evaporation of liquid. The smoother the cathode surface, the smaller the probability of dielectric breakdown (Lewis, 1968).

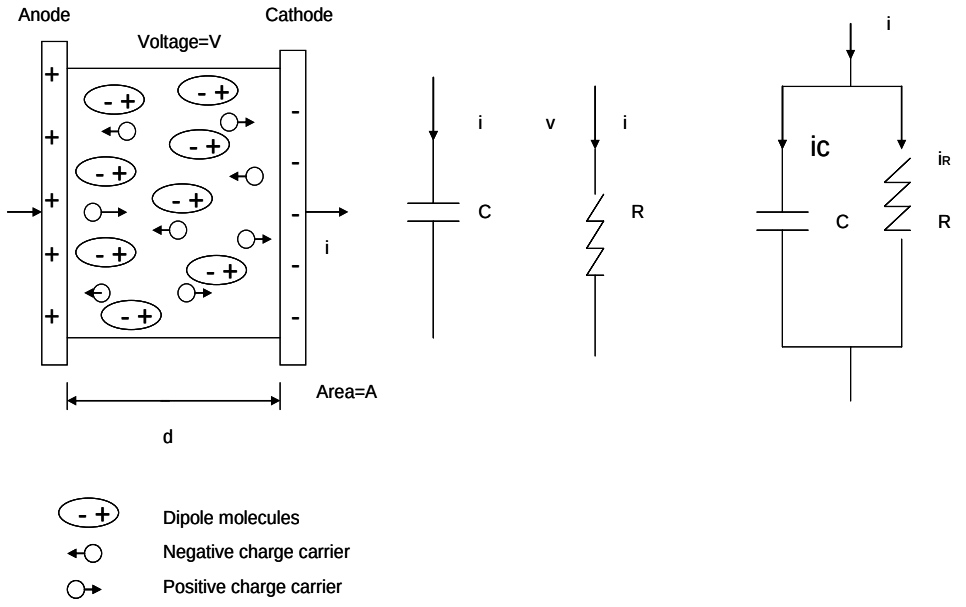

Fig. 1.7: Fluid food subject to a dynamic voltage. (a) Polarization and electronic currents. (b) Dielectric circuit model. (c) Electronic circuit model. (d) Combined circuit model for homogeneous fluid food (Zhang, Barbosa-Ca`novas & Swanson, 1995).

Dielectric breakdown is also attributed to the presence of impurities. It is more frequent to have dielectric breakdown at the gas-liquid or at the liquid-solid interface than in homogeneous liquids (Krasuchi, 1968).

At Washington State University, tests show that dielectric breakdown takes place when a gas bubble is present inside the chamber above some critical level. When the bulk temperature of the fluid food increases, the solubility of the gas in the liquid decreases, resulting in gas bubble formation. A second source of bubble formation is local heating which promotes partial vaporization.

The probability of dielectric breakdown in foods can be reduced by the use of a smooth electrode surface to minimize electron emission, the use of round

electrode edges to prevent electric field enhancement near sharp edges, designing the treatment chamber to provide uniform electric field strength so that the actual applied field strength dose not exceed the dielectric strength of the fluid foods under the test conditions, degassing prior to treatment to eliminate gas bubble formation, and pressurizing the fluid food within the treatment chamber to prevent gas bubble formation (Zhang, Barbosa-Ca`novas & Swanson, 1995)

1.4 Objectives of Research

Pulsed electric fields process is one of the emerging processes for non-thermal food preservation. This new technology is of great importance to the dairy industry and many other liquid food products. The process can accomplish food pasteurisation with short treatment times, small increase in food temperature. Extensive literature review has been done on the advantages of applying the method to food pasteurisation. The studies conducted by previous investigators include the design of treatment chambers and high voltage pulsers design. However, much more work has been done on the inactivation of specific microorganisms and on food quality using this non thermal processing technology. The following objectives were set based on our literature research and understanding of the technology:

- Design an innovative treatment chamber and investigate the effect of different geometries of the treatment chamber to concentrate the electric field intensity in the treatment zone, maintain uniform electric field distribution and minimised the change in temperature in the treatment zone

to prevent the problem of electrodes fouling , sparking and electrical breakdown during processing and to reduce the energy consumption during processing;

- Design and construct an innovative continuous pulsed electric fields system (including, high voltage pulse generator, different treatment chamber/s, cooling system, degassing unit, process monitoring and control systems and instrumentation) for non thermal pasteurization, which can provide effective treatment, which can be scale up for commercial applications;
- Experimental studies on the effect of process parameters (electric field strength, treatment time, treatment temperature) and treatment mediums on the energy requirements and on microbial inactivation rate to determine the optimal operating conditions;
- Study the mechanism under which microbial inactivation occur based on the morphological changes to Escherichia coli ATCC 25922 following PEF treatment using transmission electron microscopy (TEM);
- Evaluate the kinetics of microbial inactivation due to the combined PEF/ thermal treatment using the measured values of the inactivation rate constant, and the values of the activation energy and Arrhenius constant that we determined. The analysis will be based on the once-through process (no circulation).
- Mathematical modelling of the continuous operation taking into consideration the total recirculation of the liquid food leaving the PEF

chamber. The mathematical model includes the mass balance and the kinetic equations.

1.5 Thesis Outline

Part 1: Literature Review

Chapter One

This chapter had presented a general background of the PEF technology, overview of the basics of the pulsed electric fields processing for the non thermal pasteurisation of milk and liquid food products, key variables involved in a PEF process and electric and dielectric properties of food with some suggestions based on the literature to reduce the probability of dielectric breakdown in foods during processing. The objectives of this research were also discussed.

Chapter Two

Literature review on the work reported on PEF system design including high voltage pulse generators, treatment chambers, process monitoring and control systems, and aseptic packaging. Although PEF systems and equipment used in the different laboratories are often quite dissimilar, some important similarities in the conclusions reached are evident as will be discussed in this chapter. Explanation will be given on how the energy from a high voltage power

supply is discharged through the treated food material, which provides a starting point for understanding the mechanisms under which the PEF works.

Chapter Three

Factors that affect the pulsed electric fields process will be discussed based on information available in the literature. The main factors are classified as process parameters including (electric field intensity, treatment time, treatment temperature and pulse wave shape and frequency); microbial physiology factors (type of microorganisms, concentration, growth phase and culture temperature) and treatment medium related parameters (physical and chemical characteristics of the product) will be also discussed.

Chapter Four

This chapter reviews the principles of microbial inactivation using pulsed electric fields processing. In the literature, it was generally accepted that the main effects of PEF are produced in the cell membrane, although the disruption of internal organelles and other structural changes has also been proven. The proposed mechanism of the microbial breakdown and the different theories that explain the microbial inactivation when using PEF technology are also discussed.

The effect of PEF on food quality and enzymes activity will be also reviewed in this chapter. PEF has been shown as non thermal processes that inactivate microorganism and enzymes without significant adverse effects on vitamins, color, flavor and other nutrients of the food. The sensitivity of enzymes

to the PEF treatment varies form enzyme to enzyme, depending on the electric field parameters and enzyme structure.

Part 2: Experimental Investigations

Chapter Five

This chapter presents the design of an innovative multi pass treatment chamber/s, and investigations on different dimensions of the treatment chamber/s. The effect of using different dimensions of the electrodes and insulator on the electric field strength in the treatment zone will be discussed.

A study is conducted to assist in choosing the proper process parameters to achieve effective treatment. Experimental investigations conducted on thermal performance of the designed multi pass chamber including the variation of current when changing the voltage, total resistance across the chamber, temperature increase using different electric field intensity and flow rates and the dissipated power during the PEF application. This study was done to achieve uniform distribution of the electric field and hence uniform treatment of the food.

Chapter Six

This chapter describes the PEF experimental set-up and the design of the innovative continuous laboratory scale pulsed electric fields system that we have designed and constructed, with full description of the main components of the system. The full system is composed of a high voltage pulse generator,

continuous treatment chambers, cooling system, degassing unit, voltage and current measuring devices, control units, data acquisition systems, aseptic packaging and cleaning in place.

Chapter Seven

Microbial inactivation of gram negative Escherichia coli ATCC 25922 suspended in simulated milk ultra filtrate of different concentrations using PEF treatment is presented. The effect of the electric field intensity, treatment time, processing temperature, electrical energy input and the ionic strength of the suspension on the inactivation rate of this specific microorganism are discussed. The methods and materials used, experimental protocols, results and discussions and a summery of conclusions are presented.

When an electric field is applied to a food in a PEF chamber, it is hypothesized that microbial inactivation takes place due to biological changes. The morphological changes of E coli suspended in SMUF (50% w/w) as a result of PEF treatment were investigated experimentally under transmission electron microscopy (TEM). The mechanism in which the microbial cells are inactivated will be discussed with the assistance of some pictures taken using TEM for the treated and untreated cells.

Error analysis of the experimental work and the values that are obtained on our experiments are assessed for precision. The mean, standard deviation, %error and the confidence interval of a set of replicate measurements are presented in Appendix D.

Chapter Eight

The inactivation kinetics of Escherichia coli using combined PEF and thermal treatment based on modified Arrhenius equation is presented for the first time in the literature. Inactivation rate constant for the E coli suspended in SMUF (50, 66.6 and 100% w/w) as a function of temperature and electric field will be presented and used to calculate the parameters in Arrhenius equation.

The PEF process is carried out using a single-pass operation and sometimes recirculation operation in order to achieve a desirable microbial inactivation. Analysis of the recirculation mode is presented in this chapter and a comparison between measured (experimental) and predicted (theoretical) variation of Escherichia coli concentration with time after the PEF treatment will be discussed.

Chapter Nine

This chapter presents overall discussions and conclusions based on the major findings of this study and on literature review.

2. Chapter Two: Review of Pulsed Electric field Systems

This chapter presents the major development made in the design of the PEF system. Description of the main components that are associated within the pulsed electric fields systems is given, especially for the treatment chambers and pulse generators.

2.1 Historical View

The roots of pulsed electric field processing can be traced to Germany. Dovenspeck (1960) described a variety of pulsed electric field (PEF) equipment and methods ranging from PEF processing of sausage to specific electronic embodiments. Dovenspeck remained active for many years and collaborated on PEF development with later German investigators (Barbosa-Ca`novas & Zhang, 2001).

Sale & Hamilton (1967) and Hamilton & Sale (1967) provided important studies on microbial cellular repair after PEF treatment (Barbosa-Ca`novas & Zhang, 2001). They believed that when an external electric field is applied to a cell, transmembrane potential is induced across the cell membrane. The induced potential difference is proportional to the external field intensity. The transmembrane potential leads to membrane damage, which is the direct cause of

cell inactivation (Barbosa-Ca`novas, Gongora-Nieto, Pothakamury & Swanson, 1999). The membrane breaks down when this potential reaches a critical value (which is approximately *1 Volt* for a bimolecular lipid membrane). If the electric field is switched off, the membrane will return to the initial normal state. But if the electrical field strength exceeds the critical value (E_C) for a given length of time(t_c), permanent holes will form in the cell membrane. The resulting inactivation of microorganisms thus considered to be related to both the electric field strength and treatment time (Sale & Hamilton, 1967 and Hulsheger, Potel & Niemann, 1983).

The application of PEF to a fluid may be conducted in a batch or continuous operation. The continuous is a more interesting operation from technological point of view, in which the fluid food flows continuously through a PEF chamber where the electric field is applied. In a batch operation, the food placed in the treatment chamber where it receives several pulses (Barbosa-Ca`novas & Zhang 2001).

PurePulse Technologies owns several U.S. patents on PEF units for the treatment of liquid foods, such as dairy products, fruit juices, and fluid eggs (Dunn & Pearlman, 1987, Bushnell, Dunn, Clark & Pearlman, 1993 and Bushnell, Dunn, Lloyd & Clark, 1996). The patents described batch and continuous systems, including chamber characteristics, pulse forming network (PFN) components, and specific switching arrangements to avoid electrode fouling.

Washington State University (WSU) has also a comprehensive program to preserve foods by high intensity PEF. The PEF system designed and constructed by WSU group includes a power supply capable of delivering a peak voltage of $40kV$ and parallel plate and coaxial continuous treatment chambers. Design of the treatment chamber was based on both experimental and computer simulation (Barbosa-Ca`novas, Gongora-Nieto, Pothakamury & Swanson, 1999).

Another leading group in this technology is that of Ohio State University. They have implemented an integrated pilot plant system with aseptic packaging (Zhang, Qui & Sharma, 1997). The system has different treatment chamber configuration, with co-field treatment zones and a pulse forming network (PFN) capable of delivering energy at frequencies in the range of *kHz* that can inactivate more than 99% (2-log reduction) of a mediums bacterial spores (Barbosa-Ca`novas, Gongora-Nieto, Pothakamury & Swanson, 1999).

Several European groups have also shown interest in the application of PEF technology to food processing. Determination of the inactivation mechanisms and kinetics of several microorganisms in model and real foods is the most important contribution of these groups (Hamilton & Sale, 1967, Hulsheger, Potel & Niemann, 1983 and Grahl & Markl, 1996). In the fields of medicine and biology, PEF has been applied for the reversible or irreversible permeabilization of various biological systems (Chang, Chassy, Saunders & Sowers, 1992).

Although many publications have shown the possibility of using PEF treatment as an effective method to inactivate microorganisms, it is hard to

compare the results due to the wide variety of equipment and experimental conditions used. Comparing the performance of the different PEF systems used is one of the objectives of our work.

2.2 The Design of PEF Processing Equipment

The generation of pulsed electric fields requires major devices: a system for generating high-voltage pulses and a treatment chamber which converts the pulsed voltage into pulsed electric fields (Fig. 2.1). A PEF system also includes cooling devices, voltage and current measuring devices, a control unit and data acquisition system. The pulse power supply is used to generate high voltage from low utility level voltage, and the former is used to charge a capacitor bank with set of switches to discharge energy from the capacitor across the food in the treatment chamber. After processing, the product is cooled and packed aseptically.

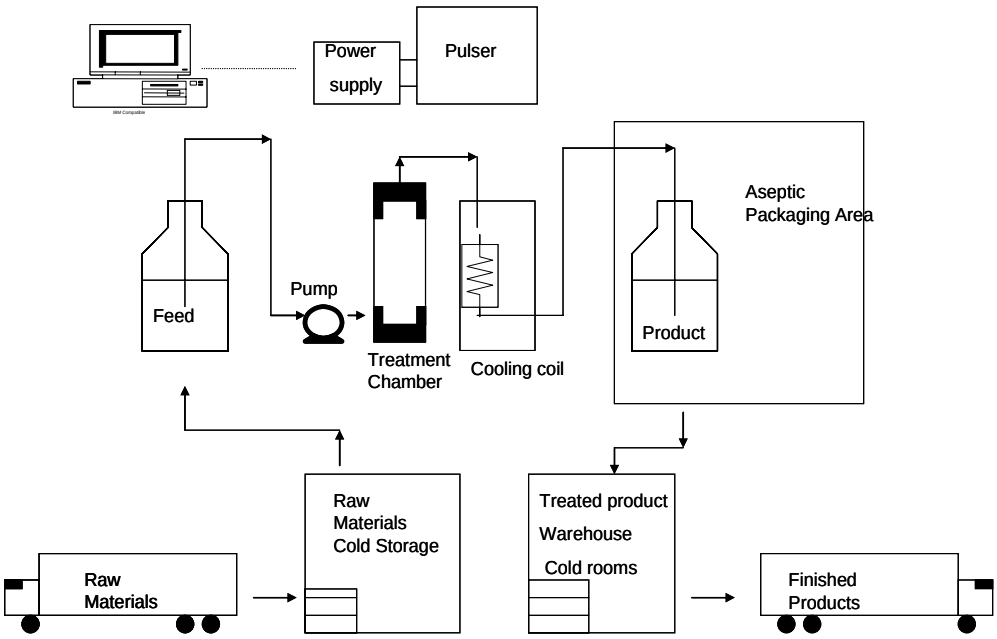

Fig. 2.1: Pulsed electric field unit operations layout (Vega-Mercado, Gongora-Nieto, Barbosa-Ca`novas and Swanson, 1999).

2.2.1 High Voltage Pulse Generators

Pulsed high voltage power supplies, as illustrated in (Fig. 2.2) converts normal utility voltage into high-intensity voltage, and can supply either direct current (D.C.) or capacitor-charging power. A capacitor bank is used to store large amounts of energy. When the capacitor is charged, high voltage is applied to the treatment chamber by using high-voltage switches to discharge the stored power (Barbosa-Ca`novas, Gongora-Nieto, Pothakamury & Swanson, 1999).

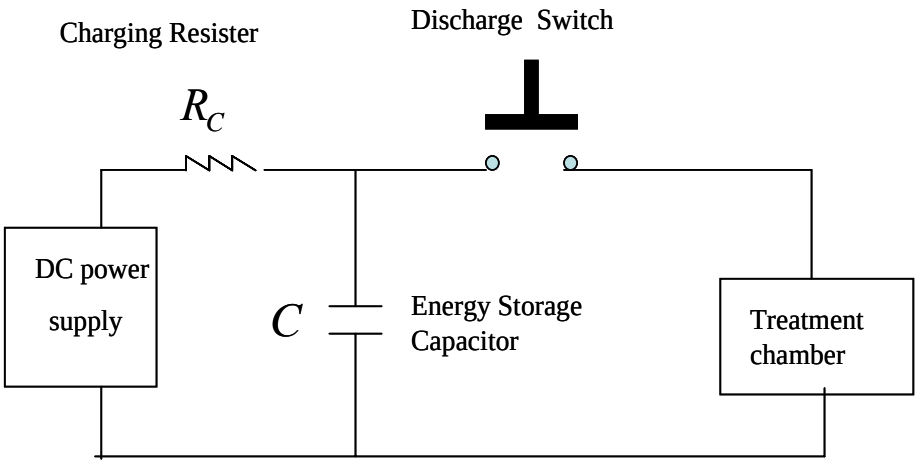

Fig. 2.2: High voltage pulse generator system (Barbosa-Ca`novas, Gongora-Nieto, Pothakamury & Swanson 1999).

The electrical circuit necessary for generating exponential pulses is illustrated in (Fig. 2.3). The circuit consists of a DC power supply and a capacitor bank connected in series with a charging resistor (R_C). The charge stored in the capacitor flows through the food in the PEF chamber when trigger signal is applied to close circuit. The resistance R_1 limits the current in case the food sparks over, and the resistance R_2 controls the decay time when the food resistivity is larger than expected. The pulse duration *(sec)* of an exponentially decaying pulse is given by equation (2.1):

$$\tau = RC_0 \quad \text{.....(2.1)}$$

Where, τ is the pulse width, R is the effective resistance and C_o is the capacitive of the energy storage capacitor.

The energy input to the food being treated must be restricted. The energy density $(Q)\left(J/cm^3\right)$ for exponential decay pulse is given by:

$$Q = V_0^2\ C_0\ n\ /2\ \upsilon\ =\ V_0^2\ t\ /2\ R\ \upsilon \quad \text{.....(2.2)}$$

Where, V_O is the initial charge voltage, C_0 is the capacitance, n is the number of pulses, t is the treatment time, R is the food resistance and υ is the volume of the treatment chamber.

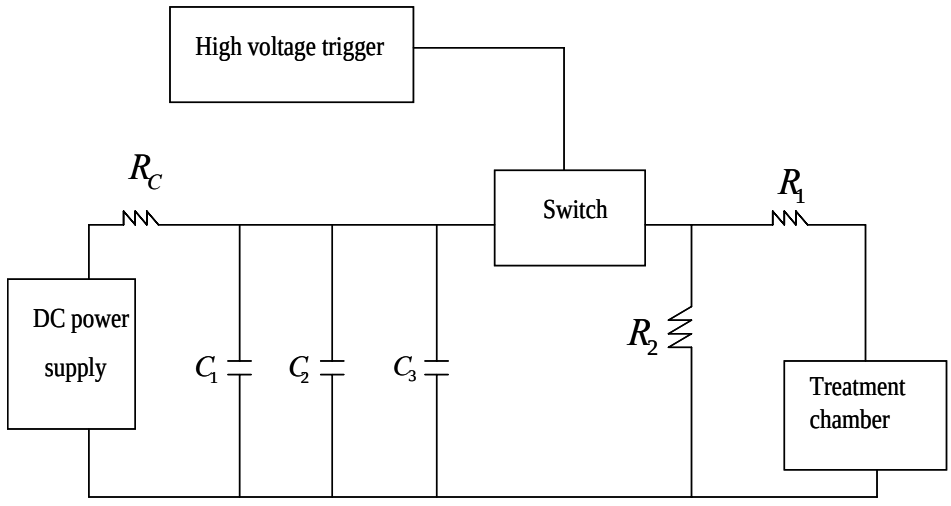

Fig. 2.3: A simplified circuit for producing exponential decay pulses and a voltage trace across a treatment chamber (Barbosa-Ca`novas, Gongora-Nieto, Pothakamury & Swanson, 1999).

To generate square waveform pulses, a pulse forming network (PFN) consisting of an array of capacitors and inductors must be used as illustrated in (Fig. 2.4).

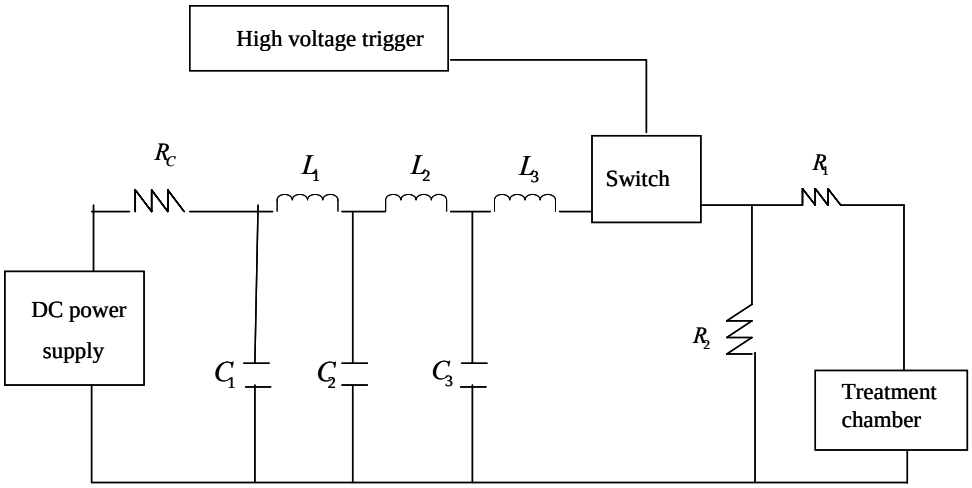

Fig. 2.4: Layout of a square generator using a pulse forming network of three capacitor-inductor units and a voltage trace across a treatment chamber (Barbosa-Ca`novas, Gongora-Nieto, Pothakamury & Swanson, 1999).

The square waveform is obtained when the treatment chamber and PFN have matching impedance. The maximum voltage delivered to the chamber (V_L) is half that of the charged voltage (V_0) on the capacitors and is a function of the impedance of the load given by:

$$V_L = V_0\left(\frac{Z_L}{Z_0 + Z_L}\right) \quad \text{.....(2.3)}$$

Where, Z_0 is the impedance of the PEN and Z_L is the impedance of the load. The voltage delivered to the chamber (V_L) can be equal to the charge voltage by superimposing two pulse forming net works. The energy density for square pulses, energy input is approximated as:

$$Q = V\ I\ \tau\ n / v = V^2 \tau\ n / R\ v = V^2\ t / R\ v \quad \text{.....(2.4)}$$

Where, V is the voltage, I is the current, υ is the treatment chamber volume, n number of pulses and τ is the treatment time (Barbosa-Ca`novas, Gongora-Nieto, Pothakamury & Swanson, 1999).

The energy required to achieve a certain level of microbial or enzymatic inactivation depends on the treatment volume of the chamber, flow rate of the product, number of pulses and their width or treatment time, and system configuration.

Many of the high-voltage pulse generators perform poorly for a number of reasons. Few of the high-voltage pulse generators can maintain the extremely high currents that result from low electrical resistance of the fluid in the treatment chamber. Some of the conventional high-voltage pulse generators can provide only unipolar pulses, these pulses causes the deposition of protein and other charge carrying particles on the surface of the electrodes. Therefore, methods and apparatuses for providing high-voltage pulses that do not suffer from these disadvantages are desirable (Qiu & Zhang, 2001).

Ho, Mittal, Cross & Griffiths (1995) demonstrated the effectiveness of a low-cost pulse generator with a maximum voltage of $30kV$ and pulse width

of $2\mu s$. Zhang, Qui & Sharma (1997) successfully used a versatile pulse-forming network capable of delivering square, bipolar and exponential wave-shape pulses with voltage up to $50kV$.

Qiu & Zhang (2001) presented a high voltage pulse generator system for effective PEF treatment. The system (Fig. 2.5) includes a power source that charges an energy storage component (either a capacitor or a pulse forming network (PFN)) for storing electrical energy from the power source. The particular composition of the energy storage component influence the shape of the high voltage pulse that can be applied (either substantially square or exponential decay pulses) as well as the values of the capacitors and inductors used are dependent on specific design characteristics (i.e. pulse duration, amplitude, etc.). The system also include a device appropriates triggers the opening and closing of plurality of switches, for opening and closing periodically to discharge the energy stored. A pulse transformer is also included for allowing a plurality of voltage and current levels to be generated at the load (treatment chamber).

The switches hold the high voltage stored in the pulse forming network (PFN) and control the current flow reliably when fired. The switches are controlled by a command signal generator, and operate at high repetition rates (0.1-5000Hz). Commonly used types of discharge switches include mercury-ignitron, gas-spark-gap, vacuum spark-gap, thyratron or magnetic, and mechanical rotary switches (Calderon-Miranda, Raso, Gongora-Nieto & Barbosa-Ca`novas, 1999).

A single thyratron can switch very large powers, but because of their high losses their frequencies are limited to a few 100*Hz*. Their main disadvantage, however, is their limited lifetime. Solid-state semiconductor switches are considered by the experts as the future technology of high power switching. New generations of the solid-state semiconductor switches is the Insulated Gate Bipolar Transistors (IGBTs), combined the best features of a metal-oxide semiconductor field-effect transistor (MOFET) input and a bipolar transistor output into a newer power-switching device. Its advantages are very rapid switching and small power consumption (Bartos F. J., 2000).

The high voltage pulse generator used in this project was designed and constructed in The University of Auckland using the modern IGBT technology, and will be presented in Chapter 6.

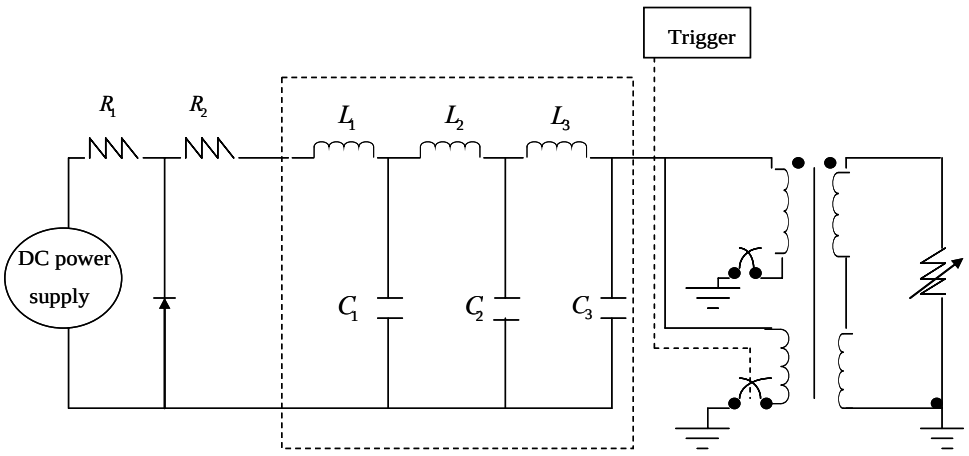

Fig. 2.5: Circuit diagram of a high-voltage bipolar pulse generator (Qiu & Zhang, 2001).

2.2.2 Design of Treatment Chamber/s

One of the key components in the PEF pasteurisation process is the treatment chamber. It must be designed to operate at high electric field intensities. When achieving high electric field intensity in the PEF treatment chamber, dielectric breakdown inside the chamber must be prevented. The breakdown may be introduced by local field enhancement on the electrodes, electrical tracking along the insulator surface, and/or gas bubbles in liquid foods. Uniform electric field distribution inside the treatment chamber minimises the electrical breakdown (Qin, 1995).

Sale & Hamilton (1967) were among the earliest researchers to study the inactivation of microorganisms in a static PEF chamber. A treatment chamber consists of two electrodes held in a position by an insulating material that form an enclosure containing the food materials. Carbon electrodes supported on brass blocks were used and placed in a U-shaped polythene spacer. Maximum electric field of $30 kV/cm$ can be applied due to the electrical breakdown caused by the presence of air above the food. Another static chamber was designed at WSU, using two round-edged disk-shaped stainless steel electrodes polished to mirror surfaces. Electrical field strengths up to $70 kV/cm$ have been applied. Treatment chambers with parallel plate electrodes offer a uniform electric field distribution along the gap axes and electrode surfaces, but create a field enhancement problem at the edges of the electrodes. Static chambers are mainly suitable for laboratory use, while for larger scale operations, continuous chambers are more efficient.

Martin, Qin, Chang, Barbosa-Ca`novas & Swanson (1997) suggested that PEF treatment in continuous systems is more effective in terms of microbial inactivation than in static systems, due to the greater treatment uniformity in the continuous systems. In 1987, Dunn & Pearlman designed a continuous chamber consisting of two parallel plate electrodes and a dielectric space insulator. In an effort to avoid product contact with the electrodes, the electrodes were separated from the food by ion conductive membranes.

Parallel plates, parallel wires and concentric cylinders were the possible electrode configurations discussed by Hofmann (1989). The electrodes can be made of stainless steel and electro-chemically inert materials such as gold platinum, carbon and metal oxides.

The parallel plates produce uniform electric field strength distribution in a large viable area, with a gap sufficiently smaller than the electrode surface, but it created a field enhancement problem at the edges of the electrodes. Disk-shaped, round-edged electrodes can minimize electric field enhancement and reduce the possibility of dielectric breakdown of the fluid food (Dunn & Pearlman 1987). Concentric cylinders provide smooth and uniform product flow, and are attractive in industrial applications (Zhang, Barbosa-Ca`novas & Swanson 1995).

To prevent direct electrolysis of the fluid food upon the application of a pulsed electric field, the electrodes may include an ion permeable membrane and an intermediate electrolyte, such that ionic electrical connection can be made with the fluid food through the ion permeable membrane rather than by direct contact with the electronically conductive electrode (Dunn & Pearlman 1987).

The attempts, which have been made by different investigations to improve the efficiency of the PEF treatment chambers, are classified as below.

2.2.2.1 Designs to Concentrate the Electric Field Intensity in the Treatment Region

The intensity of the applied electric field is one of the most important factors influencing microbial inactivation by PEF. Once the applied electric field exceeds a critical value for a given length of time, transmembrane potential is induced which results in cell death. Matsumoto, Satake, Shioji & Sakuma (1991) applied the concept of concentrating the electric field in the treatment region. They devised a converged electric field treatment chamber shown in (Fig. 2.6). An insulating plate with small holes was placed between the parallel disc electrodes to concentrate the electric field. Only the fluid inside the holes of the plate was subjected to the voltage pulsed electric field treatment. The current density at the electrode-liquid interface was held low to minimize electrolysis and reduce bubble formation. Three to six log reduction of microbial inactivation in S. cerevisiae; E. coli and B. subtilis using $30\text{-}40 kV/cm$ of electric field treatments were achieved. In this design, there exist stagnant zones in the 90^{o} corners of the cell, where microbes can build up and the liquid food may overheat undesirably. Such a stagnant zone may also prone to trap air bubbles, which could cause sparking. If the treatment outside the disc electrode was important, then the liquid in the centre of the cell would receive less electric pulses because of its shorter residence time.

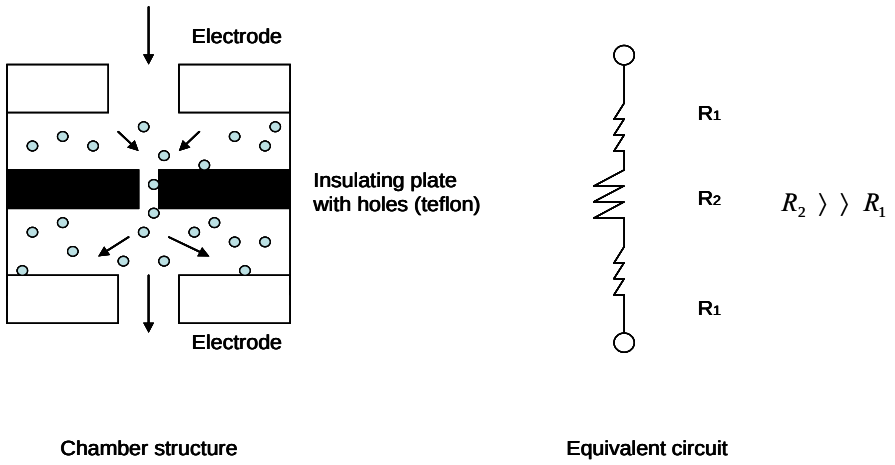

Fig. 2.6: Cross section view of treatment chamber (Matsumoto, Satake, Shioji & Sakuma, 1991).

Another design to concentrate the electric field in the treatment region was presented by Sensoy, Zhang & Sastry (1996). A continuous co-field flow PEF chamber is shown in (Fig. 2.7) with a conical insulator shape. The actual treatment volume was the small orifice as in the previously discussed design. The special conical shaped electrodes and insulators were designed to eliminate gas deposits within the treatment volume. The volume of the orifice and the conical regions were designed so that the voltage across the orifice was close to the supplied voltage. Electric field strength of $15\text{-}40 kV/cm$ was used. The problem of stagnant zone present in the previous design was minimized in this design. However, again if the treatment outside the disc electrode is important, then the

liquid in the centre of the cell will receive less treatment reducing the efficiency of the process.

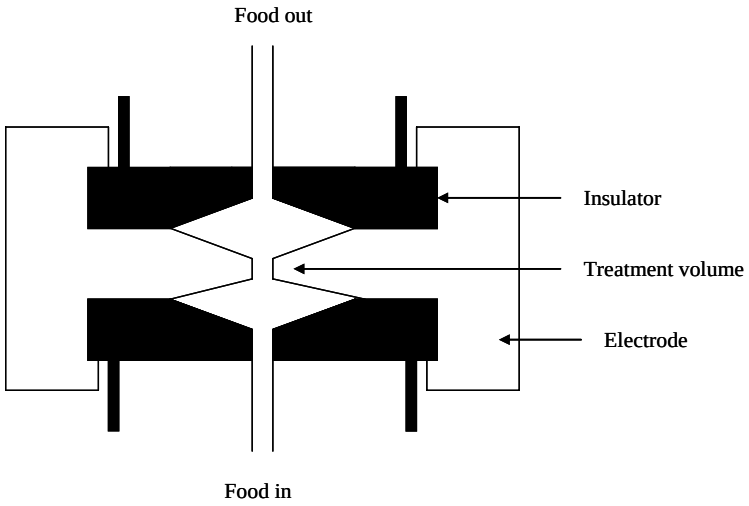

Fig. 2.7: A co-field continuous treatment chamber (Sensoy, Zhang & Sastry, 1996).

2.2.2.2 Designs to Adjust the Treatment Time

With constant pulse duration, microbial inactivation increases with the increase of the number of pulses. In general, increasing the number of pulses and/or their duration results in an increase in microbial inactivation (Barbosa-Ca`novas, Gongora-Nieto, Pothakamury & Swanson, 1999).

WSU research group modified the static parallel plate electrode chamber designed by Barbosa-Ca`novas, Gongora-Nieto, Pothakamury & Swanson, (1999) as shown in (Fig. 2.8), to increase the residence time of the liquid food

inside the treatment chamber. Baffled flow channels were added inside the chamber to provide a tortuous path of the fluid food in the treatment zone. Cooling of the chamber was provided by circulating cooling water through jackets built within the two stainless steel electrodes. Operating conditions were as follows: chamber volume 20-8 cm^3, electrode gap 0.95 or 0.5*cm*, PEF strength 35 or 70*kV/cm*, pulse width 2-15μs, pulse repetition rate 1*Hz* and food flow rate 600-1200 $cm^3/\min$. In this design the field intensity that could be applied to the food was limited because of the interface between the liquid food and the insulating spacer. This increases the chance of electric breakdown produced by electrical tracking along the insulator surface separating the two electrodes.

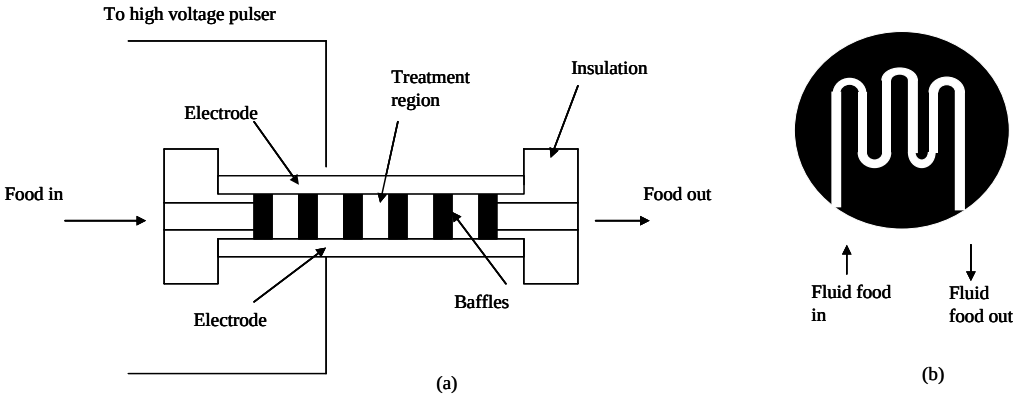

Fig. 2.8: Continuous treatment chamber with baffles, WSU group. a) Cross section view. b) Top view (Zhang, Barbosa-Ca`novas & Swanson, 1995).

Dunn & Pearlman (1987) built a continuous treatment chamber where the fluid food flow through a series of treatment chambers to provide the desired treatment time as shown in (Fig. 2.9). The chamber comprises a plurality of cylindrical shape electrode reservoir zones, which were electrically isolated from each other by intervening dielectric separating elements so that only the electrical current will pass through the fluid food product itself. The operating conditions for the system was from $5kV/cm$ to $25kV/cm$ with a square and exponentially decaying pulses having pulse width of 1 and $100\mu s$ and frequency of 0.1 and $100Hz$. The disadvantage of this cell was that the uniformity of the electric field distribution degrades with time as a result of the temperature gradient and hence, non uniform treatment and also the product had the tendency to produce arcs or current filaments (Franc, Francis, Hero & Abraham, 2001).

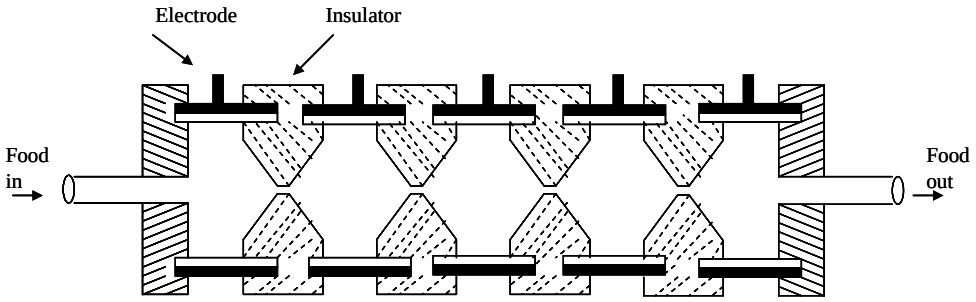

Fig. 2.9: A cross sectional side view of an embodiment of a continuous current, high electric field treatment cell assembly (Dunn & Pearlman, 1987).

2.2.2.3 Designs Based on Electric Field Distribution

Numerous researchers reported treatment chamber designs of coaxial cylinder-electrode configurations. The co-axial device was characterised by a well-controlled uniform electrical field and simple chamber structure. Coaxial chambers were basically composed of an inner cylindrical electrode surrounded by an outer annular cylindrical electrode that allows food to flow between them (Barbosa-Ca`novas, Gongora-Nieto, Pothakamury & Swanson, 1999).

The co-axial device configurations described by Boulart (1983), Hofmann & Evens (1986) and Sato & Kawata (1991), were not suitable for high intensity electric fields because of possible electrical breakdown from the insulator surface tracking or local field enhancement. The design of these chambers did not provide a uniform treatment across the treated volume since the radial geometry cause the field strength to decrease towards the outer electrode (Qin, 1995). One challenge associated with coaxial chambers was that they generally present low load resistance when used to treat most foods, and the pulser system must be able to deliver high current at the voltage employed.

Bushnell, Dunn, Clark & Pearlman (1993), designed another coaxial chamber, the major disadvantage of the chamber was the limited width of the annulus through which the product can flow and the relatively large electrode surfaces, although it provides a long tracking path on the insulator surface to assure complete treatment of the food product as it flows through the electrode region.

Qin (1995) modified the coaxial treatment chamber design to provide a uniform electric field distribution as illustrated in (Fig. 2.10). The electrode configuration in the chamber was obtained through an optimised shape design using computational fluid dynamics (CFD) analysis. The outer electrode has a protruded contour surface. A prescribed field distribution along the fluid path without electric field enhancement points was observed. The electric field region between the high voltage electrode and the grounded electrode in the coaxial treatment chamber is illustrated in (Fig. 2.11). The chamber includes cooling systems for controlling the electrode temperature. The outer electrode had a protruded contour surface obtained using CFD analysis based on theoretical simulation. In the treatment region between the two electrodes, the potential drop is nearly uniform and a strong electric field is generated. On the other hand, in the non treatment zone most of the potential drop was found inside the dielectric spacer, thus the electric field was quite weak in the liquid food path outside of the treatment region. The coaxial treatment chamber may operate at an electric field exceeding $70 kV/cm$ without dielectric breakdown (Qin, 1995).

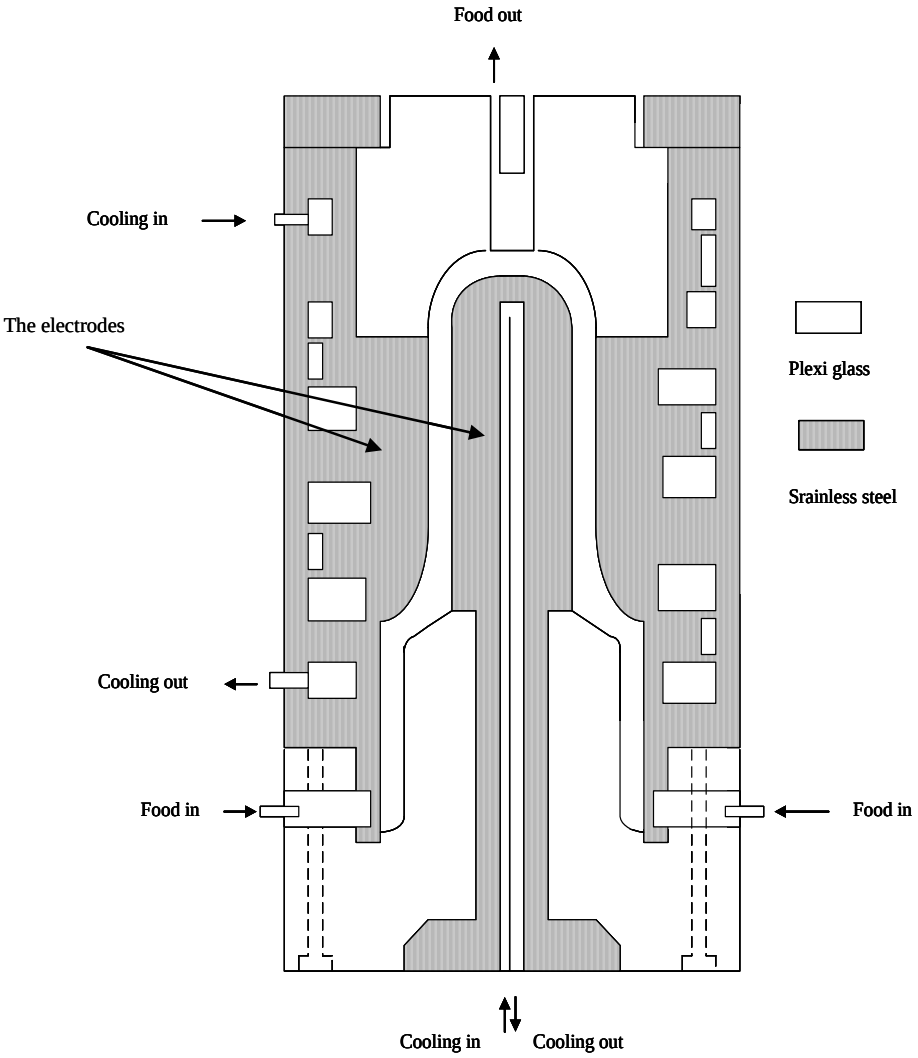

Fig. 2.10: Across sectional view of a modified coaxial treatment chambers (Qin, 1995).

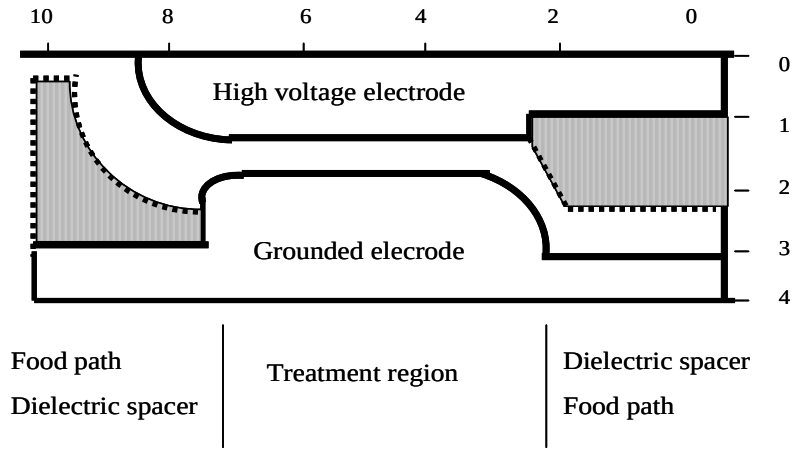

Fig. 2.11: Electric field region between high voltage electrode and grounded electrode in the coaxial treatment chamber (Qin, 1995).

Cornelis & Vincent 2000, described another modular design of PEF treatment chamber as shown in (Fig. 2.12), the chamber comprising of cylindrical shaped electrodes with an open electrode structure with a large aperture. The objective of this design was to solve the problem of non-uniformity in the electric field distribution due to a gradient in the product conductivity at steady state flow conditions. The treatment chamber consisted of several identical modules; each module had a large aperture and a small internal volume. The aperture of each module had a large cross sectional area with respect to the contained volume. By proper positioning of several of these modules the non-uniformity in the electrical field distribution of individual modules was minimised. As a result, the overall

electrical field in the treatment chamber was uniform across the volume. The peak power that was needed can be distributed over several modules which can be fed by different auxiliary pulse power supplies. The disadvantage of this design was that the electrical field distribution of the first and the last cell were slightly non-uniform. This could be due to the fact that translation symmetry in the electrode array was absent.

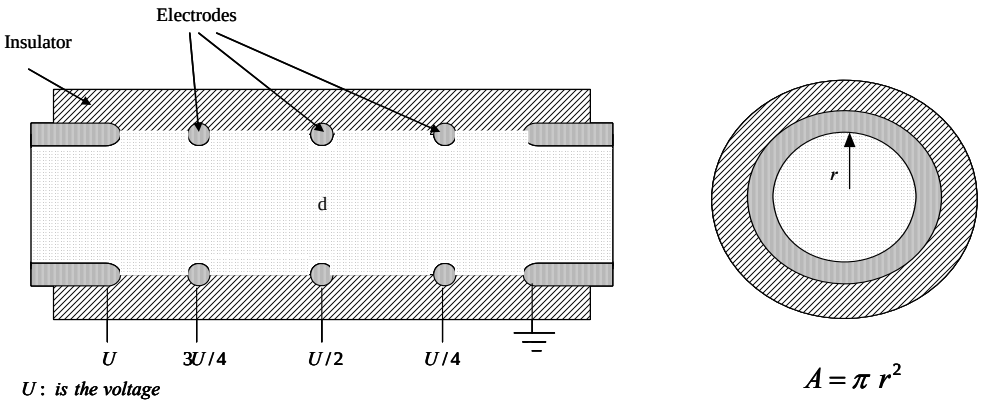

Fig. 2.12: Modular design of PEF treatment chamber (Cornelis & Vincent, 2000).

2.2.2.4 Designs Where the Electric Field Lines are parallel to the Fluid Flow

Yin, Sastry & Zhang (1997) described a co-linear treatment chamber as illustrated in (Fig. 2.13). The treatment chamber comprises of minimum two

cylindrical electrodes and a cylindrical insulator to electrically insulate the electrodes from each other. The insulator flow chamber and the electrode flow chamber were configured to form a single tubular flow chamber for accepting the flow of liquid food product. Pulsed electric field vector direction was parallel to the liquid product flow. The applied electric field intensity was $30 kV/cm$, frequency of 500-20,000Hz and pulse duration of 1 to $6 \mu s$. The non-uniform distribution of the electrical field in the treatment zone was one of the disadvantages of this design. Using small gap distances (with respect to the diameter), the electric field distribution at the entrance and exit of the treatment zone was highly non-uniform. Using larger gap distance, large temperature gradient will appear across the treatment zone at steady state conditions. The temperature of the product at the exit of the treatment zone becomes higher than at the entrance, and as a result the voltage drop over the product column will change, which results in a smaller electrical field in the region near the exit of the treatment zone. This has an adverse effect on the degree of microbiological inactivation. In addition to this temperature effect, the release of minerals and other components by cellular membrane structures would cause a similar effect. This can lead to a change in the conductivity of the product across the treatment zone (for example: due to electroporation of biological membranes intracellular contents can be released) (Cornelis & Vincent, 2000).

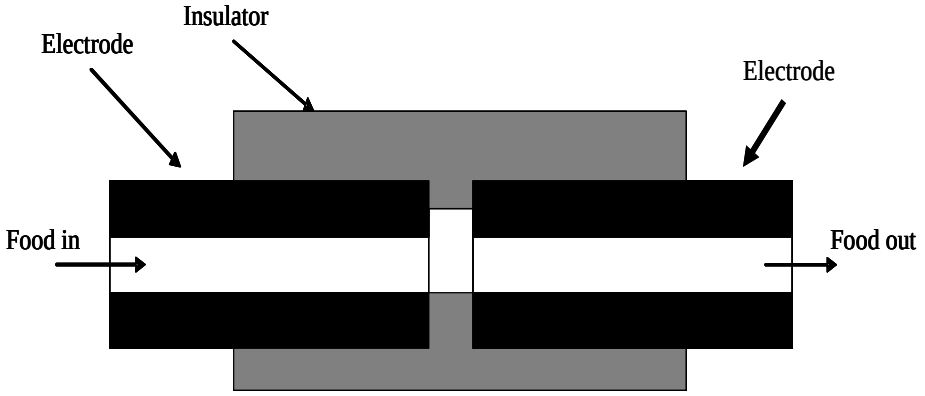

Fig. 2.13: A cross sectional side view of an embodiment of pulse dielectric field treatment device (Yin, Sastry & Zhang, 1997).

2.2.2.5 Designs to Reduce the Energy Input

Dejong & Vanheesch (1999) provided a treatment chamber with a claim of 30% reduction in energy requirements. The electrodes were included in the fluid flow channel, which results in a homogeneous electric field being generated. The residence time distribution of the fluid in the treatment unit was uniform. This in turn limited the increase in temperature of the product, minimise protein denaturation and vitamins destruction. The treatment unit illustrated in (Fig. 2.14) comprises of ten treatment chambers connected in series and the electrodes, which were composed of metal wires (5mm in diameter) to form a net. The fluid flows through the openings of the net. The flow openings have an equivalent diameter of $25mm$. It was possible to achieve electric field strength of $70kV/cm$,

pulse width of $2\mu s$ and frequency of $40Hz$ and residence time of $0.2s$ in each treatment chamber.

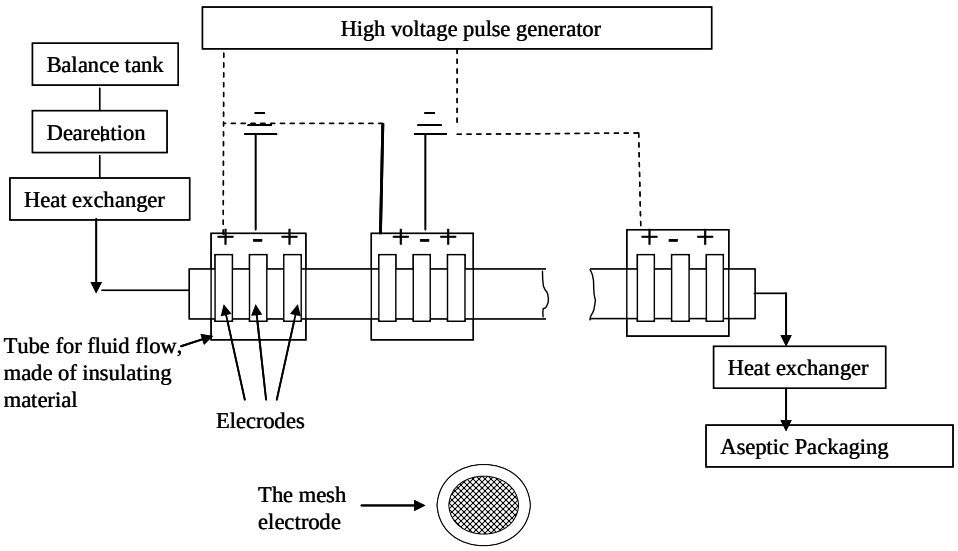

Fig. 2.14: A cross section view of a treatment unit (Dejong & Vanheesch, 1999).

2.2.2.6 Designs Based on Reducing the Risk of Dielectric Breakdowns

Barbosa-Ca`novas, Qin, Zhang, Olsen, Swanson & Pedrow (2000) improved their PEF systems by reducing the risk of dielectric breakdowns that can occur between the electrodes within the processed food that contains mixed particles.

As shown in (Fig. 2.15), the treatment chamber comprises a cylindrical rod electrode and a treatment zone that includes a curved transition face to provide an inwardly converging second electrode. Both electrodes were provided

with temperature stabilizer (heat stabilising fluids can be brought into contact with the electrodes to stabilise and control the electrode temperature). The electric field strength was relatively smaller near the product inlets, and increases up to a maximum in the principal treatment zone between the coaxially parallel faces of the two electrodes. The electric field strength also smoothly decreases in a ramp-down portion distally toward the product outflow port (Barbosa-Ca`novas, Qin, Zhang, Olsen, Swanson & Pedrow, 2000).

Another treatment chamber was presented by Barbosa-Ca`novas, Qin, Zhang, Olsen, Swanson & Pedrow (2000) for the treatment of food products that were of high electrical conductivity. These products pass higher current and hence require more electrical power input, which result in greater temperature increase during the PEF treatment process. The chamber illustrated in (Fig. 2.16) comprises specially contoured faces of electrodes to provide a treatment zone in which the electrical field strength varies between relatively high and low values. The relatively higher electric field locations were associated with the points of the primary treatment zone where the complementary electrode faces were closely spaced. The relatively lower electric field locations were associated with the points of the primary treatment zone where the complementary electrode faces were relatively further spaced. The treatment zone was provided with a longitudinally scalloped face shape. The convoluted face shapes of each side were in complementary registration with the further extension of each face in axial alignment. This electrode configuration increases the effective electrical resistance across the treatment chamber without reducing the processed fluid path

length through the treatment zone, and hence reduces the power load on the circuitry used to drive the electrodes. Additionally, the undulating electrode surfaces enhance agitation of the fluid being processed, which improve the treatment (Barbosa-Ca`novas, Qin, Zhang, Olsen, Swanson & Pedrow, 2000).

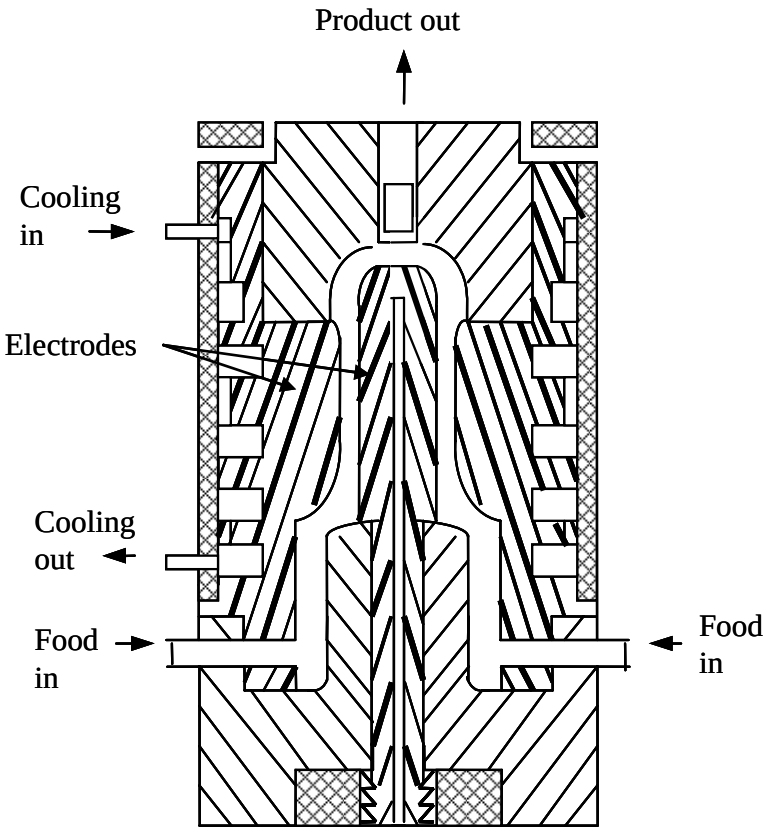

Fig. 2.15: A longitudinal sectional view of a treatment cell (Barbosa-Ca`novas, Qin, Zhang, Olsen, Swanson & Pedrow, 2000).

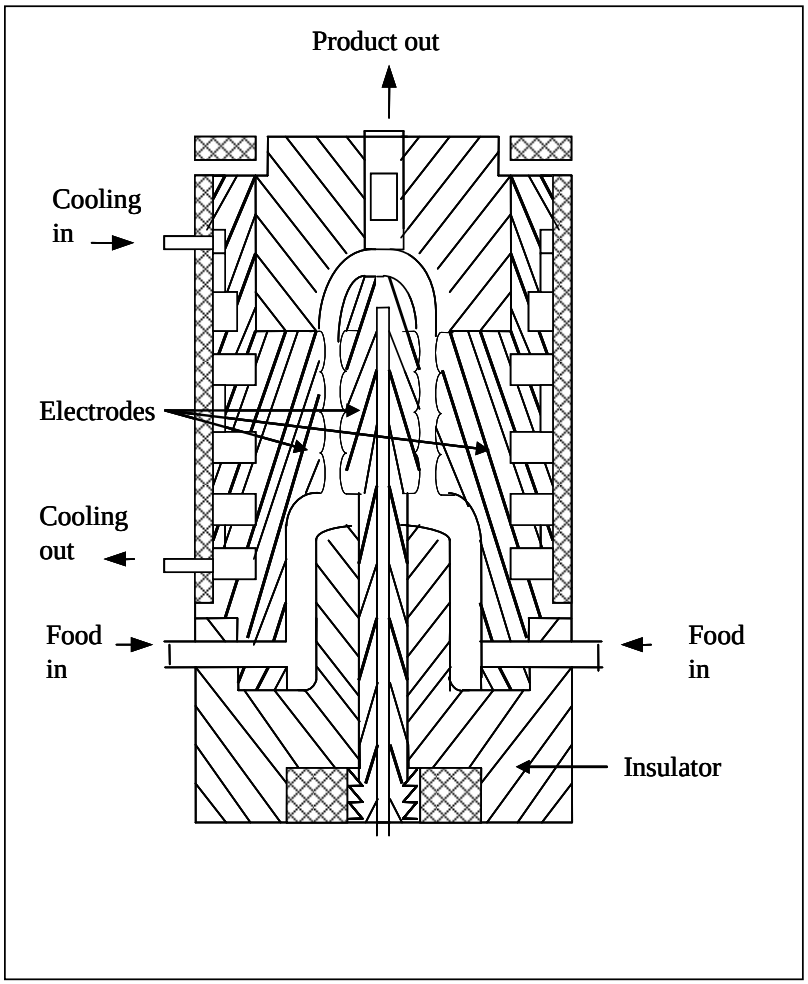

Fig. 2.16: A longitudinal sectional view of a treatment cell (Barbosa-Ca`novas, Qin, Zhang, Olsen, Swanson & Pedrow, 2000).

2.2.2.7 Designs Based on the Shape of the Insulation Spacer Separating the Electrodes

Lindgren, Galt and Ohlsson (2002) modelled and optimised the electric field strength distribution in a co-flow treatment chamber using computational

analysis based on a finite element method and genetic algorithms. The optimisation was performed with respect to the shape of the insulating space that separates the electrodes from each other. The model was based on the PEF treatment chamber design described by Yin, Sastry, & Zhang (1997). Local enhancement of the electric field strength was found in some designs of the insulation spacer. This may arise as the transition from electrode to insulator approaches an angle of 180 degree. The local enhancement arises because equipotential lines and electric field lines were mutually perpendicular everywhere, a criteria that was hard to fulfil in a greater than 90 degree intersections between electrode and insulator. The reason was that the equipotential lines also tend to be parallel to the electrodes surfaces, and that electric field lines (lines of force) tend to be parallel to the insulator surfaces. So, the electric field strength at the interception of an insulator and an electrode will deviate from the assumed electric field strength as the angle if interception deviates from 90 degrees. ‘A rule of thumb’ when designing PEF treatment chambers, is to use an angles close to 90 degree wherever an electrode intersects with an insulator.

2.2.2.8 Designs Based on the Effective Area of Flow

Franc, Francis, Hero and Abraham (2001) provided a treatment chamber without corners or alternatively with rounded corners, so that no contaminants can accumulate on the walls as in (Fig. 2.17). The chamber consists of a number of electrodes having a crescent-shaped cross section of which one side has a

radius of curvature that matches the radius of curvature of the effective area of flow. The electrodes were disposed in such away that the field lines of the electric field run parallel to one another and the potential controller is of such design that the electric field in the effective area of flow is uniform.

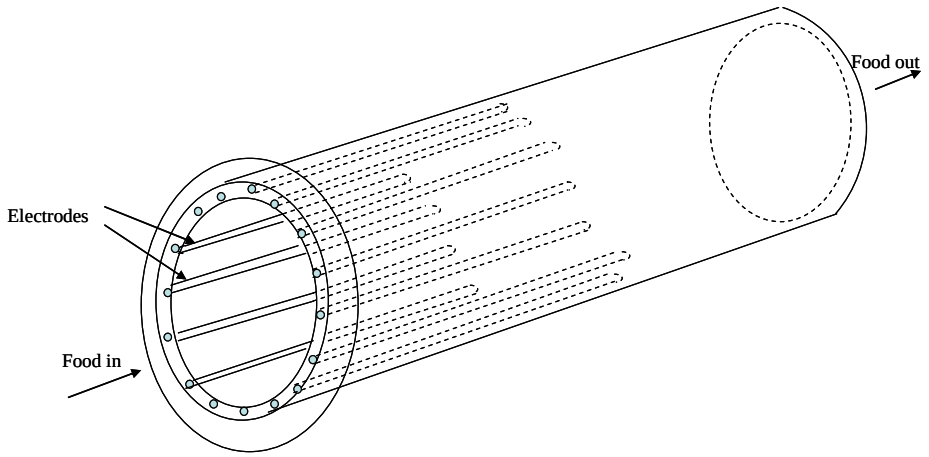

Fig 2.17: PEF treatment chamber (Franc, Francis, Hero & Abraham, 2001).

2.2.3 Cooling Systems and Temperature Control

The change of temperature during the PEF processing should be monitored and controlled to maintain non-thermal operation. The difference between the inlet and outlet temperature is due to heat deposition in the product as a result of ohmic heating.

The maximum temperature increases ΔT can be calculated using basic thermodynamics where no cooling is provided:

$$\Delta T = \frac{Q}{\rho_f C_P} \quad \text{.....(2.5)}$$

Different cooling systems were reported in the previous designs. To maintain the fluid temperature at the designed temperature during processing, intermediate cooling between chambers must be used. In order to keep the electrodes at low temperature, cooling water could be circulated in cavities around the electrodes. The fluid food product must be also cooled to refrigeration temperature (5-9ºC) using a heat exchanger following the electric field treatment, to prevent cell wall repair of the micro organisms being treated.

2.3 Conclusions:

From the previously reported PEF processing designs, the design and construction of a PEF facility for food processing requires both state-of-the-art equipment and common sense. In order to achieve efficient treatment, a number of factors must to be considered. One of the main factors is the proper design of the treatment chamber(s), where uniform electric field should be maintained so that the actual applied field strength dose not exceed the dielectric strength of the fluid foods under the test conditions. The energy required for the treatment may be reduced by a proper chamber design that can concentrate the electric field in the treatment region. Proper design of the insulator separating the electrodes, control the temperature change during processing. An optimisation was

performed in some of the published work with respect to the shape of the insulating space that separates the electrodes from each other.

The design of the insulator should minimise the local enhancements of the electric field strength. This may arise as the transition from electrode to insulator approaches 180 degree, where the equipotential lines and electric field lines are mutually perpendicular everywhere and the electric field strength at the interception of an insulator and an electrode will deviate from the assumed electric field strength as the angle if interception deviates from 90 degrees.

3. Chapter Three: Literature Review on Factors Affecting PEF Treatment

The effectiveness of microbial inactivation using pulsed electric fields treatment depends on several factors. The factors can essentially be classified as process parameters, microbial physiology parameters and treatment medium related parameters. This chapter gives an overview of the critical factors and process conditions that influences the microbial inactivation using pulsed electric fields treatment.

3.1 Process Parameters

The process parameters that are critical to the outcome of the PEF process mainly include electric field strength, treatment time, frequency, treatment temperature and pulse shape and width.

3.1.1 Electric Field Strength

Numerous research groups suggested that microbial inactivation occurs when the applied electric field exceed the critical transmembrane potential, and the degree of inactivation increases with the increase in the electric field strength (Sale and Hamilton 1967, Sale and Hamilton 1968, Hulsheger, Potel & Niemann,

1981, Wouters & Smelt, 1997, Peleg, 1995, Dunn &Pearlman, 1987, Yin, Zhang & Sastry, 1997, Reina, Jin, Zhang & Yousef, 1998 and many others).

Sale and Hamilton (1967, 1968), applied 10 pulses of $20\mu s$ width at $19.5kV/cm$ to Escherichia coli suspension at 20ºC. The results show 2 log reductions in the microbial load with a maximum temperature rise of 10ºC. Fields up to $25kV/cm$ showed membrane damage followed by lysis of erythrocytes, protoplasts and spheroplasts in the cells. Wouters & Smelt (1997) reported that the death of vegetative bacteria and yeasts was not due to temperature rise and was found independent of current density, but dependent on field strength, pulse length and number of pulses applied. The effect of current density on the microbial inactivation will be discussed later in this thesis.

The effect of PEF processing parameters (electric field strength, treatment time, and treatment temperature) on the inactivation of Listeria monocytogenes suspended in pasteurized whole milk, milk at 2% fat milk, and skim milk was studied by Reina, Jin, Zhang & Yousef (1998). A continuous co-field flow PEF system with tubular PEF treatment chambers was used at Ohio State University. The lethal effect of PEF was found to be a function of field strength and treatment time as shown in (Fig. 3.1). The treatment conditions were as follows: two levels of field strength ($35kV/cm$ and $25kV/cm$), temperature 25ºC, flow rate $7ml/s$, pulse duration $1.5ms$ and frequency of $1.7kHz$. The effect of field strength was related to the treatment time. At short times ($100\mu s$) there was no significant effect of the electric field strength between $35kV/cm$ and $25kV/cm$. For $300\mu s$

and $600\mu s$ of total treatment time, higher field strength resulted in more reduction of viable cells.

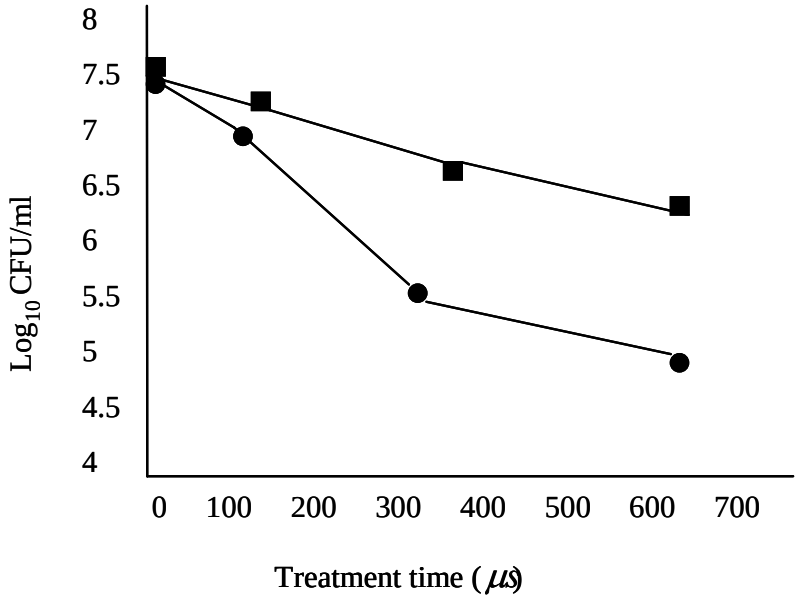

Fig. 3.1: PEF inactivation of Listeria monocytogenes in whole milk by 35 kV/cm (●) and 25 KV/cm (■) (Reina, Jin, Zhang & Yousef, 1998).

Dunn &Pearlman (1987) suggested that for an effective treatment, a substantially uniform electric field must be provided throughout the liquid food treatment zone. The uniform electric field prevents current filaments or the formation of "streamer" arcs, which may develop within the treatment zone. Accordingly, the application of higher field strength may require high precision control of field uniformity to prevent the development of such filaments or arcs and to ensure proper treatment of the fluid food products.

On bacterial spore inactivation, Yin, Zhang & Sastry (1997) studied the effect of using different electric field intensities and treatment times as shown in (Fig. 3.2). The electric field level used were 30, 37, and $40 kV/cm$, while maintaining the applied electric field frequency at $2 kHz$, pulse duration time of $3 ms$ and treatment temperature of 36ºC. The results show increase in the inactivation as the electric field intensity and treatment time increases. After the bacterial spores were exposed to electric field strength of $40 kV/cm$ for $3.5 ms$, 1.7 log microbial inactivation was achieved.

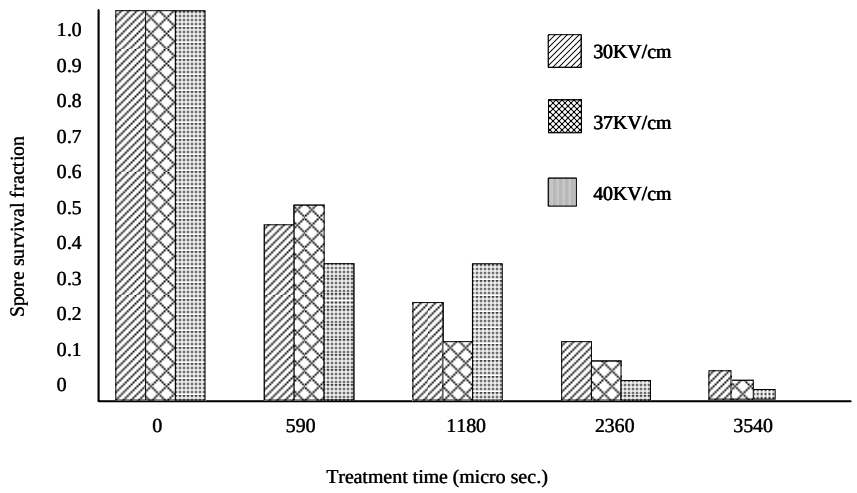

Fig. 3.2: The effect of PEF on bacterial spore inactivation at different electric field intensities as the treatment time is varied (Yin, Zhang & Sastry, 1997).

Mathematical models have been proposed to describe the relation between electric fields and microbial inactivation. For example, Hulsheger, Potel &

Niemann (1981) studied the effect of PEF on several organisms. They proposed a model that related microbial survival fraction (which is defined by the ratio of the living cells before and after treatment ($s = (N / N_o)$) with electric field strength according to the following linear relationship:

$$\ln(s) = -b_E (E - E_C) \quad \text{.....(3.1)}$$

Where b_E is the regression coefficient, E is the applied electric field, and E_c is the critical electric field obtained by the extrapolated value of E for 100% survival. The regression coefficient describes the gradient of the straight survival curves, and is dependent on experimental conditions. Different microbial inactivation kinetics and the value of kinetic constants that exemplify the use of this model are given in (Grahl & Markl 1996).

Peleg (1995), proposed another model for the percentage of surviving organisms as a function of electric fields and number of pulses. The critical field intensity corresponds to 50% survival and the kinetic constant is a function of number of pulses applied. The kinetic constants of Peleg's model for different microorganisms and different treatment conditions are given in (Peleg, 1995).

$$s = \frac{1}{1 + e^{\frac{E - E_{C(n)}}{k_{(n)}}}} \quad \text{.....(3.2)}$$

Where, $k_{(n)}$ is the kinetic constant that indicates the steepness of the survival curve around the critical electric field $E_{c(n)}$ at certain number of pulses.

3.1.2 Treatment Time

PEF treatment time is calculated from the product of the number of pulses and their duration. In general, an increase in any of these variables results in an increase in microbial inactivation (Barbosa-Ca`novas, Gongora Nieto, Pothakamury & Swanson 1999). Although most of the studies mentioned in the previous section describe the effect of treatment time together with the effect of electric field intensity, more studies on the effect of treatment time will be reviewed in this section. Sale and Hamilton (1967) reported that by increasing the number of pulses while keeping other conditions constant, the level of microbial killing increased rapidly at the first few pulses and then more slowly at later pulses. This was also reported by Aronsson, Lindgren, Johansson & Ronner (2001a), who examined the effect of treatment time on Escherichia coli, Listeria innocua, Leuconostoc mesenteroides and Saccharomyces suspended in model medium, using different number of pulses and pulse duration in a continuous PEF system. The system includes six co-field flow tubular treatment chambers, with a diameter of $2.2mm$ and electrode gap of $2.3mm$, connected in series. The pre and post temperatures were maintained at 30ºC, electric field strength was set to 25, 30, or $35kV/cm$, the square wave pulse durations selected were 2 or $4\mu s$, repetition frequencies of 250 and $500Hz$ and the flow rate through the system was adjusted to $0.683ml/\sec$. The applied number of pulses was 20 and 40 and the calculated average time in each treatment chamber was $13ms$. When the number of pulses was raised, some inactivation for all organisms was observed.

The effect of pulse durations (2 and 8 μs) was also studied by Sensoy, Zhang & Sastry (1996). They developed inactivation kinetic models of Salmonella dublin in skim milk using a co-field flow high voltage pulsed electric field (PEF) treatment system. The treatment conditions selected were electric field strength of 15-40kV/cm, treatment time of 12-127μs and medium temperature of 10-50°C. Their results are illustrated in (Fig. 3.3), showing that increasing the treatment time decreases the survival fraction logarithmically as well as positive effect when the pulse width is reduced.

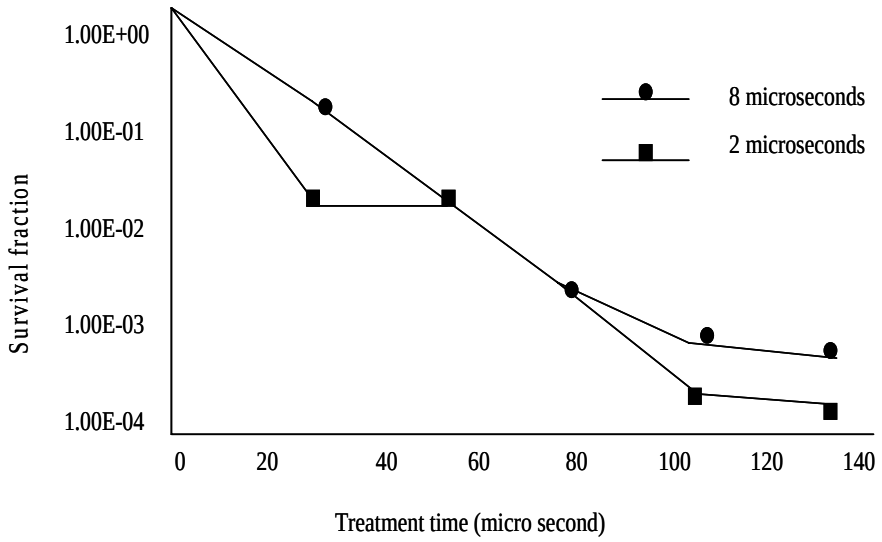

Fig. 3.3: Effect of treatment time on survival fraction using two different pulse duration (Sensoy, Zhang & Sastry, 1996).

Jeantet, Baron, Nau, Roignant & Brule (1999), applied high intensity electric fields to the Salmonella Enteritidis in diaultrafiltered egg white. They

observed a strong synergy between the electric field strength and number of pulses. The higher the number of pulses (total treatment time); higher microbial inactivation was achieved at the same intensity. This observation is in concordance with the results reported by Grahl and Markl (1996), for Escherichia coli inactivation by PEF and many others.

Mathematical models that describe microbial inactivation kinetic that combines the effect of treatment time to electric field strength were evaluated. An equation offered by Hulsheger, Potel & Niemann (1981) as follows:

$$s = \left(\frac{t}{t_c}\right)^{\left(-\frac{E-E_C}{k^1}\right)} \quad \text{.....(3.4)}$$

Where, t_c is a critical time factor that gives $s = 1$, and k^1 is a first order kinetic constant or microorganisms constant.

Although increasing the pulse frequency increases the treatment time, Yin Zhang & Sastry (1997) reported that the inactivation rate of bacterial spores decreased as the frequency increased as shown in (Fig. 3.4). Frequencies of 2,000, 3,000 and 4,000Hz, a pulse duration time of $3ms$, electric field strength of $30kV/cm$ and treatment temperature of 36ºC were selected as the operating conditions. They explained these results as there is an optimal PEF treatment frequency that may cause resonance of the bacterial spore structure. This resonance causes a loosening of the rigid bacterial spore structure such that the applied pulsed electric field can, in effect, punch through the spore structure and inactivate the spores.

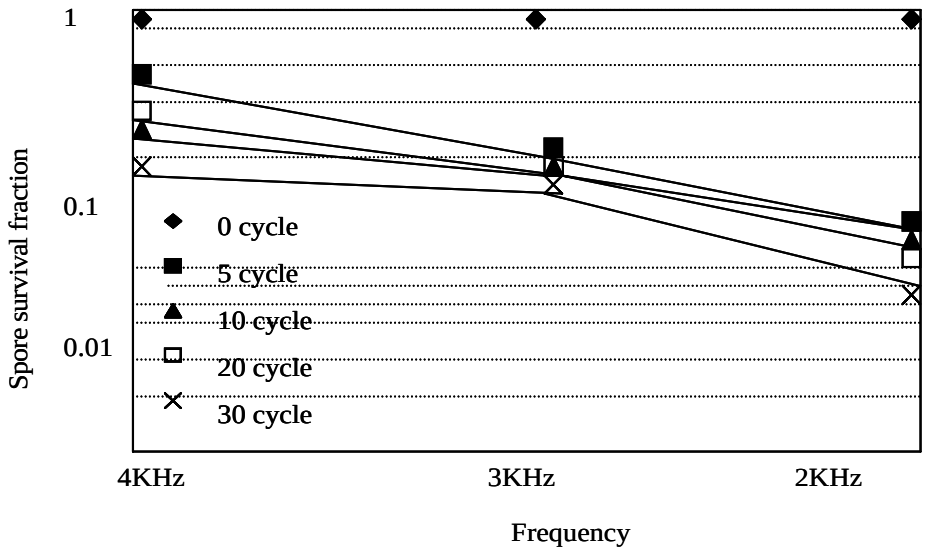

Fig. 3.4: The effect of bacterial spore inactivation as the pulsed electric field frequency is varied, (Yin Zhang & Sastry, 1997).

3.1.3 Pulse Wave Shape and Width

The exponentially decay pulse, square wave pulse and the bipolar pulse are the major waveforms used in pulsed electric field technology. Pothakamury U. R. (1996) studied the effect of pulse shape on the inactivation of E. coli inoculated in simulated milk ultrafiltrate by PEF. They found that at 7ºC and after treatment time of $100\mu s$, square-wave pulses produced 2 log reduction while exponential decaying pulses produced only 1.2 log reduction in bacterial cell population.

As mentioned earlier in Chapter one, the bipolar pulses are more lethal than monopolar pulses and also offer the advantages of reducing the deposition of solids on the electrode surface, and decreased food electrolysis (Barbosa-Ca`novas, Gongora, Nieto, Pothakamury & Swanson 1999 and Wouters &Smelt

1997). Monopolar pulses separate charged particles inside fluid foods and promote migration of charged particles to electrodes with opposite polarity. A high concentration of space charge near electrodes will cause an electric field distortion. Compared with exponentially decaying pulses and monopolar square pulses, bipolar pulses provided more efficient inactivation of microorganisms. Another form of pulses is the oscillatory decay pulses, these pulses do not expose the cell continuously to a high intensity field for an extended period, therefore allowing the cell to repair the membrane from reversible rupture (Qin, Zhang, Barbosa-Ca`novas & Swanson (1994) and Wouters &Smelt (1997).

3.1.4 Treatment Temperature

Temperature is one of the factors that have a significant effect in the inactivation of microorganisms by PEF. In general, with constant electric field strength, inactivation increases with the increase in temperature. Jayaram & Castle (1992) reported in the inactivation of Lactobacillus brevis by PEF that the temperature influence the membrane breakdown during PEF treatment, which may depend on the microorganisms tested as well as media used. Zhang, Qin, Barbosa Canovas & Swanson (1995a) found that increasing the suspension temperature from 7 to 20°C significantly increased the PEF inactivation of E coli in simulated milk ultra filtrate when subjected to 16 pulses of $2\mu s$ width at $35kV/cm$. Additional increase in temperature from 20 to 33°C did not result in an increase in PEF inactivation. In comparison with thermal treatment, the decimal reduction time ($D\ value$) for E coli 11229 suspended in milk solution

(10%, 30% and 42%w/w) at $58^{o}C$ as reported by Tomlins & Ordal, (1976) were 1.4, 2.4 and $7.3\,\text{min}$ respectively.

Reina, Jin, Zhang & Yousef (1998) applied electric field intensity of 30 kV/cm to L. monocytogenes suspended in milk using a continuous PEF system with co-field flow, tubular PEF chambers. They reported 3 to 4 log reductions at 50°C after $300\mu s$ and $600\mu s$ of treatments respectively as shown in (Fig. 3.5). The treatment conditions were as follows: flow rate of $7ml/cm$, pulse duration time of $1.5ms$ and frequency of $1{,}700Hz$. The D *value* for the thermal treatment of L. monocytogenes at $65^{o}C$ is $0.1\,\text{min}$ (Walstra, Geurts, Noomem, Jellema & van Boekel, 1999). Hence, microbial inactivation using PEF treatment can be achieved at lower treatment temperatures and less treatment time.

On spore inactivation, Yin, Zhang & Sastry (1997) reported that there is an optimum treatment temperature in which bacterial spores tend to germinate as a first step then followed by inactivation. They applied electric field strength of $30kV/cm$ at frequency of $1{,}500Hz$ and pulse duration time of $2ms$ to bacterial spores at 20, 30 36, 40 and 50ºC. In (Fig. 3.6) the existence of an optimum temperature of 36ºC for inactivating bacterial spores by PEF treatment is shown. The inactivation rate increased with total PEF treatment time at 30 and 36ºC. However, the inactivation rate did not exhibit significant change after $540ms$ of total treatment time at 20, 40 and 50ºC treatment temperatures.

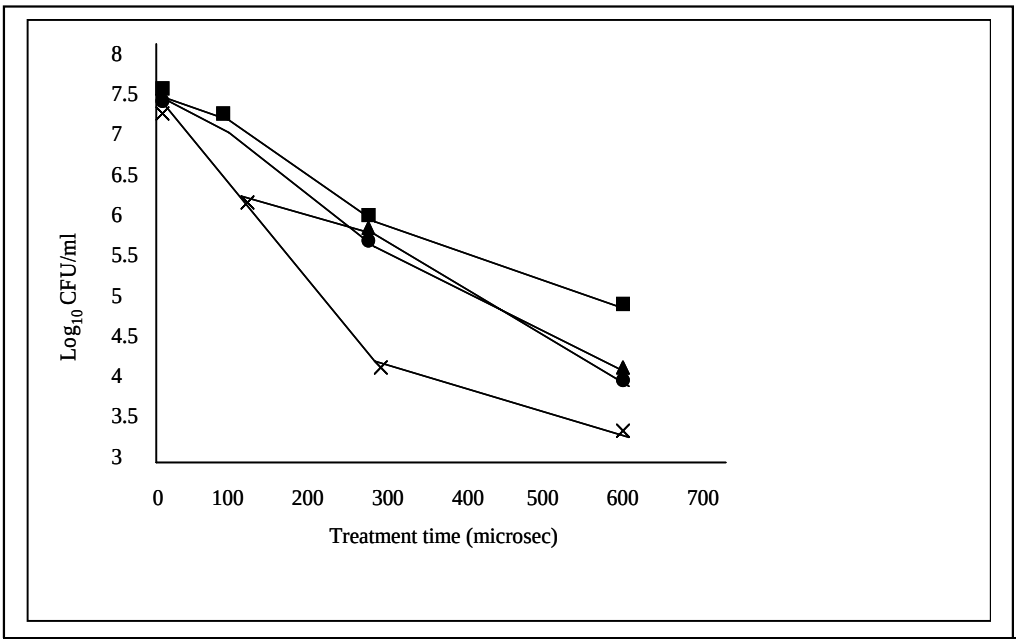

Fig. 3.5: PEF inactivation of Listeria monocytogenes in whole milk at 10ºC (●), 25º (■), 30ºC (◆), 43ºC (▲), and 50ºC (X), (Reina, Jin, Zhang & Yousef, 1998).

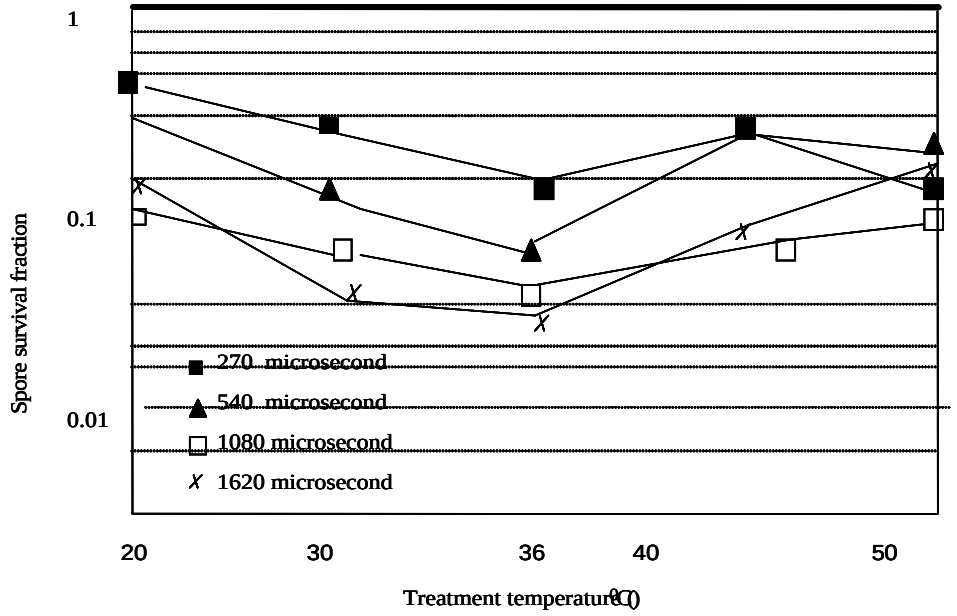

Fig. 3.6: Effect of temperature variation on bacterial spore inactivation (Yin, Zhang & Sastry, 1997).

3.2 Microbial Physiology Factors

The microbial physiology factors include the type of microorganisms (such as gram positive, gram negative, yeast or spore form), their concentration, and growth stage of the microbial entity as some changes can take place during the microbial growth.

3.2.1 Type of Microorganisms

The different types of microorganisms vary in their sensitivity to PEF treatment due to different membrane structure and cell wall construction. Sale and Hamilton (1967, 1968) reported that the various species differed in their sensitivity to the electric field, the yeasts being more sensitive than the vegetative bacteria.

The size and shape of microorganism play a part an important role. This can be explained as the induced voltage across the cell membrane is proportional to the geometric size of the cell. Numerous of other similar studies have been published that indicate the dependence of the lethal effect of PEF pulses on the shape and size of the microbes (Mazurek & Ludicki, 1995 and Qin, Barbosa-Ca`novas, Swanson Pedrow & Olsen, 1998a). The later applied repetitive high-voltage pulses with an exponential decaying wave shape to different microorganisms, Escherichia coli, Staphylococcus aureus and Saccharomyces cerevisiae suspended in model media. Continuous PEF system comprises of modified coaxial treatment chamber was used. Their results show the induced

voltage across the cell membrane is proportional to the geometric size of the cell. As a consequence, yeasts are more sensitive to electric field pulses than bacteria due to their large size as in Fig. (3.7).

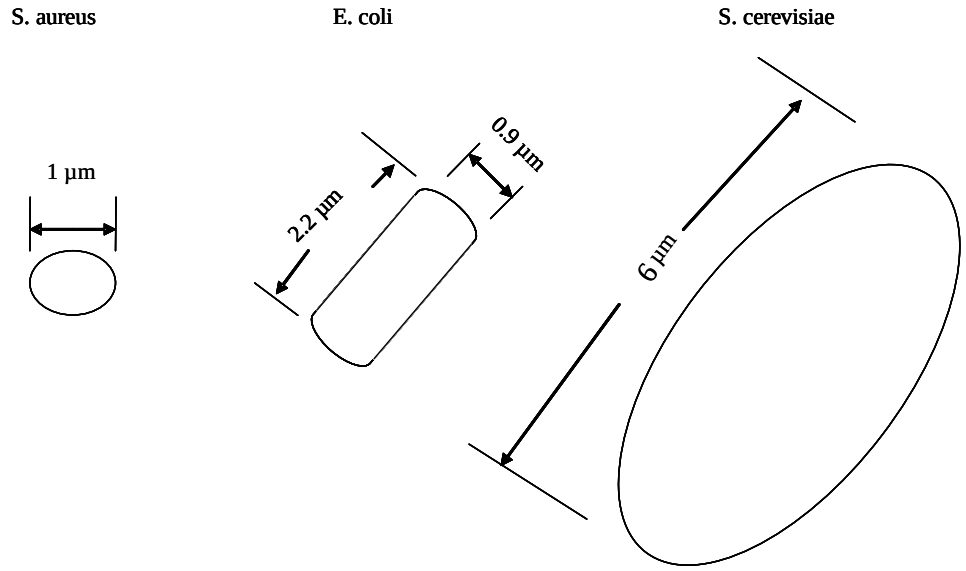

Fig. 3.7: A cell size comparison (Qin, Barbosa-Ca`novas, Swanson Pedrow & Olsen, 1998a).

Similar findings were obtained by Aronsson, Lindgren, Johansson & Ronner (2001a) on the PEF sensitivity of Escherichia coli, Listeri innocua, Leuconostoc mesenteroides and Saccharomyces cerevisiae suspended in model medium. The microorganisms tested differ in size, shape and cell wall construction. Cells were treated in a continuous PEF system, the electrical field strength, the number of pulses and pulse duration, were varied. Their results show

that gram-positive bacteria were more resistant than gram-negative bacteria and the yeast (S. cerevisiae) tested was the most sensitive.

The microbial cells when subjected to an electric field, the induced membrane potential V_m for spherical cells surrounded by non-conducting membranes is given by Hulsheger, Potel & Niemann (1983) as follows:

$V_m = f`aE_C$ ……...(3.5)

Where, $f`$ the shape is factor (1.5 for spheres), a is the cell radius and E_c is the critical external field strength. If the cell is a cylinder with hemispheres at either end, the shape factor f is given by Zimmermann, Pilwat & Riemann (1974):

$f = L/(L - 0.33d)$ ……..…….....(3.6)

Where, L is the length and d is the diameter of the cylinder.

3.2.2 Concentration of Microorganisms

The concentration of microorganisms in a product may have an effect on its electric field-induced inactivation (Barbosa-Ca`novas, Gongora-Nieto, Pothakamury & Swanson, 1999). The significant negative effect of the inoculum indicated that the higher the inoculum size, the lower the bactericidal effect of PEF. Hulsheger & Niemann (1980) reported that when inoculum size was high, cells could aggregate together and consequently enhance the effect of PEF on these aggregated cells.

Zhang, Monsalve-Gonzalez, Qin, Barbosa-Ca`novas & Swanson (1994b), designed an experiment to reveal whether microbial survival fraction is affected by the initial inoculum level of yeasts. Initial concentration of yeast were set at 10^6, 10^5 and 10^4 *cuf/ml*, and subjected to pulsed electric field of $2.5V/\mu m$ and pulse width of $25\mu s$. The survival fraction of S. cerevisiae was inversely correlated with the initial concentration under the selected treatment conditions. This issue will be further discussed in this thesis.

3.2.3 Effect of Growth Phase

Properties of microbial cell membrane are different at different stages of microorganism growth, and logarithmic phase cells are more sensitive to PEF treatment than cells in lag or stationary phase (Barbosa-Ca`novas, Gongora-Nieto, Pothakamury & Swanson, 1999). Microbial growth in logarithmic stage is characterized by a higher number of cells in a state of proliferation, during which the area between the mother and the daughter cells and sensitive parts of the cell envelope are susceptible to the applied electric field. Therefore, the tender cells in the logarithmic phase are more sensitive to the electric field. This was also reported by Gaskova, Sigler, Janderova & Plasek (1996), who found that the deactivation effect of PEF during exponentially growing cells is greater by more than 30% of those in a stationary stage.

Pothakamury, Vega-Mercado, Zhang & Swanson (1996) also studied the effect of the growth phase on the inactivation of E. coli inoculated in simulated milk ultra filtrate by PEF as shown in (Fig. 3.8). Cells harvested at lag,

logarithmic growth phase and stationary phases were subjected to 2 and 4 pulses with electric field strength of $36kV/cm$ at 7ºC. The workers concluded that the cell membrane properties are different at different stages of microorganism growth, and logarithmic phase cells were more sensitive than lag and stationary phase cells to the pulsed electric field treatment. Jacob, Forster & Berg (1981) have reached similar findings, they investigated the inactivation of yeast cells in different growth phases by PEF and concluded that the cells of Saccharomyces cerevisiae in the logarithmic growth phase are more sensitive to an electrical discharge than cells in the stationary phase.

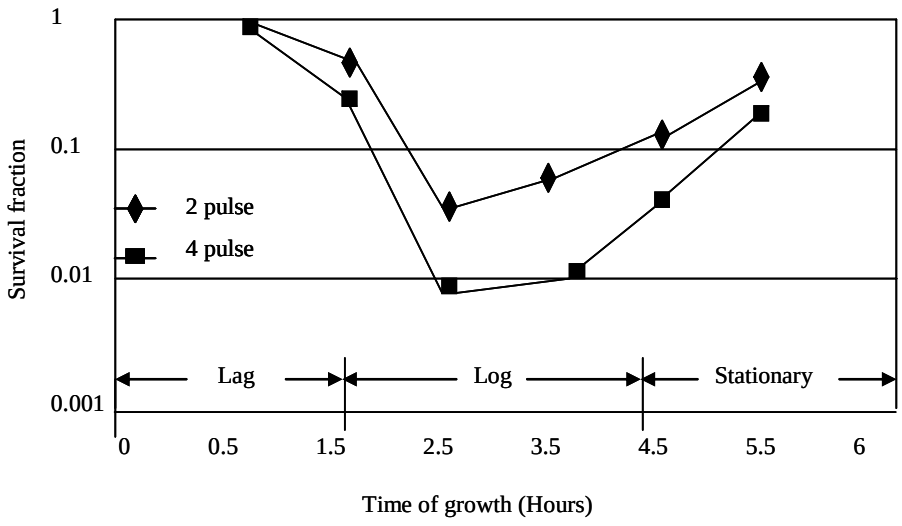

Fig. 3.8: Cells of E coli harvested at different growth stages suspended in SMUF and subjected to an electric field of 36 KV/cm at 7ºC, (Pothakamury, Vega-Mercado, Zhang & Swanson, 1996).

3.2.4 The Effect of Culture Temperature

During microbial culturing, the bacteria incorporate saturated and long-chain fatty acids into phospholipids. The resulting phospholipids are in a rigid gel structure at low temperature, but turns into a less ordered or liquid crystalline structure as the temperature increases. PEF can thus be determined as being effective in liquid crystalline structures but not at phase transition temperatures (which is 10ºC lower than the culture temperature) (Ohshima, Sato, Terauchi & Sato, 1997 and Ho & Mittal, 1997). The phase transition from gel to liquid crystalline is dependent on temperature. Hence, it can affect the physical stability of the cell membrane (Stanley, 1991).

Ohshima, Okuyama & Sato (2002) studied the effect of culture temperature of Escherichia coli on PEF treatment. The E coli cells were cultivated at 20, 30, 37 and 42°C, and subjected to electric field of $16 kV/cm$ and frequency of $50 Hz$. Results showed that when E coli cells were cultivated at 20°C or 42°C, cells were more easily sterilized by the PEF sterilization than the E coli cells cultivated at 37°C, which is the optimal culture temperature of E coli.

On the other hand, Gaskova, Sigler, Janderova & Plasek (1996) explained how lower post pulse temperatures allow higher inactivation due to the lifetime of induced pores in lipid membranes and/or the lateral mobility of membrane components. They clarify that low post pulse temperatures should therefore aid in maintaining enhanced membrane permeabilities which promote irreversible cell

damage. They also reported that the high killing efficiency of PEF at low postpulse temperatures is lost at higher strength electric fields.

3.3 Treatment Medium Related Parameters

The treatment medium parameters are related to the physical and chemical characteristics of the product, in which the microorganisms are present.

3.3.1 Medium Conductivity

The conductivity might be one of the most important parameter in the PEF treatment. Decreasing medium conductivity increases the difference in conductivity between the microbial cytoplasm and the medium and hence, increases the flow of ionic substances across the microbial membrane. This causes additional pressure on the membrane due to osmotic forces and makes it more sensitive to the PEF treatment. However, the presence of ions appears to be necessary to increase the transmembrane potential (Bruhn, Pedrow, Olsen, Barbosa-Ca`novas & Swanson, 1997) as will be discussed in chapter 4.

Furthermore, a low conductivity enhances allows the application of higher electric field, enhancing the PEF treatment. Using food with lower electrical conductivity minimise ohmic heating of the suspension being treated. As temperature increases, due to the energy transfer during treatment, the conductivity of the food increases and hence the energy input needed to achieve a

dosage level increase. There is a dynamic relationship between electrical conductivity, energy input, and temperature increase.

Vega-Mercado, Pothakamury, Chang & Swanson (1996) examined the effect of PEF in relation to the ionic strength of the treatment medium on the inactivation of E. coli ATCC 11229 suspended in simulated milk ultra filtrate using static chamber. The ionic strength of SMUF was adjusted with KCL to 0.168 M and 0.28 M. The electric field intensities applied were from 20 to $55 kV/cm$. There were 2.5 log cycles difference between the 0.168 M and 0.28 M. They reported that the suspension with the lower electric conductivity reduced the stability of the cell membrane when exposed to a medium with several ions.

The survival of L. brevis cells in suspensions of phosphate buffer solutions of different conductivities ($17 mS/m$ to $223 mS/m$) using electric pulses was investigated by Jayaram, Castle & Margaritis (1993). More reduction was observed in liquids with lower conductivities primarily due to conductivity influencing the membrane permeability.

Dutreux, Notermans, Wijtzes, Gongora-Nieto, Barbosa-Ca`novas & Swanson (2000a) applied PEF treatment of E. coli suspended in distilled water (conductivity of the distilled water was $0.2 mS/cm$). The treatment led to a total bacterial inactivation within 35 pulses, whereas in fat-free milk and in phosphate buffer surviving organisms were still present after 63 pulses when treated under similar conditions. This conclusion is similar to those of other workers (Vega-Mercado, Pothakamury, Chang & Swanson, 1996, Jayaram, Castle & Margaritis, 1993 and Hulsheger, Potel & Niemann, 1981 and many others.

Ruhlman, Jin & Zhang (2001) measured some physical properties for different liquid food products including milk at different compositions for PEF treatment. They found products that have low electrical conductivity and viscosity, and high density, will be the easiest and most energy efficient to process using PEF. The increase in energy input and the change in temperature as shown in (Fig. 3.9) and (Fig. 3.10) are directly proportional to the increase in electrical conductivity. Temperature change during the PEF treatment also increases when the food density decreases.

Viscosity of the product determines Reynolds number and a uniform velocity profile during the PEF treatment is likely to be achieved at turbulent flow.

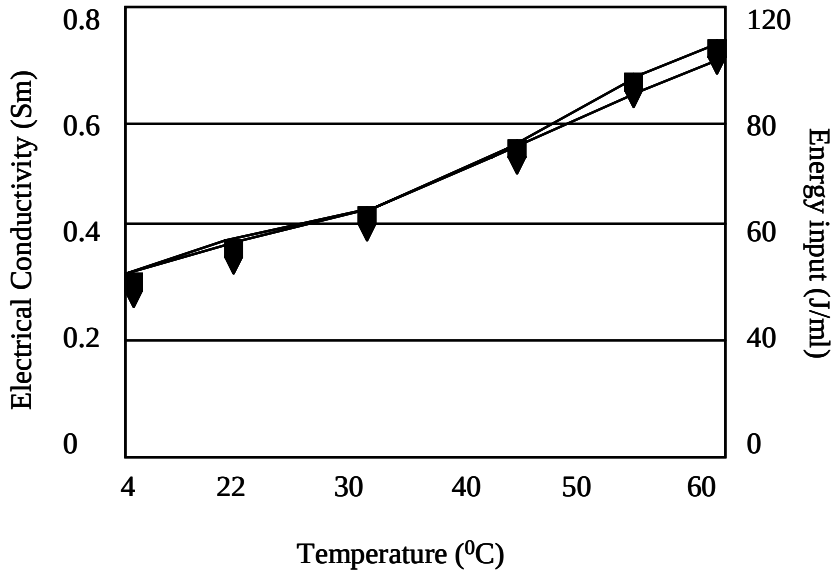

Fig. 3.9: Electrical conductivity and calculated temperature change per pair of chambers vs. input temperature for orange juice (Ruhlman, Jin & Zhang 2001).

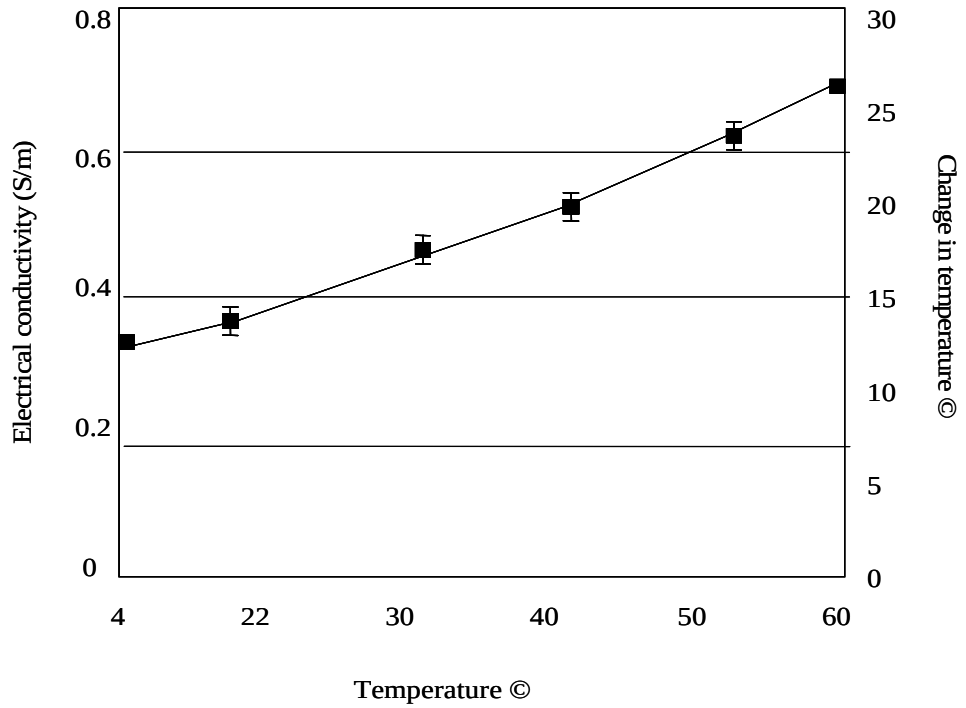

Fig. 3.10: Electrical conductivity and calculated temperature change per pair of chambers vs. input temperature for orange juice (Ruhlman, Jin & Zhang, 2001).

3.3.2 Media Composition

It is more difficult to reduce the survival fraction of microorganisms present in complex food materials (for example milk) than in buffer solutions and usual model foods. It was reported that an increase in the concentration of protein (Martin, Qin, Chang, Barbosa-Ca`novas &Swanson, 1997), lipid (Grahl & Markl, 1996), or xanthan (Ho, Mittal, Cross & Griffiths, 1995) appears to increase microbial resistance to electric pulses. These substances diminish the lethal effect of PEF in microorganisms because foods are rich in ionic species

(Martin, Zhang, Castro, Barbosa-Ca`novas & Swanson, 1994). Food can generate small peak electric fields across the treatment chamber (Barbosa-Ca`novas, Gongora-Nieto, Pothakamury & Swanson, 1999).

Dutreux, Notermans, Wijtzes, Gongora-Nieto, Barbosa-Ca`novas & Swanson (2000a) carried out inactivation of Escherichia coli and Listeria innocua suspended in milk and phosphate buffer, with the same pH and conductivities. The results showed the inactivation of E coli in milk was lower by less than I log compared to that found in phosphate buffer. It seems doubtful that the components of milk have an effect against the lethal action of PEF.

However, Reina, Jin, Zhang & Yousef (1998) inoculated pasteurized whole, 2% fat content, and skim milk with Listeria monocytogenes Scott A to study the effects of milk composition (fat content) on the inactivation of Listeria monocytogenes Scott A. Using electric field of $25-35kV/cm$ at 25ºC, no significant differences were observed in the inactivation of Listeria monocytogenes Scott A in the three types of milk. It seems that there is no effect due to the presence of fat globules, but the presence of protein, either as casein or whey protein has a protective effect.

3.3.3 Addition of Agents

Yin, Zhang & Sastry (1997) studied the effect of PEF on bacterial spore inactivation in two treatment mediums, a 0.02% NaCl and a 0.02% NaCl+0.01% L-alanine. L- alanine is considered as a germination agent for many strains of bacterial spores. The PEF treatment parameters selected were electric field

strength of $30kV/cm$, frequency of 1,000Hz, pulse duration time of $6\mu s$ and a treatment temperature of 36ºC. As shown in Fig. (3.11), the difference between the inactivation rate of bacterial spores in two treatment mediums during the first

$120\mu s$ of treatment time was small. However, when the treatment time reaches 300μs, the suspension medium seems to influence the PEF treatment significantly.

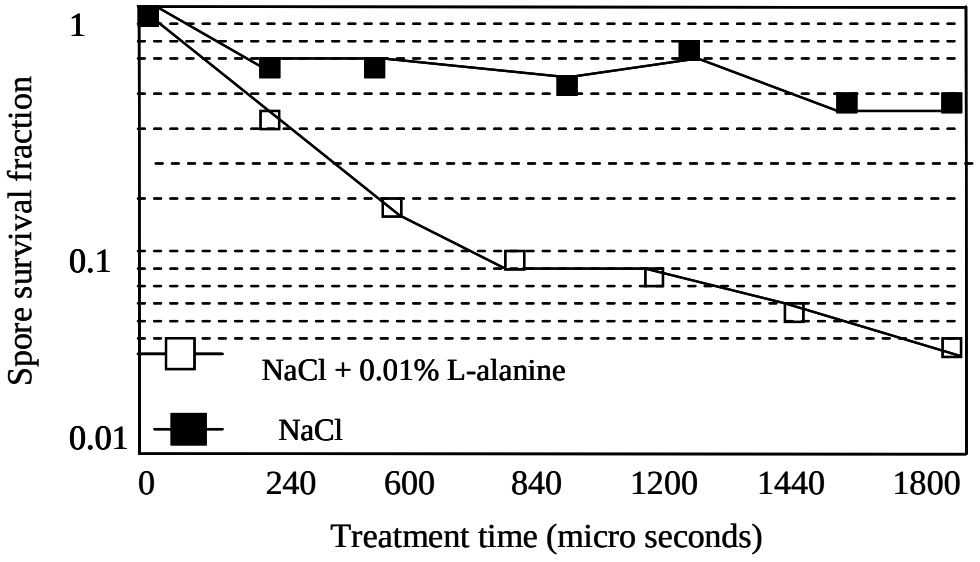

Fig. 3.11: The effect on bacterial spore inactivation in two treatment mediums as the treatment time is varied, (Yin, Zhang & Sastry, 1997).

Calderon-Miranda, Barbosa-Ca`novas & Swanson (1999) studied the inactivation of Listeria innocua suspended in skim milk by PEF as well as the sensitization of PEF treated L. innocua to ninsin. The selected electric field strength was 30, 40 and $50 kV/cm$ and the number of pulses applied were 10.6, 21.3 and 32. The sensitization exhibited by PEF treated L. innocua to nisin was assessed for 10 or 100 IU nisin/ml. The exposure of L. innocua to nisin after PEF had an additive effect on the inactivation of the microorganisms as that exhibited by the PEF alone. The reduction of L. innocua accomplished by exposure to 10 IU nisin/ml after 32 pulses was 2, 2.7 and 3.4 log cycles for an electric field intensity of 30, 40, and $50 kV/cm$, respectively. Population of L. innocua subjected to 100 IU nisin/ml after PEF was reduced by 2.8-3.8 log cycle for the selected electric field intensities and number of pulses.

3.3.4 Effect of pH

In early studies, pH was found to have no effect on the inactivation of microorganisms (Sale & Hamilton, 1967; Hulsheger, Potel & Niemann, 1981). However more recently, it has been demonstrated that pH plays an important role in the inactivation kinetics of PEF. In general, microorganisms are the most resistant to all kinds of treatment at their optimum pH for growth. When the pH is raised or lowered from this value, there is a consequent increase in sensitivity to the treatment. Both acidic and alkaline pH values induce additional stress to microbial cells, and consequently increase their susceptibility to physical and chemical treatments (Aronsson & Ronner, 2001).

Corlett & Brown (1980) explained that the role of pH in the survival of microorganisms is related to the ability of the organisms to maintain the cytoplasm pH near neutrality. Tsong (1990) also explained the effect of pH on the survival of microorganisms. The membrane permeability increase due to formation of pores in the cell wall during PEF treatment and the rate of transport of hydrogen ions may also increase due to the osmotic imbalance around the cell. Thus, a reduction in cytoplasm pH may be observed because a higher number of hydrogen ions are available than at a neutral pH. The change in pH within the cell may induce chemical modifications in fundamental compounds such as DNA or ATP (Rahman, 1999). On the other hand, Alvarez, Raso, Palop & Sala (2000) stated that the influence of pH on microbial inactivation by PEF is still unclear, since they observed that Salmonella senftenberg was more resistant at pH 3.8 than 7.

Jeantet, Baron, Nau, Roignant & Brule (1999), achieved a higher inactivation level of Salmonella enteritidis at pH 9.0 than at pH 7.0. Vega-Mercado, Pothakamury, Chang, Barbosa-Ca`novas & Swanson (1996) observed that inactivation of E. coli was greater at pH 5.7 than at pH 6.8. Wouters, Dutreux, Smelt & Leeveld (1999) reported increased survival of L. innocua at pH 6.0, in comparison with pH 4.0 more than six log reduction of L. innocua was achieved. Raso, Calderon, Gongora, Barbosa-Ca`novas & Swanson (1998) observed greater inactivation by pulsed electric fields of mold ascospores (Neosartoria fischeri) in fruit juices with lower pH values.

Aronsson & Ronner (2001) examined the influence of pH, water activity and temperature on the inactivation of E coli and S. cerevisiae suspended in model media in a continuous PEF unit. Electric field parameters were set at $25kV/cm$ for S. cerevisiae and $30kV/cm$ for E coli, 20 pulses with 4 μs duration. As shown in (Fig. 3.12), when the pH value was decreased from 7.0 to 6.0, the inactivation level of E coli was increased from 1.7 to 3.6 log reductions. Further decreases in pH to 5.0 and 4.0 increased the inactivation level to 4.6 and 5.7 log reductions, respectively.

The yeast pattern of inactivation was very different from that of E coli, showing a minimum inactivation at approximately pH 5.0 instead of pH 4.0. In general, yeasts possess broader pH growth ranges than bacteria and S. cerevisiae was reported to grow in pH range of 2.5-8.0 (Praphailong & Fleet, 1997) with optimum growth at approximately pH 5.0, which could explain relatively higher survival at pH 5.0. As illustrated in (Fig. 3.13), 3.8 log reduction of S. cerevisiae was achieved at pH 4.0 and water activity of 1.00, while at pH 5.0, the log reduction was only 1.8. Further increase in the pH value to 7.0 resulted in greater inactivation.

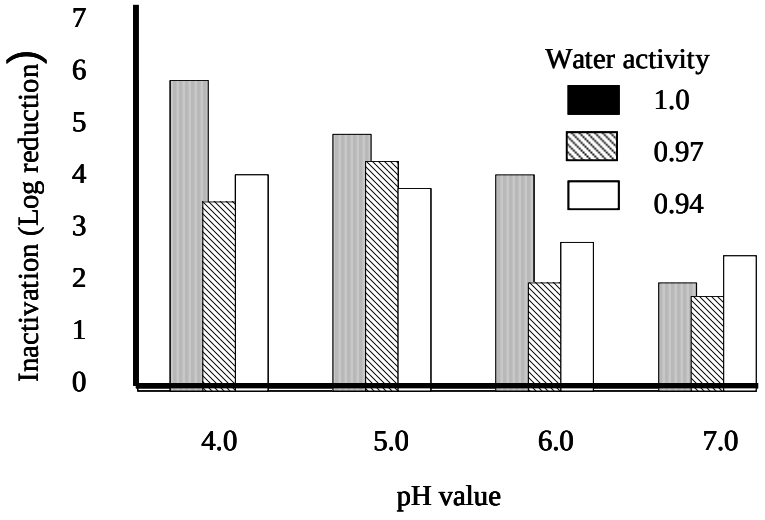

Fig. 3.12: Inactivation of E coli ATCC 26 in NTM at inlet temperature 30ºC with varying pH and a_w, after exposure to PEF (Aronsson & Ronner, 2001) .

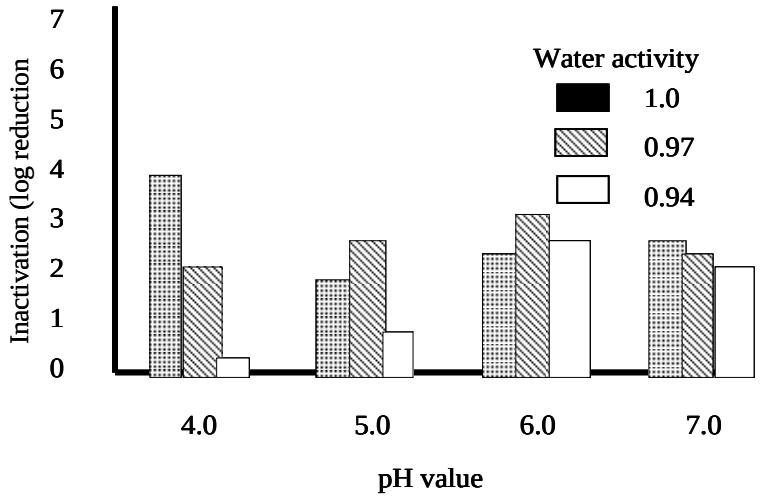

Fig. 3.13: Inactivation of Saccharomyces CBS 7764 in NTM at inlet temperature 30ºC with varying pH and a_w, after exposure to PEF (Aronsson & Ronner, 2001).

More results were observed by Vega-Mercado, Pothakamury, Chang, Barbosa-Ca`novas & Swanson (1996) for the inactivation of E. coli suspended in modified simulated milk ultra filtrate (SMUF) in a static chamber. The pH of the SMUF was adjusted by adding HCl (5 ml of 1 M solution) to pH 5.7 or 6.8. The electric field varied from 20 to $55 kV/cm$. Low intensity ($20 kV/cm$) resulted in a nonsignificant inactivation of microorganisms independent of temperature and pH. Meanwhile, the inactivation of E. coli increased with an increase in the number of pulses and with the increase in the electric field intensity from $40-55 kV/cm$. The results indicate that the inactivation was more significant at pH 5.69 than 6.82.

Heinz & Knorr (2000) investigated the effect of medium pH (in the range of 5.5 to 7) and composition as well as the simultaneous application of high hydrostatic pressure (200 Mpa) on the inactivation of vegetative Bacillus subtilis when exposure to high intensity pulsed electric fields. The variation in pH was found to have minor influence on the treatment. They explained the independence of pH might be due to the action of homeostatic regulation mechanisms, which maintains the proton concentration and prevent changes of the ionic composition in the protoplasma. Hence, the ohmic resistance of the cell interior is not affected by the variations in medium pH within the investigated range.

More recently, García, Gómez, Raso & Pagán (2005) evaluated and compared the pulsed electric field (PEF) resistance of four Gram-positive bacteria (Bacillus subtilis, Listeria monocytogenes, Lactobacillus plantarum, Staphylococcus aureus) and four Gram-negative bacteria (Escherichia coli, E. coli

O157:H7, Salmonella serotype Senftenberg 775W, Yersinia enterocolitica). They found that microbial inactivation depends on the treatment medium pH. L. monocytogenes showed the highest PEF resistance at pH 7.0 and was one of the most sensitive at pH 4.0. The other PEF resistant strains at pH 4.0 were the Gram-negatives E. coli O157:H7 and S. Senftenberg. The workers also reported that a subsequent holding of PEF-treated cells in pH 4.0 for 2 h increased the degree of inactivation up to 4 extra log reductions depending on the bacterial strain investigated.

3.3.5 Water Activity

A decrease in water activity of the suspension medium represses the killing effect of the PEF treatment. Aronsson & Ronner (2001) investigated the role of water activity (a_w) in the survival of E. coli and S. cerevisiae as presented previously in (Fig. 3.13) and (Fig 3.14). Lowering (a_w) seems to protect both E coli and S. cerevisiae from PEF inactivation.

The effect of lowering the water activity was more pronounced at lower pH values. 4.6 log reduction was observed on E coli at a_w 1.00, 30ºC and pH 5. When the a_w value was lowered to 0.94, an apparent decrease of 1 log reduction in inactivation level was observed.

The inactivation level was also the greatest for S. cerevisiae at a_w 1.0, pH 4.0 and 30^{o}C. When a_w decreased from 1 to 0.97, the inactivation level was

decreased from 3.8 to 2.1 log. Additional reduction in a_w to 9.4 decreased the inactivation to only 0.4 log, and at pH 6 and 7 there was no distinctive trend.

3.4 Conclusions

Microbial inactivation by PEF depends on many factors that are critical to the outcome of the process. An adequate knowledge of the factors affecting microbial inactivation is necessary to obtain quality PEF inactivation data, as well as to prevent experimental artefact and to avoid misinterpretation of results.

The electric field intensity, pulse width and number of pulses were the main process parameters that affect microbial inactivation using PEF treatment. Increasing the electric field intensity leads to an increase in the cell membrane conductance and permeability. Further more, the PEF inactivation is a function of the type of microorganism, bacterial growth stage, initial inoculum size, preculture condition, ionic concentration, and conductivity of the suspension medium.

The PEF treatment and the ionic strength are more likely to be responsible for the poration and compression of microbial cells while the pH surrounding the cell affects the cytoplasm when the poration is completed. It is possible that the high acid environment of the food product may prevent sub-lethally damaged cells from recovery.

As noted in the investigations on the effect of the electric fields intensity on the microbial inactivation, the average input power was changed as well due to

the higher current passing through the chamber as a result of increasing the voltage, the higher input power could also effect the inactivation, and it is difficult to distinguish if the microbial killing is affected by the power as well as the electric field. In our work, we will investigate the effect of the electric field intensity at constant electric power in Chapter 7.

The effect of electric fields intensity needs to be assessed more accurately. Changing the electric field will change the current passing through the treated solution and hence, the power absorbed by the treated sample. In order to sustain the power consumed, the treatment solution of lower conductivity must be selected when the electric field is increased, or the same solution (same electrical conductivity) at different inlet temperature. This is one of the objectives of this project.

4. Chapter Four: Literature Review - Principles of PEF Microbial Inactivation Mechanism and Food Quality Changes

Each preservation technology has its own inactivation mechanisms, and PEF researchers have been trying to explain the mechanism of microbial inactivation. This chapter review the proposed mechanisms of microbial inactivation when subjected to pulsed electric field treatment. It was believed that PEF treatment can cause electroporation and/or electrofusion of the cell membrane due to the increase in transmembrane potential, electromechanical compression of the membrane and enhancement of structural defects and conformational changes in the lipid or protein molecules.

Selected studies published on the effects of PEF on the quality attributes of food will be also addressed in this chapter.

4.1 Mechanism of Microbial Inactivation

PEF can cause electroporation, the permeabilization of the membranes of cells and organelles, or electrofusion, the connection of two separate membranes into one (Qin, Pothakamury, Barbosa-Ca`novas & Swanson, 1996). There are several theories to explain how pores are formed but it is still unclear whether it occurs in the lipid or in the protein matrices (Barbosa-Cánovas, Gongaro-Nieto,

Pothakamury & Swanson, 1999). The electric field induces structural changes in the microbial cells and membranes of micro-organisms (Pothakumury, Vega-Mercado, Zhang, Barbosa-Ca`novas, Zhang & Swanson, 1997).

Some studies postulate the cell membrane as the site of critical effect of reversible or irreversible loss of membrane function as the semi permeable barrier between the cell and its environment. An external electric field of short duration was assumed to induce an imposed transmembrane potential above a critical electric field intensity, which may produce a dramatic increase of membrane permeability (more details in section 4.1.1). Exchange or loss of cell contents, cell lysis and irreversible destruction may occur as secondary mechanisms. This will limit the ability of cells to repair themselves, which adversely affect permeable cells through osmotic pressure differences between the medium and the interior of the cell.

Pothakamury, Barbosa-Ca`novas, Swanson & Spence (1997) treated cells of S. aureus suspended in SMUF by both thermal treatment and PEF treatment with 64 pulses at 20, 30 and $40 kV/cm$. Using electron microscopy, they observed that cells treated by PEF exhibited rough surfaces, and that the ones treated under more severe conditions showed small holes in the membrane and leakage of cellular contents. The mechanism of inactivation was different from that of thermal treatment, since heat treatment (66°C for 10 min) resulted in a great damage of the cell organelles, whereas they do not suffer cell wall rupture as induced by PEF treatments.

Chang (1989) investigated another mechanism of the cell membrane damage due to electromechanical coupling effect. In this mechanism, the intense electric field within the cell membrane forces the charged groups of the membrane, such as the phosphate groups of phospholipids molecules and ions absorbed by the membrane, to move in the direction of the applied electric field. Molecular and ion movement produces mechanical stresses in the membrane. When this stress is large enough, it mechanically disrupts the membrane structure.

4.1.1 Transmembrane Potential

The biological cell membrane acts as an insulator shell to the cytoplasm. The electrical conductivity of the cytoplasm is six to eight orders greater than that of the membrane (Chen & Lee, 1994). When an electric field is applied, free charges generated on the membrane surfaces attract each other since they are of opposite signs. This attraction gives rise to a compression pressure that causes the membrane thickness to decrease as shown in (Fig. 4.1). The accumulation of more surface charges increases the electromechanical stress or transmembrane potential (Kinosita & Tsong 1977 and Zimmermann 1986). The electric forces and compression pressure increases with the decrease in the membrane thickness. A further increase in electric field intensity leads to a critical membrane potential and subsequently to a reversible membrane breakdown or pore formation as shown in (Fig. 4.1-c). With much greater field strengths, larger and larger areas of the membrane are subjected to breakdown as in (Fig. 4.1-d). If the size and

number of pores become large in relation to the membrane surface, irreversible breakdown associated with mechanical destruction of the cell occurs (Zimmermann, 1986).

The induced potential is greater in a large cell, which means larger cells are more susceptible to damage than smaller cells (Chen & Lee 1994).

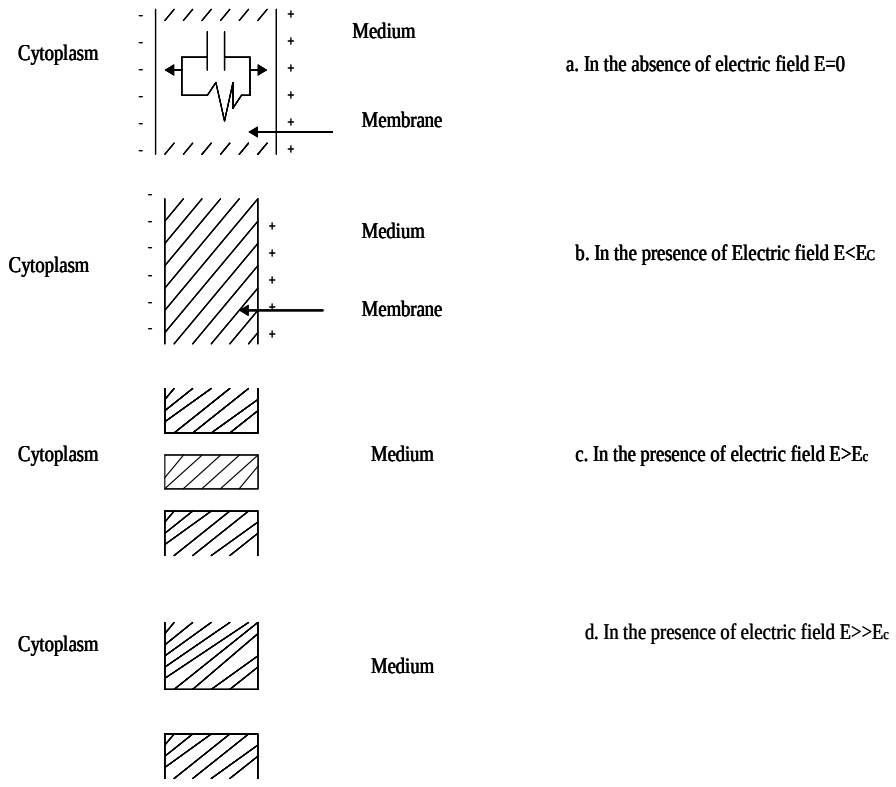

Fig. 4.1: Electroporation of the cell membrane by compression when exposed to high-intensity electric fields (the membrane considered a capacitor and represented by the hatched area; E_c *represents the critical electric field intensity (Zimmermann, 1986).*

4.1.2 Electromechanical Compression and Instability

Ho & Mittal (1996) presented a review of the electromechanical instability theory, which is based on structural changes of the membrane. The mechanism considers membrane breakdown to occur as a consequence of the decrease in the membrane thickness due to a compressing stress, where the membrane undergoes an increase in area per lipid, which destabilizes the bilayer.

The electric compressive force (P_e) per unit area of the membrane is given by Coster & Zimmermen (1975):

$$P_e = \frac{\varepsilon \varepsilon_0 V^2}{2\delta^2}$$

Where, V is the potential difference across the membrane, ε is the dielectric constant or relative electric permittivity, ε_O is the electric permittivity of free space and δ is the membrane thickness.

Zimmermann, Pilwat, Beckers, & Riemann (1976) reported that one of the drawbacks of the electromechanical instabilisation theory is the assumption that the membrane acts as a capacitor containing a perfectly elastic dielectric. It fails to take into account the subsequent behaviour of transmembrane voltage, membrane conductance, electropores and molecular transportation.

4.1.3 Osmotic Imbalance

In another theory, the rupture of the microbial membrane is believed to be due to the osmotic imbalance generated by the leakage of ions and small molecules after being exposed to an electric field. Permeation then causes swelling and the eventual rupture of the cell membrane as shown in (Fig. 4.2).

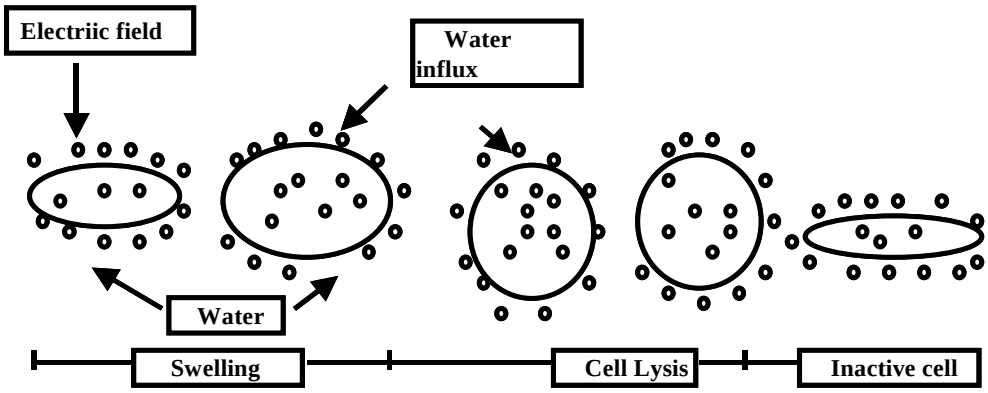

Fig.4.2: Electroporation of a cell membrane based on colloid osmotic swelling (Tsong, 1990).

4.1.4 Viscoelastic Model

The viscoelastic model (Fig. 4.3) is based on the surface tension and the viscosity of the membrane. It considers the membrane as a thin viscoelastic film with fluctuating surfaces bound by two semi-infinite bulk phases. The membrane

behaves as a viscoelastic, isotropic material represented as a standard solid model (Fig. 4.3-a). The model composed of a Kelvin body with an elastic modulus (G') and viscosity (μ') in series with a linear spring with its own elastic modulus (G_0'). This model is equivalent to the three-element Maxwell fluid model with a viscosity and elasticity being in parallel with a restoring spring (Fig. 4.3-b). It is an extension of the electromechanical model of membrane breakdown because it takes into account surface tension and membrane viscosity. The time (τ) at which membrane breakdown occurs is given by Dimitrow (1984) as:

$$\tau = \alpha \frac{\mu / G}{\left(\frac{\varepsilon_m^2 \varepsilon_0^2 V^2}{24 \sigma G h^3} - 1 \right)}$$

Where, α is the proportionality constant, μ is the viscosity, G is the elasticity, ε_m is the relative dielectric constant of the membrane material, ε_0 is the permittivity of free space, V is the voltage, σ is the surface tension, and h is the membrane thickness. The critical breakdown potential U_c is given by:

$$U_c^2 = \left(\frac{8\sigma}{hE} \right)^{0..5} \frac{Eh^2}{\varepsilon_m \varepsilon_0}$$

Where, E is the Young's modulus. The membrane stability decreases with decreasing the surface tension. The average membrane thickness may not be changed significantly, but the amplitude of the local shape perturbations can increase and thus lead to local breaking of membrane and pore formation (Barbosa-Ca`novas, Gongaro-Nieto, Pothakamury & Swanson, 1999).

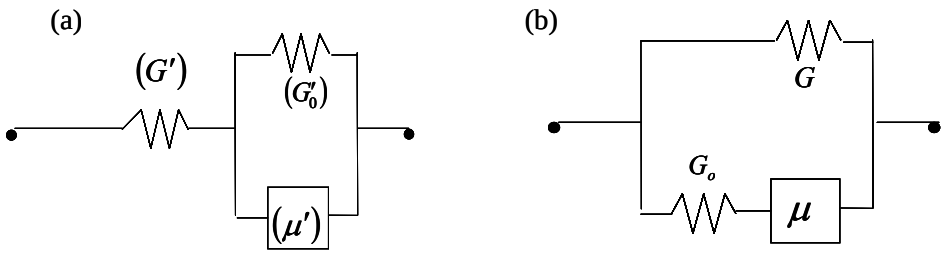

Fig.4.3: Two viscoelastic models to represent membrane dynamics: a) a Kelvin body $(G_O^{`}, \mu^{`})$ *in series with a spring* (G) *and b) a Maxwell body* (G_O, μ) *in parallel with a spring* (G) *(Dimitrow, 1984).*

4.1.5 Hydrophobic and Hydrophilic Pores

Exposure of bilayer lipid membranes to PEF causes the formation of pores in the membrane in two-stage process. The first stage is the formation of hydrophobic pores which formed due to thermal motion of lipid molecules in the lipid layer. The life time of the hydrophobic pores is in the order of lipid molecule fluctuation (Barbosa-Ca`novas, Gongaro-Nieto, Pothakamury & Swanson, 1999).

The second stage of the pore formation process is the formation of hydrophilic pores, which have a wall of reoriented lipid molecules with polar heads. The formation of hydrophobic or hydrophilic pores based on pore energies at different pore radii. A hydrophobic pore of zero radius with zero energy

represents the membrane in normal state. When the pore radius exceeds a critical value (r^*), the pore energies of both hydrophobic and hydrophilic pores become equal. If the radius increased further, in this case the energy of hydrophilic pore is lower than that of hydrophobic pores, and hydrophilic pores will form and mechanical break down of the membrane will induce (Qin, Pothakamury, Barbosa-Ca`novas & Swanson, 1996).

4.1.6 Theories Based on Conformational Changes

The membrane reorientation theory proposed by Neumann & Rosenheck (1972) is based on the electric field-induced displacements of membrane components such as lipids or protein. Furthermore, due to the transient characteristics of the reoriented polar heads of the pores and the associated increase in membrane conductance, both disappear as soon as the external electric field. Schwarz (1978) described these changes as conformational transitions of integral macromolecular structures such as lipid or protein components of a membrane. Schwarz also agreed that these alterations may lead to an electrical and/or mechanical breakdown of the membrane (Barbosa-Ca`novas, Gongora-Nieto, Pothakamury & Swanson, 1999).

4.1.7 Electric Field-Induced Structural Changes

Early researchers pointed out that PEF treatment causes conductivity changes and loss of the ability to plasmolyze without any global damage to cells. This could be due to the low electric field intensities that have been applied.

However, at higher electric field intensities structural damage has been reported in Lactobacillus, Saccharomyces, Escherichia coli, and Staphylococcus (Barbosa-Ca`novas, Gongaro-Nieto, Pothakamury & Swanson, 1999).

Calderon-Miranda, Barbosa-Ca`novas & Swanson (1999) investigated under transmission electron microscopy (TEM), the morphological changes on Listeria innocua as a result of PEF treatment in both skimmed milk with and without 37IU nisin/ml. L. innocua was subjected to PEF at selected electric field intensities of 30, 40, and $50 kV/cm$. Their results show that L. innocua treated by PEF in skimmed milk exhibited an increase in the cell wall roughness, cytoplasmic clumping, leakage of cellular material, and rupture of the cell walls and cell membranes. L. innocua subjected to PEF in skimmed milk containing 37 IU nisin/ml exhibited an increased cell wall width. At the highest electric field intensity ($50 kV/cm$), elongation of the cell length was observed. The morphological changes observed on L. innocua as a function of the electric field intensity are presented in (Fig. 4.4). As the electric field strength increases, the cell wall of L. innocua looses smoothness and uniformity. Their results also suggested that there were no morphological differences between cells treated by PEF in skimmed milk with or without nisin. The inactivation of L. innocua was a consequence of rupture of the cell membrane and loss of cell membrane functionality.

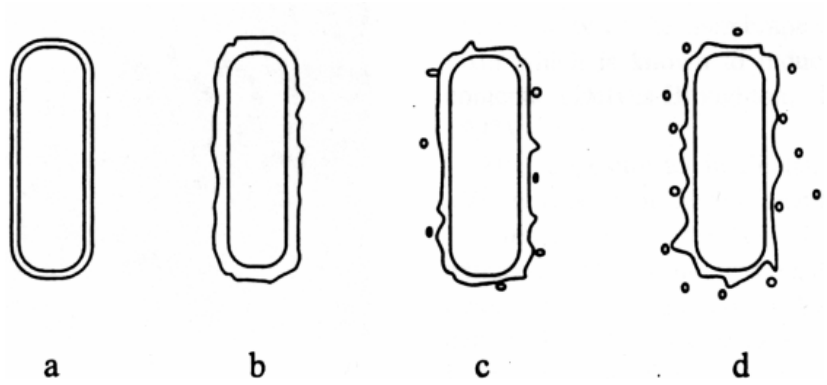

Fig. 4.4: Effect of an increase in PEF intensity on a L. innocua cell wall; (a) normal cell; (b) cell subjected to 30 KV/cm; (c) cell exposed to 40 KV/cm; and (d) exposure to 50 KV/cm (Calderon-Miranda, Barbosa-Ca`novas & Swanson, 1999).

Additional research conducted by Harrison, Barbosa-Ca`novas & Swanson (1997), with the use of transmission electron microscopy (TEM) revealed that electric field treatment caused important structural changes to S. cerevisiae cells (ATCC 16664) suspended in apple juice. Cell elongation, surface roughening and massive damage in the form of a cell wall hole were noticed. Most of the cellular organelles, such as the nuclear membrane and ribosomes were partially or completely disintegrated. Furthermore, it was concluded that TEM dose not support the electroporation mechanisms as the major mode of yeast inactivation. The conclusion was that PEF treatment result in cytological disruption of a large portion of the yeast cellular organelles (especially ribosomal bodies), which is the primary mode of S. cerevisiae inactivation; with electroporation is acting as a secondary mechanism of inactivation.

4.2 Effect of PEF Treatment on Food Quality

Despite the extensive knowledge available on food preservation by heat treatment and despite continued attempts to improve the quality of processed foods (Durance, 1997), there is still a need for technologies that minimize the destructive influence of heat on valuable food compounds (Knorr & Heinz, 2001).

The use of high intensity pulsed electric fields processing in food such as fruit juices and milk is gaining popularity as a research area, since it is claimed to represent one of the promising non-thermal alternatives to conventional pasteurisation methods. The PEF treated food should remain fresh and possess a satisfactory ambient shelf life. There have been only few studies published on the effects of PEF on the quality attributes of food. In this chapter, the effect of pulsed electric fields on enzymes, vitamins, color, and flavor of different food are reviewed.

4.2.1 Effect of PEF Treatment on Enzyme Activity

An enzyme has to maintain its native structure to prevent denaturation or degradation. Enzymes are stabilized by weak non covalent forces, such as hydrogen bonds, electrostatic forces, van der Waals forces and hydrophobic interactions; internal salt bridges; and in some cases, disulphide bonds (Price & Stevens, 1991). A change in the magnitude of any of these could cause denaturation. The application of high electric field pulses might affect the forces

involved in maintaining the three-dimensional structure (secondary, tertiary and quaternary structure) or conformation of the globular protein (Ho, Mittal & Cross, 1997). Where: secondary structure refers to local interactions between amino acids in close proximity in the sequence to generate regular structural features, such as helices and turns, tertiary structure refers to the overall folding of the polypeptide chain so as to cause interactions between amino acids remote from one another in the sequence, and quaternary structure refers to the arrangement of polypeptide chains of an enzyme which contains more than one such chain (Price & Stevens, 1991).

Enzyme activity is affected by high electric field pulses as reported by several researchers (Vega-Mercado, Powers, Barbosa-Ca`novas & Swanson 1995, Vega-Mercado, Pothakamury, Chang, Barbosa-Ca`novas & Swanson 1996, Ho, Mittal & Cross 1997, Castro 1994, Bendicho, Estela, Fernandez-Molina, Barbosa-Ca`novas & Martin 2002b, Loey, Verachtert & Hendrickx 2002 and Yeom, Zhang & Dunne 1999). According to the literature, the factors that influence PEF enzymatic inactivation are the electric parameters, enzymatic structure, treatment temperature and suspension medium.

Castro (1994) reported that reduction of Alkaline phosphatase activity (APL) in milk by PEF is a function of field intensity, fat content of milk and concentration of APL. The activity of ALP was decreased with the increase in field intensity. A reduction of 43-59% in APL activity was reported when the enzyme was suspended in 2% milk and exposed to 70 pulses of $0.40-0.45ms$ duration at$14.8-18.8kV/cm$. SMUF with $2mg/ml$ APL was subjected to

seventy pulses of $0.74ms$ duration with field strength of $22kV/cm$, the ALP activity was reduced by 65%. The activity of APL dissolved in UHT pasteurized 2% and 4% milk was reduced by 59% when exposed to 70 pulses of 0.40 ms at $18.8kV/cm$, while higher reduction of 65% was observed in non fat milk. APL suspended in milk (1 ml of raw milk dissolved in 100 ml 2% milk) treated with $13.2kV/cm$ and 43.9ºC after 70 pulses showed a reduction of 96% in activity, while heat treatment at 43.9ºC for 17.5 min showed only a 30% reduction. The inactivation of APL was reported to be attributed to conformational changes induced by PEF. Castro (1994) also achieved a reduction of 90% in the inactivation of the proteolytic enzyme plasmin when exposure to 30 and $45kV/cm$ and 10-50 pulses of $2\mu s$ duration. The process temperatures were 10 and 15ºC respectively.

Vega-Mercado, Pothakamury, Chang, Barbosa-Ca`novas & Swanson (1996) investigated the effect of presence of calcium on the inactivation of the protease from P. fluorescens M3/6 when exposed to PEF. The inactivation was the same for the three solutions containing 0, 10, and $15mM$ calcium.

Ho, Mittal & Cross (1997) tested eight enzymes of wide range of molecular weight, 14.3-186 kDa, and quaternary structure using a compact bench-top PEF system. 30 pulses were applied to the eight different enzyme solutions using $13-87kV/cm$ field, $0.5Hz$ pulse frequency, $2\mu s$ pulse width and 20ºC processing temperature. For some enzymes, activities were reduced after the pulse treatments. Lipase, glucose oxidase and heat stable α-amylase exhibited a vast reduction of 70-85%, peroxidase and polyphenol oxidase showed

a moderate 30-40% reduction while alkaline phosphatase displayed only a slight reduction under the conditions employed. Their results suggest that the degree of denaturation varied from enzyme to enzyme. Secondary structure or more likely, tertiary structure of the enzyme may play a more important role.

Vega-Mercado, Powers, Barbosa-Ca`novas & Swanson (1995) examined the feasibility of using pulsed electric field in the activation of Plasmin in simulated ultra filtrate. Plasmin activity was reduced by 90% following treatment with 50 pulses of $2\mu s$ width at $50kV/cm$ and at $45kV/cm$ field intensity, and at process temperature of 15ºC. Reducing the process temperature to 10ºC decreased the activity by 60% only. The susceptibility of casein to proteolysis varies as a function of treatment conditions.

The change in the proteolytic activity of plasmin was determined as the change in its ability to hydrolyse casein in a 'Bio-Rad Protease Activity Gel' contained in a petri dish. The enzymatic ability was related to the width of the clear zone in the gel. The reduction in proteolytic activity was estimated as the percentage reduction in clear zone (%RCZ):

$$\%RCZ = (D_{in} - D_{tr})/(D_{in} - D_{well}) \text{.....(5.1)}$$

Where, D_{in} is the clear-zone diameter of a $100\mu g/ml$ plasmin control, D_{tr} is the diameter of the clear zone for treated plasmin after 24hr incubation, and D_{well} is the well diameter (not treated).

Yang, Li & Zhang (2004) subjected a group of enzymes to continuous PEF treatments for $126\mu s$ treatment time. 51.7% and 83.8% of pepsin was

inactivated at $37 kV/cm$ and $41.8 kV/cm$ respectively. Enzyme activity of polyphenol oxidase decreased by 38.2% when treated at $33.6 kV/cm$ for $126 \mu s$. Enzyme activity decreased by 18.1% and 4% for peroxidase treated at $34.9 kV/cm$ and chymotrypsin treated at $34.2 kV/cm$, respectively. However, Lysozyme activity was not affected by PEF treatment below $38 kV/cm$. The researchers indicated that inactivation was a function of electric field strength and electrical conductivity of media. Both the PEF and the induced heat contributed to the observed inactivation effect, depending on the properties of enzymes and test conditions.

Loey, Verachtert & Hendrickx (2002) studied the sensitivity of Lipoxygenase, Polyphenoloxidase, Peroxidase, Pectimmethylesterase, Alkaline phosphate and Lactoperoxidase towards PEF treatment when dissolved in distilled water. Electric field strengths of 10, 20, and $30 kV/cm$, pulse widths of (5 and $40 \mu s$) at frequencies of 1 and $100 Hz$ were applied. The inactivation was less than 10% in all the enzymes tested. However, at longer processing times, inactivation of Lipoxygenase and Polyphenoloxidase was increased to 64 & 12% respectively. The researchers suggested that the inactivation was caused by electrochemical reactions that could take place at the electrode surfaces during long treatment times, because the Lipoxygenase and Polyphenoloxidase are oxidoreductases that contain iron and copper atoms at their active site.

The observed effects of pulsed electric field treatment on enzyme by different research groups appear to depend, besides on the enzyme on the characteristics of the PEF system used and on the electric process parameters.

Because the technology has not yet been standardized, PEF process and equipment specifications are varying from study to study, which makes a comparison of results between different studies very difficult. The experimental conditions such as electric field strength, number of pulses, wave shape, pulse width and batch, step-wise or continuous circulation processing should be considered.

Enzymes might also have beneficial effects on food. In this case knowledge of enzyme stability under the relevant processing conditions is required for process design to enhance the enzymatic activity during processing.

4.2.2 The Effect of Pulsed Electric Fields on Vitamin Retention

Generally some vitamins present in milk are more sensitive to heat treatments than others. However, all thermal processes affect the nutrients originally present in raw milk. Ascorbic acid, thiamine and riboflavin are water soluble vitamins and cholecalciferol and tocopherol are fat-soluble. Bendicho, Espachs, Arantegui & Martin (2002) investigated the effect of high intensity pulsed electric fields and heat treatments on water-soluble (thiamine, riboflavin, ascorbic acid) and fat-soluble vitamins (cholecalciferol and tocopherol) of milk. Samples of SMUF were subjected to PEF treatments up to $400\,\mu s$ at field strengths from 18.3 to $27.1 kV/cm$ and also to heat treatments of up to $60\,\text{min}$ at temperatures from 50 to 90°C. They observed no changes in vitamin content after PEF or thermal treatments except for ascorbic acid. Milk retained most of the ascorbic acid (93%) after a $400\,\mu s$ treatment at $22.6 kV/cm$. Thermal

pasteurisation at (63^{o}C and 30min) and at (75^{o}C for $15\,\text{sec}$) retained 49.7% and 86.7% of the vitamin. Their results also show that skim milk retained more ascorbic acid than SMUF after PEF treatments as shown in (Fig. 4.5). They reported that this could be due to the higher complexity and protective effect of natural milk components such as proteins, mainly casein. On the contrary, thermal processed SMUF retained more vitamins than milk. This difference gives PEF a real advantage over thermal treatments when products with complex matrices have to be processed.

Yeom, Streaker, Zhang & Min (2000) investigated the effects of PEF on the quality of orange juice when exposed to field intensity of $35 kV/cm$ for treatment time of $59 \mu s$ and compared the results with those of heat pasteurisation at 94.6ºC for $30\,\text{sec}$. No significant difference was found in the concentration of ascorbic acid when treated with PEF unlike the heat treated orange juice.

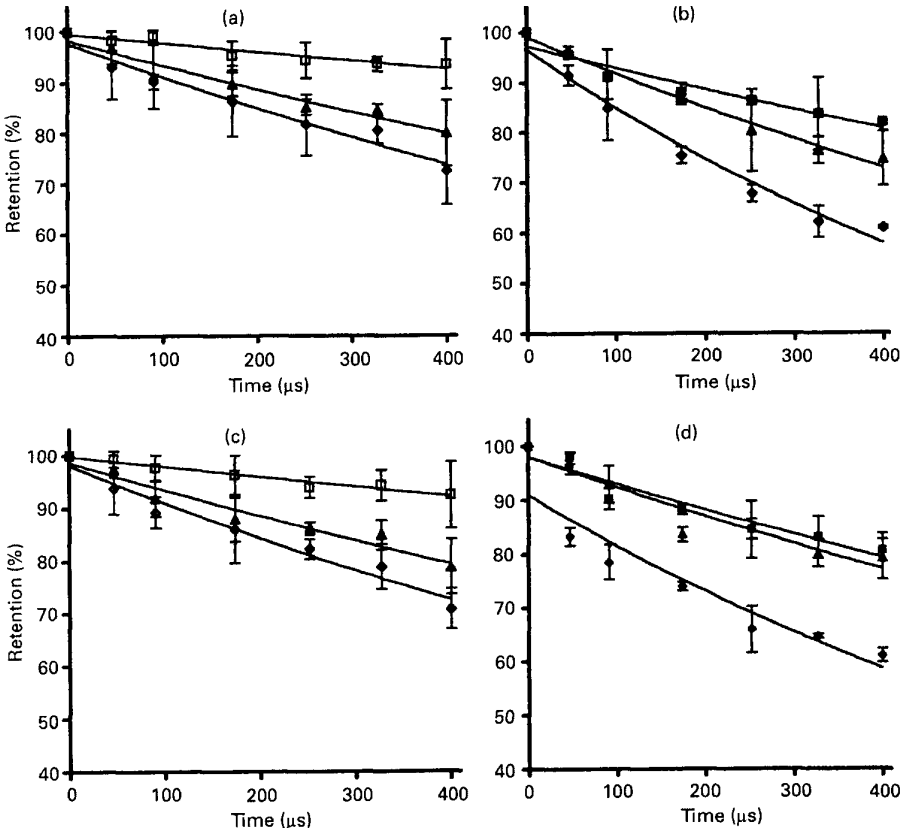

Fig. 4.5: .Retention of ascorbic acid in milk or simulated milk ultrafiltrate (SMUF) exposed to PEF treatments. (a) milk exposed to PEF at 20-25°C; (b) SMUF exposed to PEF at 20-25°C; (c) milk exposed to PEF at 50-55°C; (d) SMUF exposed to PEF at 50-55°C, (Yeom, Streaker, Zhang & Min, 2000)

4.2.3 Effect of PEF Treatment on Colour

Jin (1999), investigated the effects of high voltage pulsed electric field on colour stability of cranberry juice after PEF treatment and during storage, in comparision to thermal pasteurisation. Cranberry juice contains anthocyanin pigments. Anthocyanin containing products are susceptible to colour deterioration during processing and storage, resulting in combined anthocyanin degradation

and brown pigment formation (Albers & Wrolstad, 1979 and Markakis, 1982). The Cranberry juice samples were treated at The Ohio State University in a bench scale continuous PEF system (Sensoy, Zhang & Sastry, 1997) at $20kV/cm$ for $50\mu s$, $20kV/cm$ for $150\mu s$, $40kV/cm$ for $50\mu s$ and $40kV/cm$ for $150\mu s$.

The thermal treatment of the cranberry juice was based on the conditions for a typical commercial HTST process for treating cranberry juice (Wightman & Wrolstad, 1995). The juice sample was heated rapidly to 90ºC and kept for $90s$, and then rapidly cooled to 8ºC.

Anthocyanin pigment content was determined by the pH differential method of (Fuleki & Francis, 1968). Their results did not show any significant differences in the content of anthocyanin pigments between PEF treated samples and controlled samples. However, heat treatment significantly reduced the anthocyanin pigment content. The loss of the pigments for all samples increased in a similar manner during the 14 day storage period at 4^oC. While thermal treated samples retained the lowest anthocyanin content after two week storage. Mackinney & Chichester (1952) indicated that the loss of anthocyanin pigments in strawberry juice by heating may be a result of the sensitivity of anthocyanin pigments to oxidation.

4.2.4 Effect of PEF Treatment on Flavour

The effect of PEF and heat treatment on the retention of flavor compounds in orange juice was studied by many researchers. Jia, Zhang & Min (1999) processed fresh squeezed orange juice by pulsed electric field at

$30kV/cm$ for 240 and $480\mu s$ and by heat treatment at 90ºC for $1\min$. The effect of PEF and thermal processing on five flavor compounds (ethyl butyrate, α-pinene, octanal, limonene, and decanal) were studied. The flavour compounds in the headspace of orange juice were analysed by a combination of SPME (Buchholz & Pawliszym, 1994) and gas chromatography. The losses of volatile flavour compounds in orange juice samples treated with PEF for $240\mu s$ and $480\mu s$, and heat treated were 3%, 9% and 22%, respectively. Ethyl butyrate has the lowest molecular weight and boiling point and hence experienced maximum loss. The boiling points of octanal and limonene are similar, but the losses of octanal and limonene by PEF treatment for $480\mu s$ were 0% and 7.5%, respectively. Decanal was not lost by PEF, but experienced loss through thermal treatment of 41%. The researchers indicated that the flavour loss by PEF process was mainly due to PEF vacuum degassing system. The most volatile compounds with small molecular weight were removed to a greater extent by the vacuum system. However, the change in the orange juice flavour was more pronounced by thermal processing (Shaw, 1986). Irreversible damage to the citrus juice flavor results from chemical reactions initiated or occurring during the heating process (Braddock, 1999).

4.3 Conclusions

Several theories have been discussed to explain how pores are formed. Although some researchers postulate that pores are formed due to electroconformational changes in lipid or protein molecules, it has been also

suggested that the mechanical breakdown of the cell membrane may not be caused by electroporation, but by secondary effects such as osmotic imbalances between cells and their environment.

The microbial inactivation principle seems to vary from microorganism to microorganism. In addition, the same species apparently undergo different PEF effects, which may be caused by different inactivation mechanisms depending on treatment conditions and suspending media.

In this work, TEM studies were carried out to study the mechanism of E coli inactivation using PEF treatment. Details will be presented in Chapter 8.

In the effect of PEF on food quality, enzymatic activity was affected by high intensities electric field pulses. Enzymes have different sensitivity to PEF treatment. Many factors can influence PEF enzymatic inactivation. The electrical parameters includes electric field strength, treatment time, number of pulses and pulse duration, and also the biological parameters including enzymatic structure such as the secondary and tertiary structure, treatment temperature and suspension medium.

According to the literature, enzymes are generally more resistant to electric pulses than microorganisms. This requires further investigation, especially on the effect of pH, temperature, resistivity, and composition of the enzyme-containing medium or food. However, this is not part of the investigation of this thesis.

PEF treatment of different foods provides many advantages with regards their flavour and nutrients level, which results in a fresh like products. PEF processed orange juice retained more flavour compounds than the heat pasteurised orange juice. Milk treated using PEF also proved to retain more ascorbic acid than milk treated with heat.

The loss of flavour compounds by PEF process could be minimized by improving the degassing system in the PEF apparatus.

The anthocyanin pigment lost by heat is probably due to the sensitivity of anthocyanin pigments to oxidation, as heat creates an opportunity for oxidative reactions, which cause a degradation of the pigments.

5. Chapter Five: Experimental Studies on PEF Treatment Chambers Design, Optimisation and Operation

The design of pulsed electric fields process and the associated processing equipment is the key element to control microbial inactivation and food quality in non thermal pasteurisation. The PEF unit consist of number of equipments as will be discussed in the next chapter.

One of the key components in the design of the PEF pasteurisation process is the treatment chamber. It is of interest to find how to achieve uniform electric field distribution, uniform treatment time, maximise the electrical resistance of the treatment region, and maintain a minimum treatment temperature.

This chapter discusses the effect of using different geometries (electrodes and insulator) of continuous multi pass treatment chamber/s on the electric field strength in the treatment zone. The second section presents experimental investigations conducted on thermal performance of the designed chamber with regards variation of current, total resistance across the chamber, temperature increase using different electric field intensity and flow rates and dissipated power during the PEF application.

5.1 Experiments to Determine the Design of the Multi Pass Treatment Chambers

Multi pass treatment chambers were designed based on some theoretical and experimental investigations as will be presented in this chapter.

The treatment zone and electrodes dimensions were determined to provide maximum electric field strength in the treatment zone. In order to achieve maximum possible electric field strength in the treatment zone, the electrical impedance of the treatment zone must be maximised, while minimising the voltage loss in the outlet and inlet of the treatment chamber.

5.1.1 Materials and Methods

5.1.1.1 Treatment Chamber

A batch treatment chamber was designed based on theoretical predictions. Theoretically increasing the electrodes diameter decreases the voltage loss in the inlet and outlet of the treatment region, and decreasing the distance between the two electrodes also decreases the voltage loss in these regions.

The chamber consists of two mesh electrodes and an insulator element configured to form a narrow treatment region as shown in (Fig. 5.1). The chamber was made of many parts that can be changed so that different treatment zone diameters ($6.9mm$ and $3.9mm$) and different electrode diameters ($50mm$ and $25mm$) can be tested. The treatment zone depth was fixed at $5mm$ while the

distance between the electrodes was $15mm$. The opening of the mesh electrodes was $0.85mm$ with wire diameter of $0.42mm$. The chamber was made of Plexiglass that can hold temperature up to $60^{o}C$.

The electric field is complex in this kind of chamber. Accordingly, experiments were conducted on this chamber using low voltage in the range of (0.4-11.6V) applied to SMUF of 100% concentration. Low voltage was applied for the testing to avoid significant increase in solution temperature, which in turn could affect the current passing through the treated solution. The experimental results were analysed to determine the proper dimensions of the chamber, where maximum strength of the electric field was concentrated in the treatment zone.

5.1.1.2 Electrical Circuit

The electrodes were connected to the power supply through an isolating transformer and a variac to provide different voltages, as shown in (Fig. 5.2). The voltage was measured using PM 2519 automatic multimeter (Philips) and the AC current of $50Hz$ was measured by Escort EDM 117 meter.

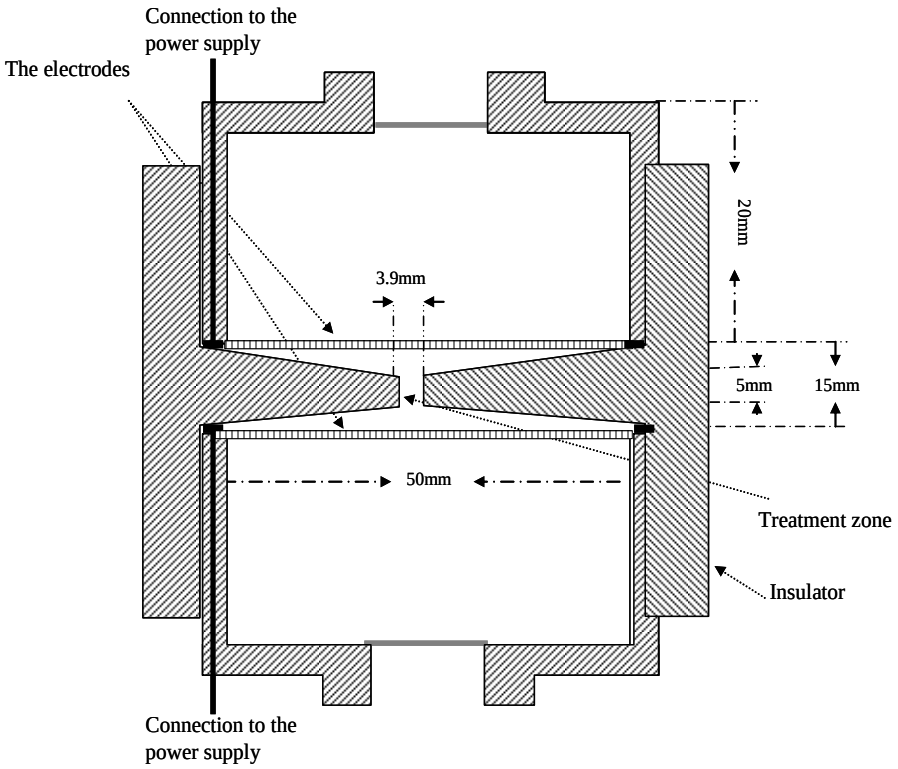

Fig. 5.1: Batch Treatment chamber (The University of Auckland, 2004).

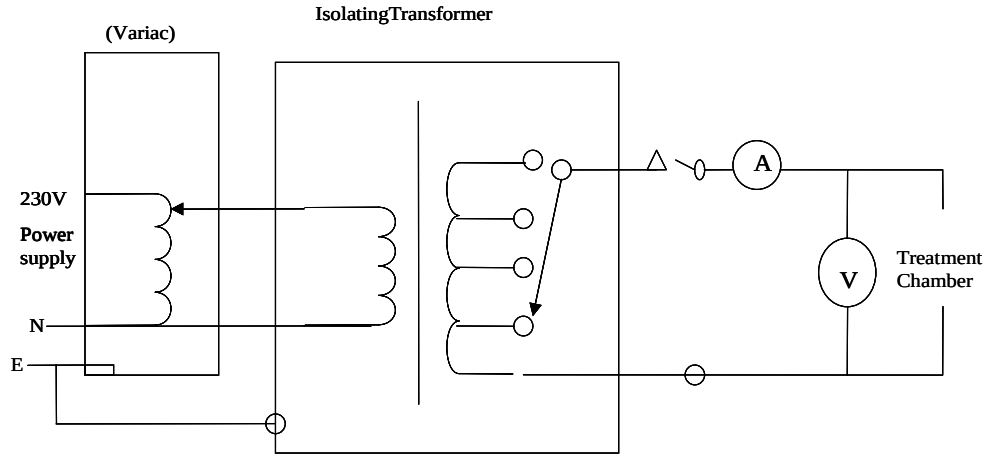

Fig. 5.2: Electric circuit to generated low voltage.

5.1.1.3 Suspension Medium

Simulated milk ultra filtrate (SMUF) was used as the suspension medium. SMUF is a formulated system of milk constituents used in non thermal processing research to study the potential changes during non thermal processing. It is a salt solution with a selected composition (Table 5.1) encountered in milk ultra filtrates.

Table 5.1: Simulated milk ultrafiltrate (Pothakamury, Monsalve-Gonzalez, Barbosa-Ca`novas & Swanson, 1995).

Components	*Concentration (g/L distilled water)*
Lactose	50.00
Potassium phosphate monobasic	1.58
Tripotassium citrate	0.98
Trisodium citrate	1.79
Potassium sulphate	0.18
Calcium chloride dehydrate	1.32
Magnesium citrate	0.38
Potassium carbonate	0.30
Potassium chloride	1.08

5.1.2 Experimental Protocols

The experimental procedures were as follows:

1. The effect of cross sectional area of the treatment zone and electrodes diameter on total resistance across the treatment chamber was studied. Experiments were conducted using treatment zones of different diameters (3.9

and 6.9*mm*) and electrodes of different diameters (50 and 25*mm*). The treatment chamber was filled with SMUF at room temperature, and then the voltage was raised gradually. The variation in current was measured and the total resistance across the chamber was calculated using Ohm's law.

2. Experiments to determine the ohmic resistance of the inlet and outlet regions of the treatment chamber were conducted to minimise the voltage loss in these inlet and outlet regions. To achieve this objective, a stainless steel disk (act as a second electrode) was placed in the outlet of the treatment zone, as shown in (Fig. 5.3).

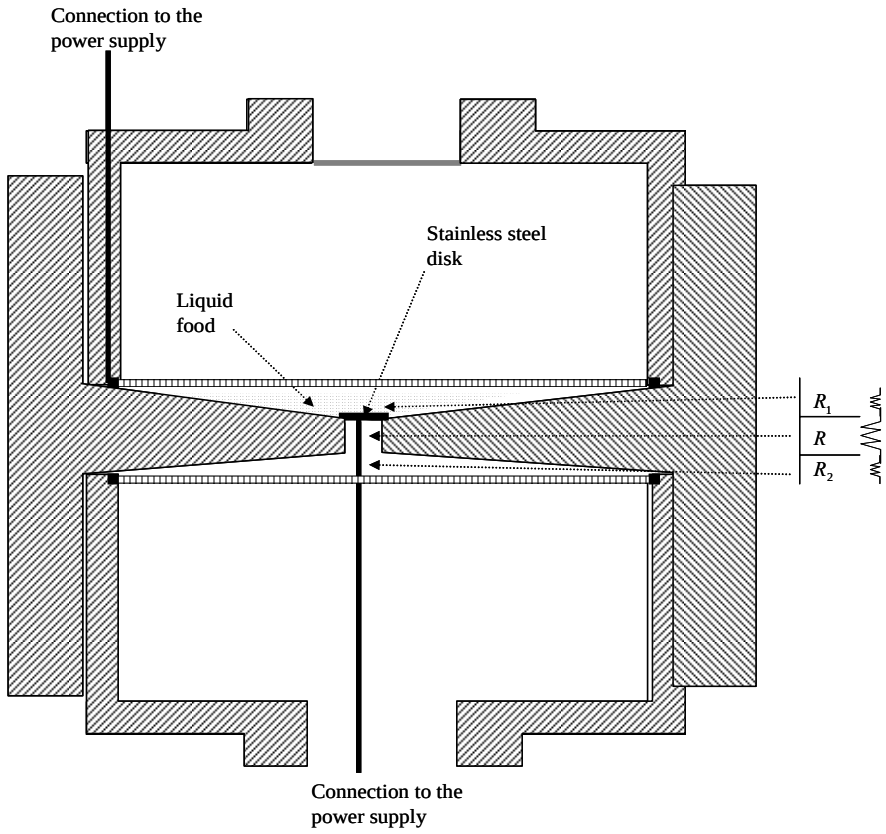

Fig. 5.3: The treatment chamber with the disk placed on the top of the treatment zone.

The cavity representing the outlet of the treatment zone was filled with SMUF, and its impedance was measured using Ohm's law. The impedance of the outlet and inlet of the chamber maybe assumed equal and the impedance of the treatment zone can be calculated by subtracting the impedance of the inlet and outlet regions from the total impedance.

Experiments were repeated using different dimensions of the treatment zone (diameter of 3.9 and $6.9mm$) and different dimensions of electrodes (diameter of 50 and $25mm$). The experimental results were analysed to determine the proper dimensions that minimise voltage loss.

5.1.3 Results and Discussion

Decreasing electrode diameter from $50mm$ to $25mm$ did not affect the total resistance across the treatment chamber as shown in (Fig. 5.4) and (Fig. 5.5). The significant increase in the impedance of the treatment zone is due to the reduction in its diameter as expected according to equation (1.4). The slight decrease in the measured impedance as voltage increase is probably due to minor increase in the SMUF temperature due to ohmic heating.

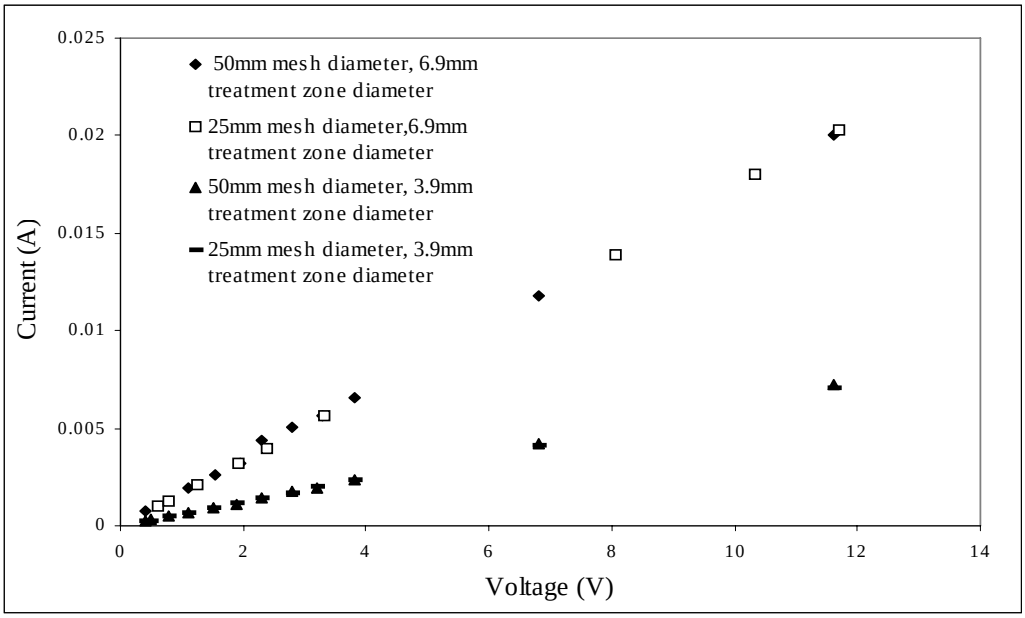

Fig.5.4: Dependence of the discharge current on applied voltage. SMUF in batch treatment chamber using the different diameters of the treatment zone (3.9 and 6.9mm) and two different dimensions of electrodes (50 and 25 mm).

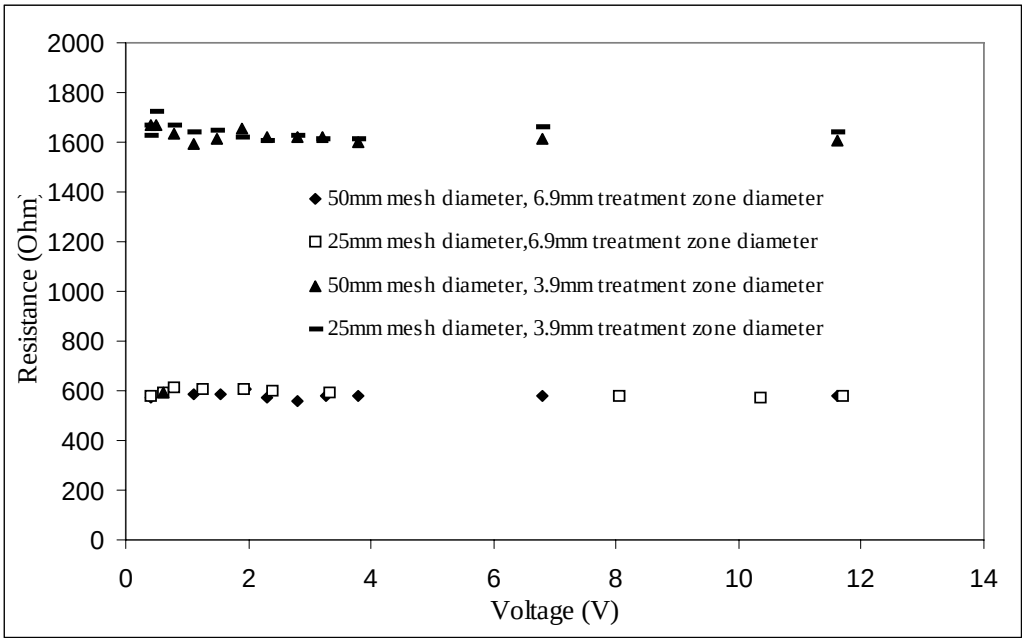

Fig. 5.5: Resistance across the treatment chamber. SMUF (100%w/w) in batch treatment chamber using the different diameters of the treatment zone (3.9 and 6.9mm) and two different dimensions of electrodes (50 and 25 mm).

The resistance of the outlet/inlet of the treatment chamber was the largest using the 50*mm* mesh electrode diameter and 3.9*mm* diameter of treatment zone as shown in (Fig. 5.6). Although this resistance was the largest, the voltage loss was minimum because the percentage of this resistance to the total resistance across the treatment chamber was minimum as illustrated in (Fig. 5.7). The voltage loss in the outlet region was about 10.5-12.4% and expected to be the same in the inlet region, which shows that the combined voltage loss in both sides was in the range of (21-28.8%).

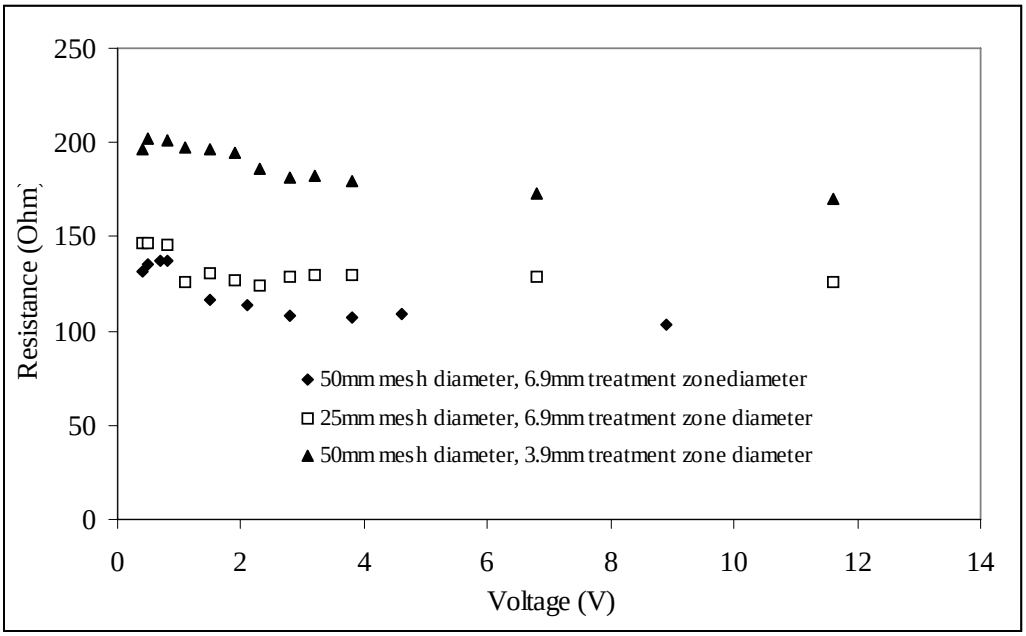

Fig. 5.6: Resistance in the outlet of the treatment chamber, using the different diameters of the treatment zone (3.9 and 6.9mm) and two different dimensions of electrodes (50 and 25 mm).

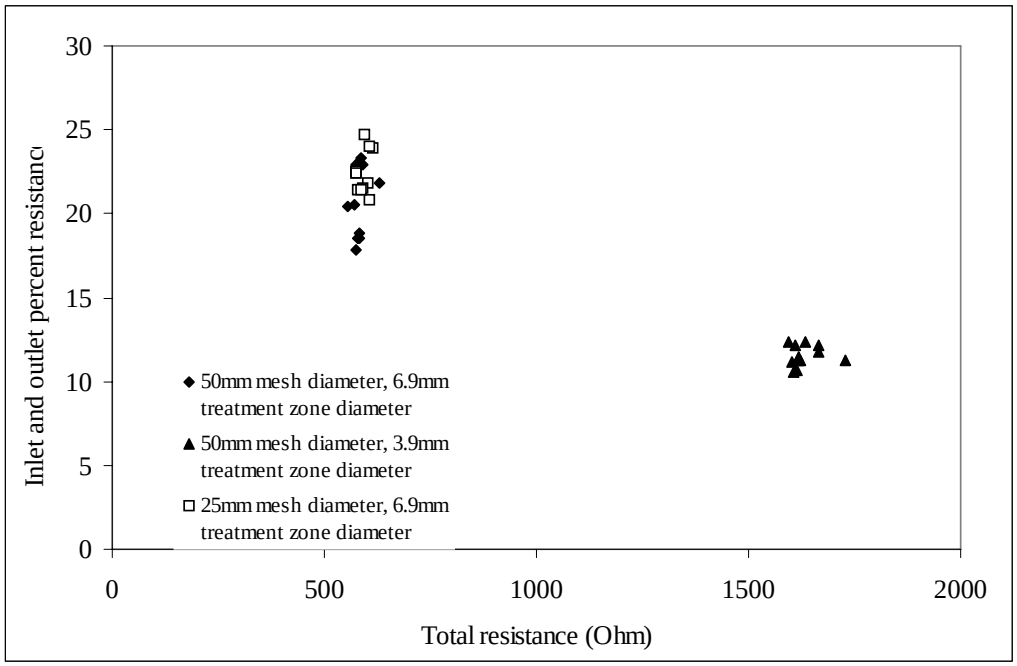

Fig. 5.7: The percentage of the resistance in the outlet of the treatment chamber to the total resistance across the treatment chamber, using the different diameters of the treatment zone (3.9 and 6.9mm) and two different diameters of electrodes (50 and 25 mm).

According to the mentioned experimental investigations, and according to the specification of the high voltage pulse generator used in this work (details of the generator will be discussed in chapter 6), it was decided to design four treatment chambers, where the diameter of the cross sectional area of flow was $3.9mm$ and the electrode diameter was $50mm$. Increasing the number of chambers to four, allowed the total current passing through the chambers to be within the acceptable limit (*150A*). The thermal performance of the multi pass chambers is described in the next section.

5.2 Preliminary Testing of the Multi Pass Treatment Chambers

Static testing at low voltage provides only rough testing of the treatment chamber. The chamber must be tested with flow system using high voltage pulses similar to normal operation as will be discussed in the following sections.

5.2.1 Materials and Methods

5.2.1.1 Treatment Chambers

Four multi pass treatment chambers were constructed according to our previous experimental investigations. The chambers were connected in parallel in terms of electrical power, and connected in series with regards the flow of the liquid.

5.2.1.2 Suspension

Different concentrations of simulated milk ultra filtrate (SMUF) were used as the suspension medium. SMUF was diluted with deionised water to 66.66% and 50% (w/w). The electrical conductivities of the different solutions were measured at different temperatures using a conductivity meter (specification as in Appendix A) as shown in (Fig. 5.8).

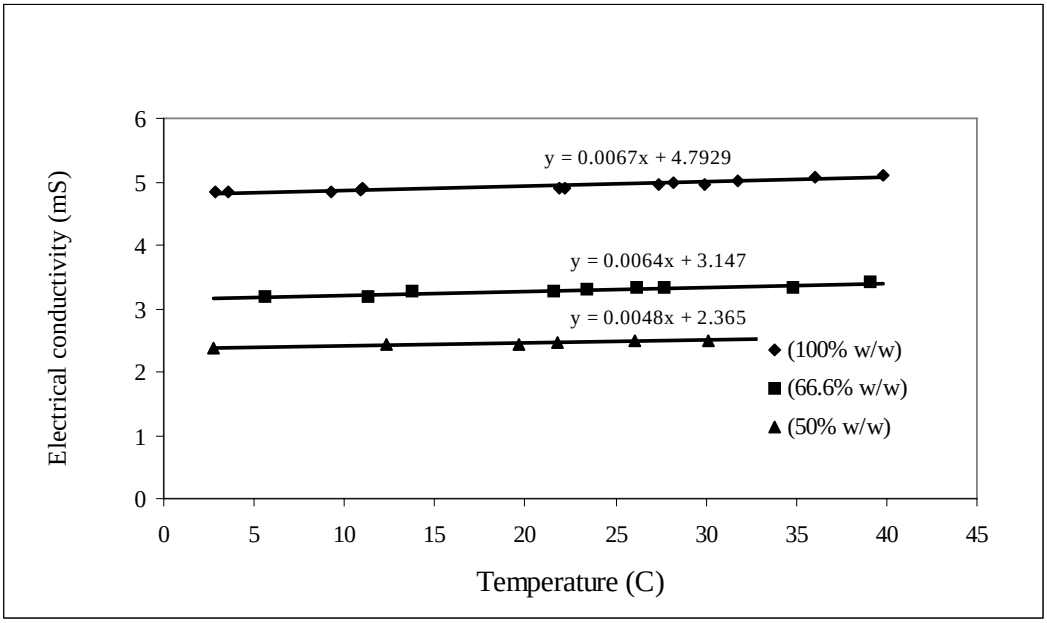

Fig. 5.8: Electrical conductivity of SMUF (100w/w, 66.66w/w and 50%w/w) at different temperatures.

5.2.2 Experimental Protocols

1. SMUF of 100%, 66% and 50% concentrations at flow rate of $2.5 ml/s$ and inlet temperature of 20^{o}C was subjected to square bipolar pulses at frequency of $190 Hz$ with different electric field intensities. The electric field intensity was calculated across the treatment gap based on our previous experimental findings (77% of the electric field strength across the treatment chamber). The variation of current due to voltage increase was measured and the total resistance across the treatment chambers was calculated. The change in temperature due to ohmic heating was recorded. Variation of the dissipated power

when increasing electric field intensity using different concentrations of SMUF was also calculated according to equations (5.1) and (5.2).

Energy per pulse $(kJ/pulse) = V \times I \times \tau$.....(5.1)

Where, V is the voltage, I is the current and τ is the pulse width.

Dissipated power $Q(kJ/s) = Energy\ \ per\ \ pulse\ \ (J/pulse) \times f\ \ (pulse/s)$

.....(5.2)

2. The effect of SMUF concentration on the energy density was studied by subjecting SMUF of 100 %, 66.66% and 50% concentration at flow rate of $6.66 ml/s$ to electric field intensity of $28.6 kV/cm$ and frequency of $190 Hz$. The energy densities were calculated according to the equation below.

$$Q^{`} = \frac{VI\tau\ n}{\upsilon} \text{.....(5.3)}$$

Where, $Q^{`}$ is the energy density, V the voltage, I the current, τ the pulse width, n the number of pulses and υ is the treatment volume.

3. When selecting a proper flow rate of the suspension medium, sufficient number of pulses should be received while maintaining the product temperature within a preselected value. SMUF (100% concentration) at different flow rates (1.66, 2.16, 2.5, 3.33 and $4.16 ml/s$) was subjected to square bipolar pulses at frequency of $190 Hz$ and different electric field intensities ($4.8 - 38.2 kV/cm$). The change in temperature was recorded and the dissipated power was calculated according to equations (5.1) and (5.2).

5.2.3 Results and Discussion

Increasing the electric field intensity increases the current passing through the treated solution as expected. SMUF with lower electrical conductivity shows less increase in the current during the application due to its lower conductivity (Fig. 5.9) and (Fig. 5.10). The impedance slightly decreased as the voltage increased using the different concentrations of SMUF, which could be due to the increase in the conductivity of the solution due to ohmic heating. The measured current is the total current across the four PEF units and hence, the measured impedance is the total impedance across the four units.

It is always desirable to have maximum impedance in the treatment zone to minimise the current passing through the solution, which minimise ohmic heating and decreases the probability of dielectric breakdown of foods. Therefore, foods with lower electrical conductivity is more suitable for PEF treatment as been noted by Vega-Mercado, Pothakamury, Chang & Swanson (1996), Jayaram, Castle & Margaritis (1993), Dutreux, Notermans, Wijtzes, Gongora-Nieto, Barbosa-Ca`novas & Swanson (2000a), Vega-Mercado, Pothakamury, Chang & Swanson (1996), Jayaram, Castle & Margaritis (1993), Hulsheger, Potel & Niemann (1981) and Ruhlman, Jin & Zhang (2001).

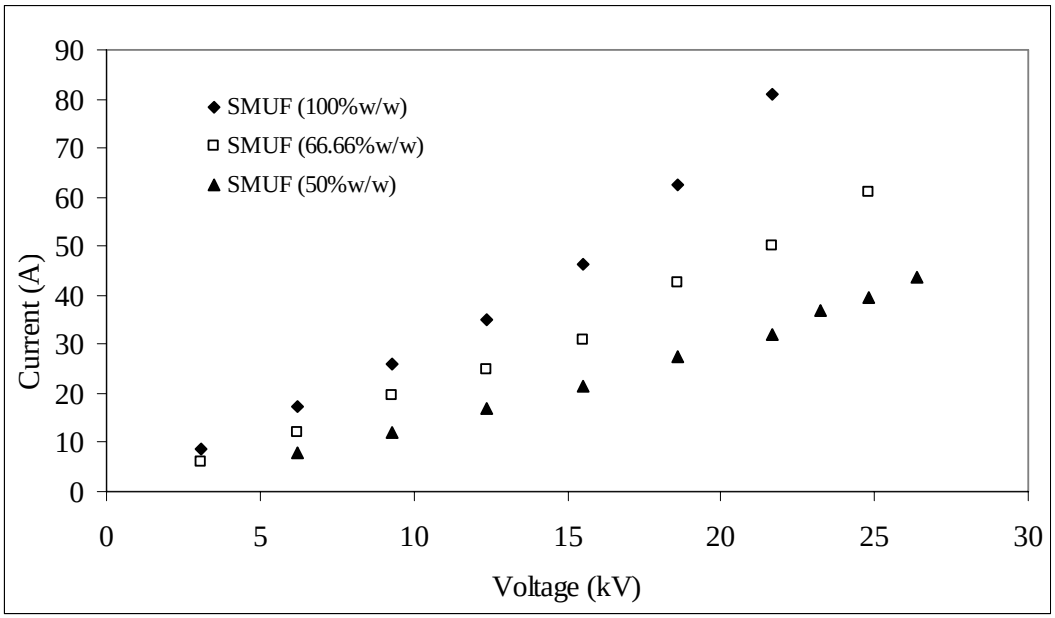

Fig. 5.9: Variation of current vs. voltage using multi pass treatment chambers, different concentrations of SMUF and flow rate of 2.5ml/s.

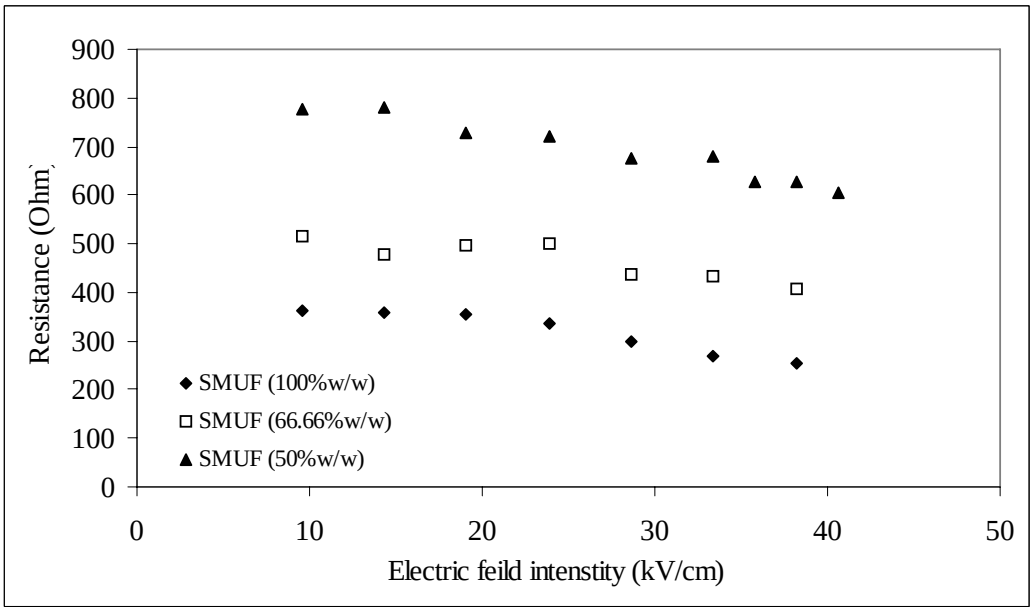

Fig. 5.10: Total resistance across the four multi pass treatment chambers using SMUF of different concentrations, flow rate 2.5ml/s.

The dissipated power and hence the treatment temperature increased with increasing electric field strength and conductivity of the solution as shown in (Fig. 5.11) and (Fig. 5.12). There is a dynamic relationship between electrical conductivity, energy input, and temperature increase, which suggest the need of accurate control of these parameters during this non-thermal pasteurisation (Ruhlman, Jin & Zhang 2001).

The energy density calculated according to equation (5.3) using SMUF of 100 %, 66.66% and 50% concentration at flow rate of $6.66 ml/s$ and subjected to electric field intensity of $28.6 kV/cm$ at frequency of $190 Hz$, was higher using SMUF with the higher concentration as shown in (Fig. 5.13).

Decreasing the flow rate of SMUF (100% concentration) when subjected to different electric field intensities increased the dissipated energy as shown in (Fig. 5.14). At lower flow rate, ohmic heating would cause the electric conductivity to increase leading to higher current and higher dissipated power.

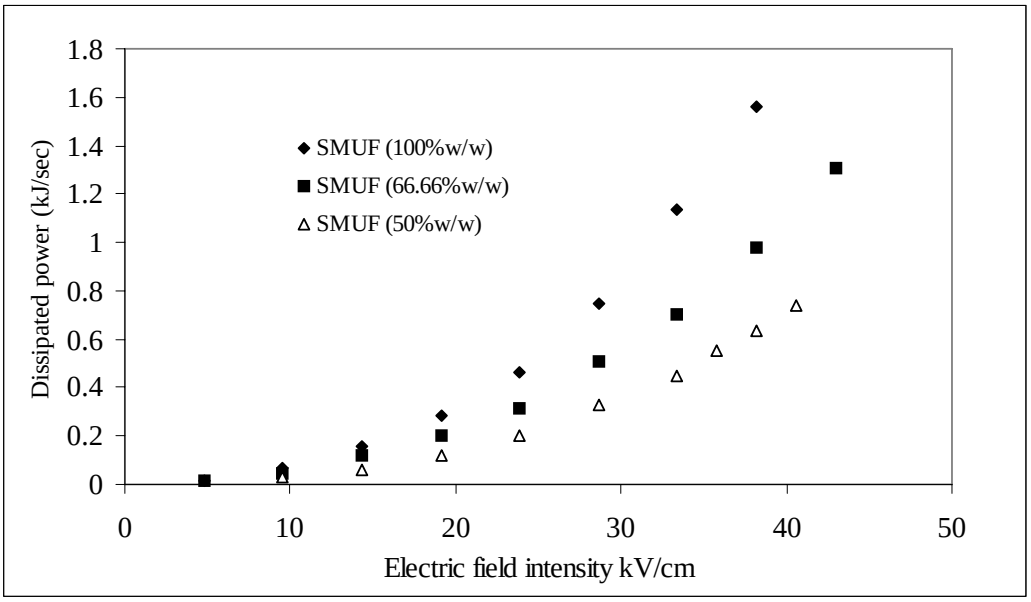

Fig.5.11: Variation of the dissipated power with increasing electric field intensity using the different concentrations of SMUF at flow rate of 2.5ml/s and 20°C inlet temperature.

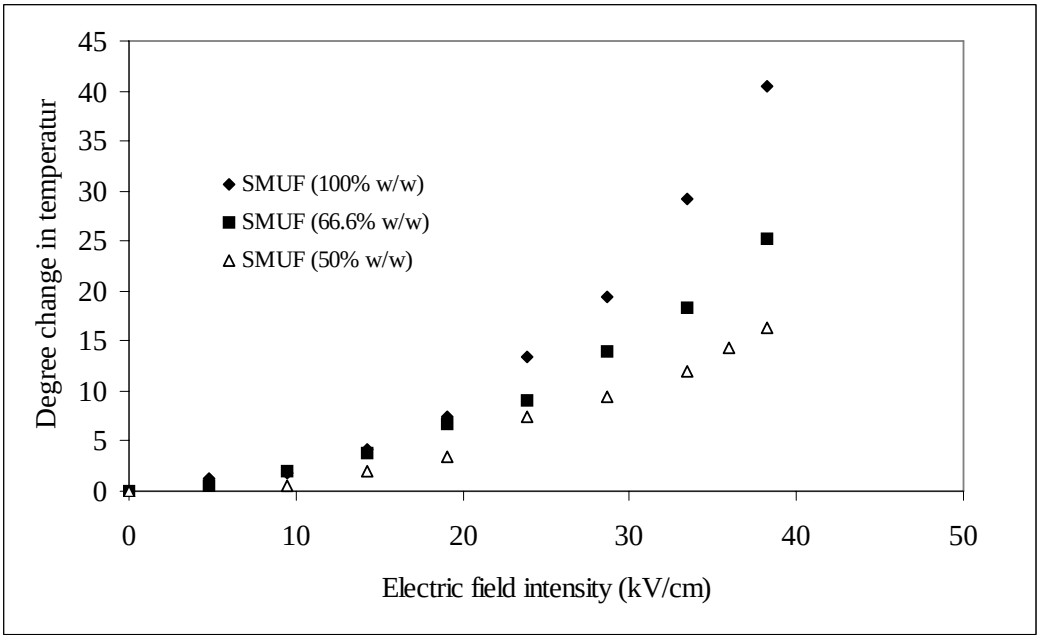

Fig. 5.12: The temperature change of SMUF (different concentrations) at flow rate of $2.5ml/s$ *when subjected to different electric field intensities.*

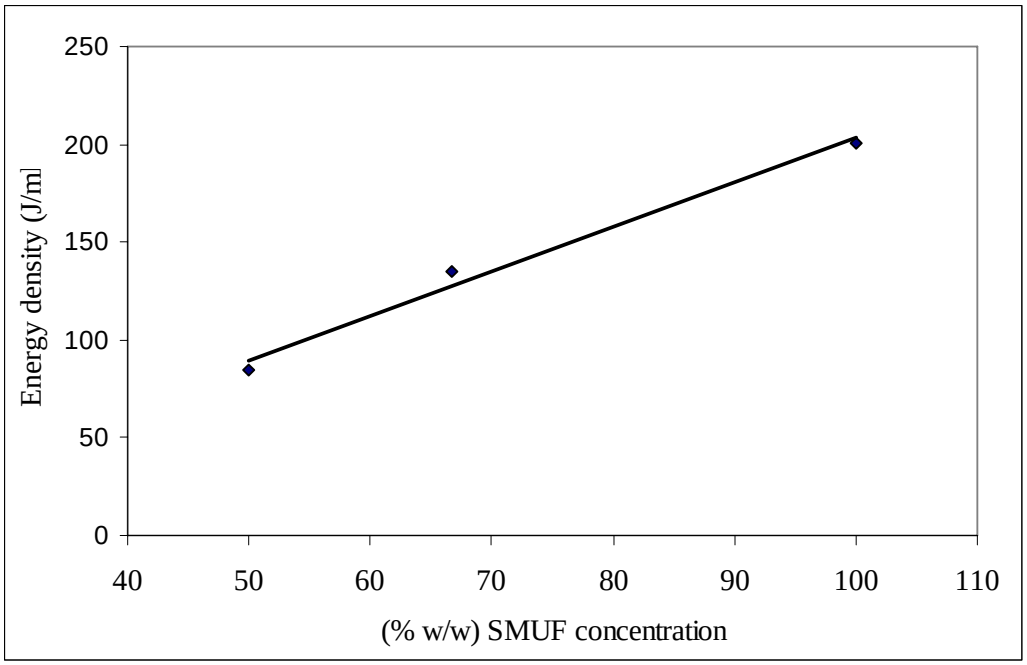

Fig. 5.13: The effect of SMUF concentration on the energy density, 28.6kJ/cm, flow rate 2.5ml/s.

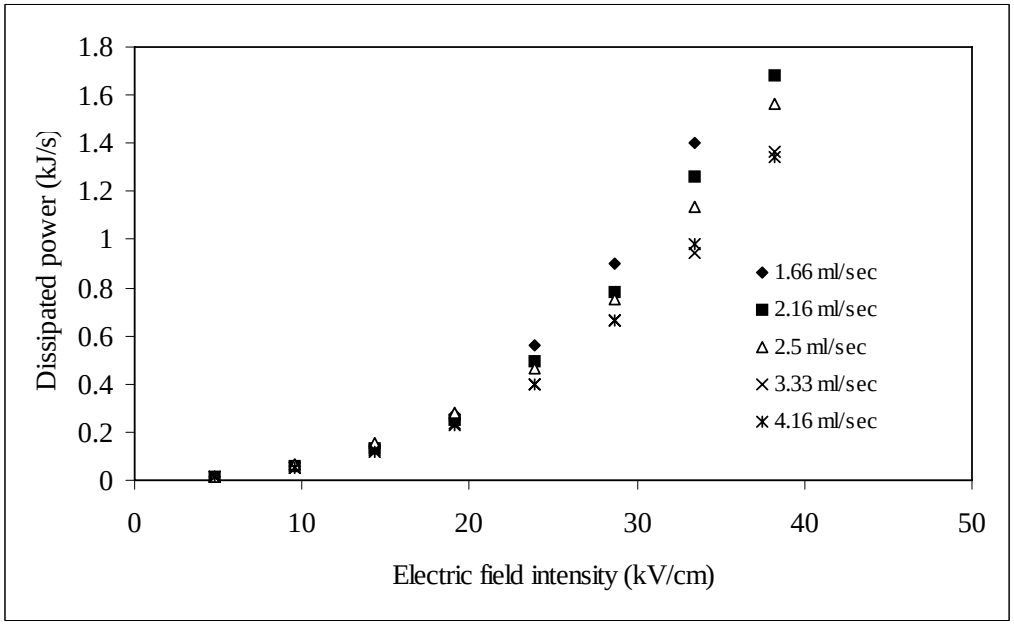

Fig. 5.14: Variation of the dissipated power with increasing electric field intensity using SMUF of 100% concentration at different flow rates.

5.4 Conclusions

Electric field strength is a function of the geometric design of a PEF chamber. Proper dimensions of the PEF chamber should be determined to concentrate the electric field in the treatment zone, to minimise voltage loss in the outlet and inlet of the treatment chamber and to maintain the treatment temperature within preselected values. Electric field strength in a treatment zone of a PEF chamber is inversely proportional to the cross sectional area of the fluid flow and to the gap distance between the two electrodes.

The dissipated power is lower when using solutions with lower electrical conductivity as the increase in the treatment temperature is directly proportional to the electrical conductivity of the product. Because it is important to maintain a treated food temperature at low values during the PEF treatment, the multi pass treatment chambers were designed with intermediate cooling.

6. Chapter Six: Experimental Study on Pulsed Electric Fields System Design

This chapter presents an innovative PEF continuous laboratory scale pulse electric field system that we have designed and constructed using modern technology. The system offers a high treatment efficiency in terms of microbial inactivation and energy saving. The experimental set up, design of the treatment chambers and the main components that are involved are described in this chapter.

6.1 Pulsed Electric Field Unit

The PEF unit (Fig. 6.1) was designed and constructed for non thermal treatment of liquid foods. The system is composed of a number of components, including a high voltage pulse generator, continuous treatment chamber/s, cooling system, degassing unit, voltage and current measuring devices, data acquisition systems, aseptic packaging and cleaning in place.

During start up, the system was sterilised (cleaning and sterilisation procedure are described later in this chapter). Then a sterile solution matched in electrical properties with the product to be treated was deareated in the degassing unit to ensure no bubble formation during the PEF process. The sterile solution was pumped through a flow meter and into the PEF unit using a piston pump. The

pump was provided with in-line pulse suppressor to eliminate possible pulsation in the flow (specification of the pump and flowmeter are in Appendix A). A valve was used to control the flow rate, which determines the number of pulses the fluid receives to achieved significant destruction of the specific microorganisms under investigation.

Four treatment chambers were connected in series, details of the design of the treatment chambers is given in section (6.1.2). In each treatment chamber, the liquid food was subjected to a plurality of high voltage electric pulses at field strength in the range of $15-46 kV/cm$ by using a high voltage pulse generator connected to the electrodes in each chamber. The treated liquid food leaving each chamber was passed through a cooling coil immersed in a controlled temperature refrigerated water bath to lower its temperature to a pre selected temperature prior to entering the next chamber. The liquid food was also pressurised by the pump during processing to prevent dielectric breakdown of gas bubbles entrapped in the liquid food.

In order to avoid using large number of treatment chambers, the treated liquid was recirculated through the four chambers more than once to achieve the desired microbial deactivation as shown in (Fig. 6.1). Samples of the product leaving the PEF unit and samples of the liquid food in the feed tank were drawn at different times using a pipette and introduced to an aseptic packaging in small containers for microbial analysis.

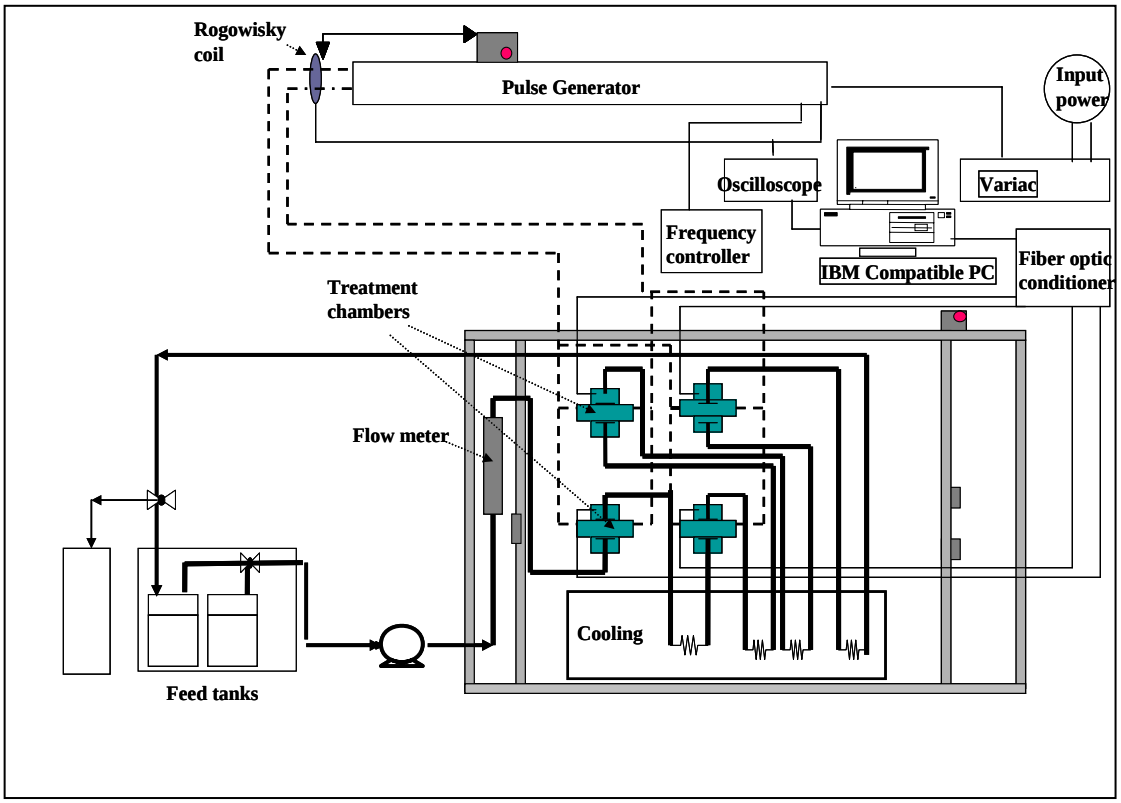

Fig. 6.1: The PEF unit (The University of Auckland, 2004).

6.1.1 High Voltage Pulse Generator

The high voltage pulse generator (Fig. 6.2) was constructed to our specifications by H. F. Power Ltd (Auckland, New Zealand). The pulse generator provides high voltage up to $30kV$ and square bipolar pulses having $1.7\mu s$ duration, pause duration of $2.5\mu s$, frequency up to $200Hz$ and a short pulse rising time of less than $0.5\mu S$. The generator peak power is $4.5MW$ (more technical specification of the PEF generator are given in Appendix A).

The pulse generator is composed of *DC* power supply that charges an energy storage component (an array of capacitors and inductors). The generator

includes 31 identical modules (Fig. 6.2 (a)). Each module (Fig 6.2 (b)) includes a set of four switches, designed using the modern Insulated Gate Bipolar Transistors *(IGBTs).* The switches operates in pairs for opening and closing periodically to discharge the current stored in the energy storage component, this results in the application of bipolar pulses across the treatment chamber.

The generator further includes an input transformer and a rectifier designed to raise the voltage to $1.0kV$ and another transformer to increase the out put voltage to $30kV$, so that the nominal output impedance can be changed by changing the turns ratio of the transformer without having to alter the switching circuits. The cell can be configured to operate symmetrically around ground potential, i.e. one terminal of the cell does not have to be grounded. This solution reduces unwanted ground currents through the processed liquid. Safety of the system was also enhanced, as the maximum voltage to ground is now equal to only half the maximum voltage between the cell electrodes.

The output voltage can be varied by means of a three phase input variac connected to the high voltage generator. A *DC* voltmeter connected to a *DC* bus bar was used to measure the voltage across the electrical pulse delivery system for verification of consistent energy delivery. An oscilloscope and Rogowski coil were used to measure the output current and to illustrate the pulse shape. The frequency of the output pulses can be varied by means of a signal generator and was monitored to determine consistent energy per unit volume of medium/product.

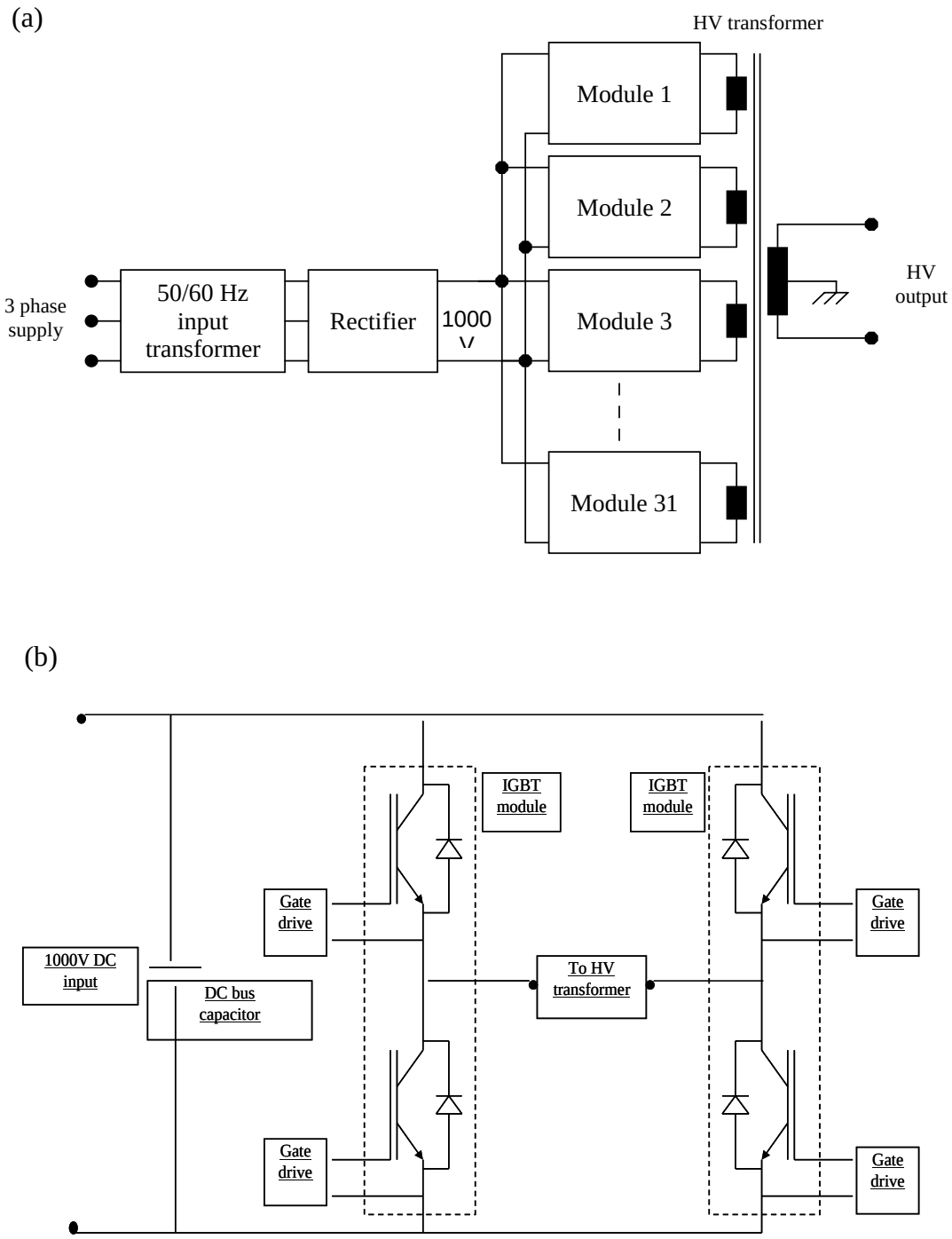

Fig. 6.2: a) High voltage pulse generator, Sketch of full system. b) Sketch of each model (The University of Auckland, 2004).

The bipolar square wave pulse generated in this work (Fig. 6.3) maintains a pulse with a flat top, and provides very fast pulse rise and fall times. The variation in pulse to pulse voltage was minimized and hence, the energy provided to each volume of liquid is highly uniform. The square shape of the pulse was achieved when the cell impedance was in the range of (200 - 600 *Ohm*). The increase or decrease of cell resistance cause significant distortion to the shape of the square pulse as shown in (Fig. 6.4) and (Fig. 6.5). The circuit must be adjusted for any major change in cell resistance to insure perfect square pulse.

The ohmic heating caused by the unwanted low voltage associated with other types of pulses was minimised in our design. The use of bipolar pulses causes extra stress in the microbial cell membrane, enhancing its electric breakdown. It also offers better energy utilization, reduced deposition of solids on the electrode surface and decreased food electrolysis and corrosion.

The short rising time increases the efficiency of the PEF treatment as the efficiency slightly depends on the pulse rising time. At very short rising time, all the power supplied is utilised for microbial inactivation with minimum heating (Alvarez, Raso, Sala & Condon, 2003).

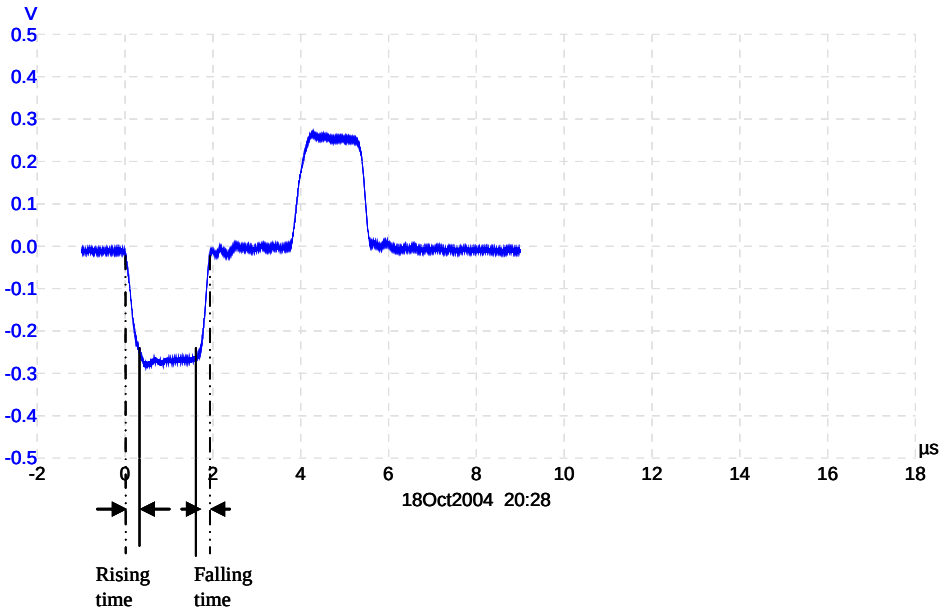

Fig. 6.3: The square bipolar pulse generated by the PEF unit (The University of Auckland), the cell resistance in the range of (200-600Ohm).

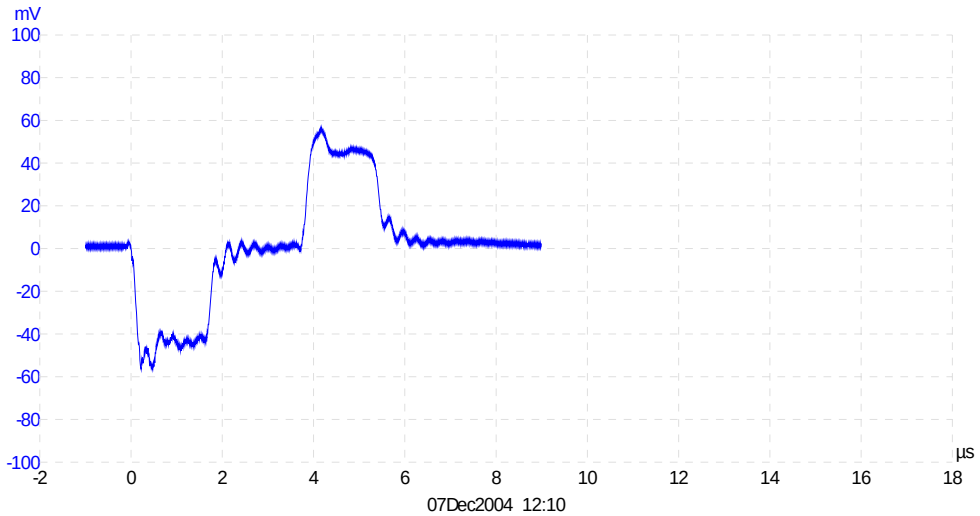

Fig. 6.4: The square bipolar pulse generated by the PEF unit (The University of Auckland), the cell resistance is 〉 600 *Ohm.*

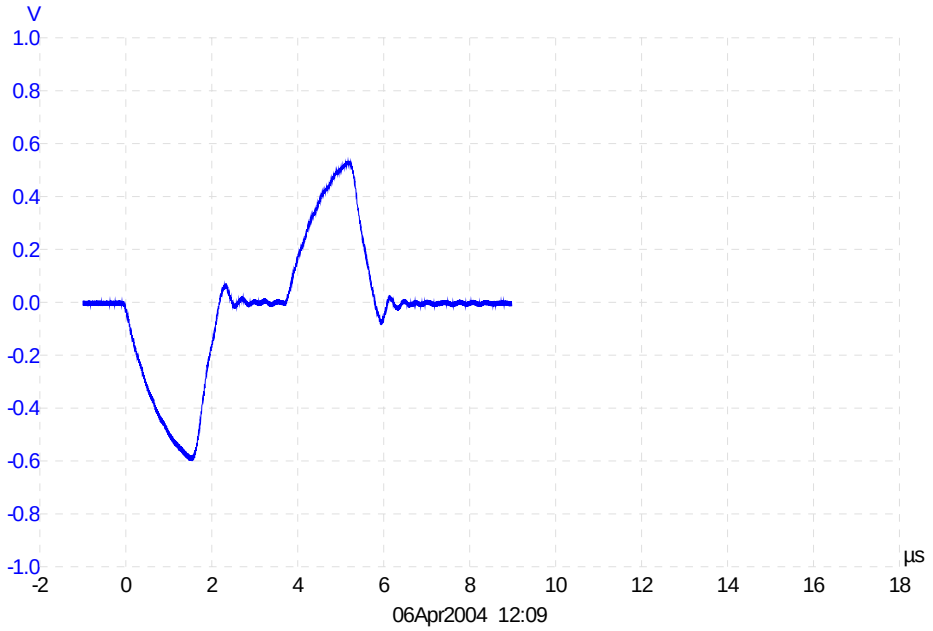

Fig. 6.5: The square bipolar pulse generated by the PEF unit (The University of Auckland), the cell resistance 〈 200 Ohm.

6.1.2 Treatment Chambers

Innovative PEF treatment chambers were designed and constructed to operate at high electric field intensities with uniform electric field distribution. The chambers were designed to reduce the possibility of electric breakdown during the PEF process and to minimise the increase in the product temperature and prevent electrodes fouling. Different factors were considered in these designs to prevent these undesirable phenomena.

Multi pass treatment chambers (electrically connected in parallel, where the total current is the summation of currents passing in all chambers and should

not exceed the limitation of the generator *150A*) were designed according to the specifications of the high voltage generator. The chambers were designed with electrical resistance across the treatment chamber/s match the allowable current. Multi pass chambers were designed with dimensions selected to obtain highest electrical resistance across the chamber and hence lower current.

The multi pass treatment chamber/s was designed to provide maximum possible electric field strength in the treatment zone, while maintaining minimum increase in the liquid food temperature. This was achieved by proper design of the electrodes and by controlling the maximum thermal excursion below pre-selected values, which was achieved by the inter-cooling between the treatment chambers. The chambers were also designed to insure that all microbes are exposed to a uniform electric field and receive similar number of pulses. The system includes four treatment chambers connected in parallel in terms of the electric power connections and in series in terms of the fluid flow. The treatment chamber design is illustrated in (Fig. 6.6) with the electrodes shown in (Fig. 6.7). The details and dimensions of each part of the chamber are shown in Appendix B, and may be described as follows:

1. Each chamber includes two electrodes, an insulator element configured to form the treatment region and the inlet and outlet parts. The electrodes are stainless steel mesh, with the fluid flow-through openings formed between the wires of the net. The electrodes were connected to the high voltage pulse generator and to the ground respectively and were electrically isolated from each other by an insulator element designed to form an orifice where the

voltage across the orifice was close to the supplied voltage. The liquid food flow through the openings of the first electrode and through the orifice where the electric field was concentrated, then through the opening of the second electrode. The electrodes were included in the fluid flow channel, which results in a homogeneous electric field. The residence time distribution of the fluid in the treatment zone was uniform.

2. This design ensured no fouling on the electrodes because the electrodes were placed apart from the treatment gap where the electric field was concentrated. This will prevent accumulations of materials such as protein molecules on the surface of the electrodes, which could result in inadequate microbial inactivation. Fouling of processor electrodes will cause high temperature spots at the electrodes surface and eventually sparking which will lead to further fouling, this was also reported by (Barbosa-Ca`novas, Qin, Zhang, Olsen, Swanson & Pedrow, 2000).

3. The surface area of the mesh electrodes and the distance separating them were determined to obtain maximum electric field strength in the treatment region and hence, minimize voltage loss in the inlet and outlet regions (before the fluid enter and after it leave the orifice). The electrode diameter was $50mm$ with a mesh opening diameter of $0.85mm$ and wire diameter of $0.42mm$. The surface area of the wires conforming the net was calculated and designed to achieve maximum electric field in the treatment region.

4. The cross sectional area and depth of the orifice were determined to provide the maximum impedance in the treatment zone and hence maximum voltage

across the orifice. The orifice diameter and the gap depth were $3.9mm$ and $5mm$ respectively as discussed in the experimental investigations presented earlier in chapter 5.

5. The insulator element separating the two electrodes as was designed wider than the other parts of the chamber (the inlet part and the output part) to avoid any short circuiting that can be introduced between the two electrodes on the outside of the chamber. Increasing the thickness of the middle part of the chamber, increases the path for any current that can be introduced between the two electrodes.

6. The chamber was also designed to attain a relatively uniform high-intensity electric field to achieve maximum and uniform microbial inactivation, by minimizing the local field enhancements to prevent the probability of electrical breakdown inside the chamber along the insulator surface separating the two electrodes. The potential for electrical breakdown at the insulator can be minimized by removing the triple point (i.e. the interface between the electrode, insulator, and liquid or pumpable food) from the high field region, increasing the length of the insulator, placing the insulator at an angle with the electric field, and reducing electric enhancement by appropriate design of the shape of the electrodes.

7. The chamber was placed in a vertical position, to ensure accurate control of the fluid residence time distribution, temperature distribution and hence product local conductivity (which changes with temperature). Liquid stratification will occur if the treatment chambers were placed horizontally.

The warm liquid accumulating at the top of the treatment chamber is more electrically conductive and therefore will heat faster, while the cooler liquid at the bottom will have lower electrical conductivity and thus heats slower. The consequence is that part of the product is treated more severely than others if the treatment units are placed horizontally. A vertical alignment also helps with degassing the cells at start up.

8. The mesh electrodes aid in mixing the fluid food when flowing through them. The fluid food was mixed when entering and leaving each chamber. This insures all the fluid receives equal number of pulses.

9. The choice of mechanical construction and materials of the chamber were also studied. The chamber is made of Teflon which can hold temperature up to 260^{o}C without deforming. The electrodes were made of 316 food grade stainless steel. Rubber washers were used to seal the chamber and prevent leaking.

10. The chambers made of removable components. The components were clamped together by using wooden clamps as shown in (Fig. 6.8) and (Fig. 6.9) to prevent the possibility of short current circuits that can be formed through the holes in case of using bolts of any material for example steel or plastic.

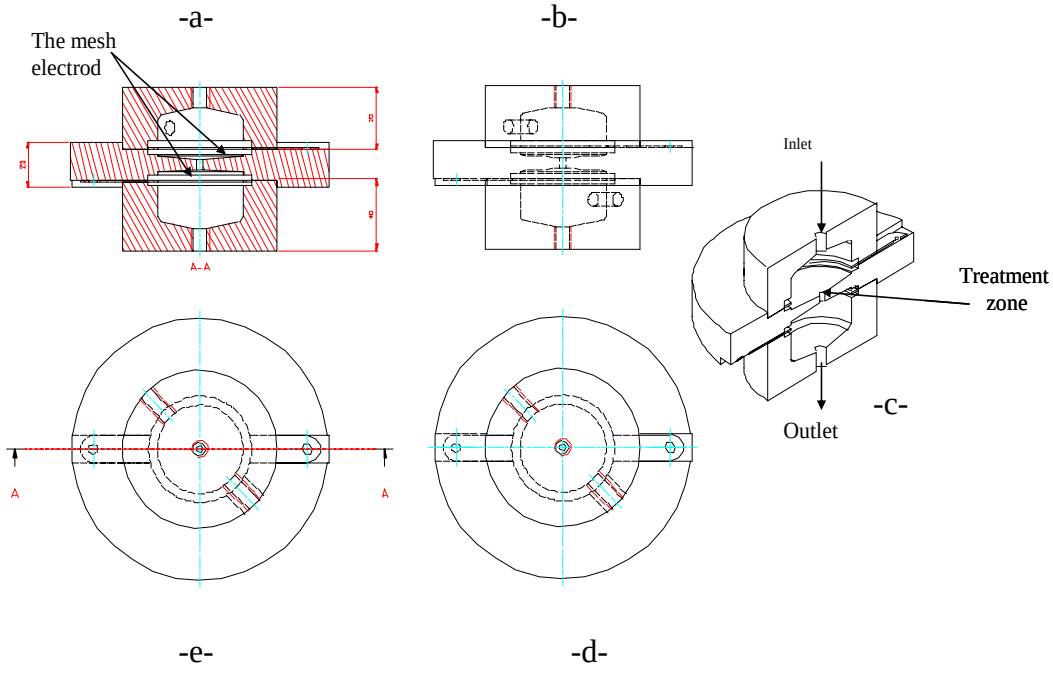

Fig. 6.6: a) A cross sectional view of the multi pass treatment chamber. b) Side view of the chamber. c) Three dimensional section of the chamber. e) Top view of the chamber. d) Bottom view of the chamber.

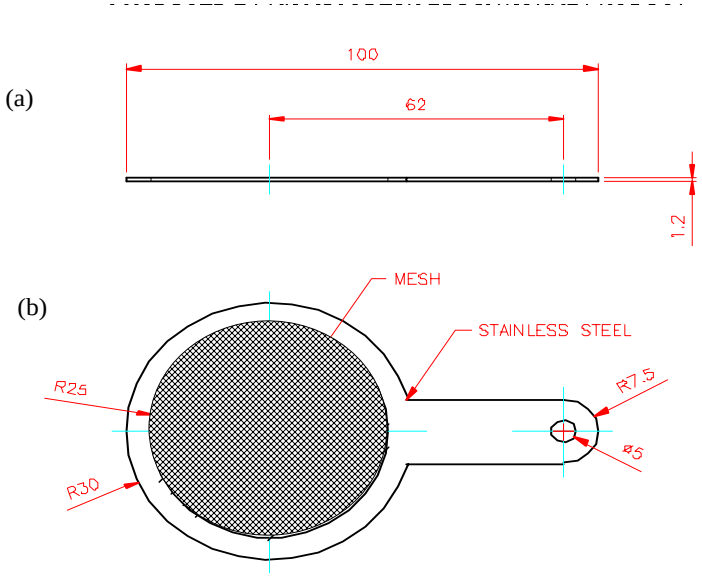

Fig. 6.7: a) Side view of electrode. b) Top view of the electrode.

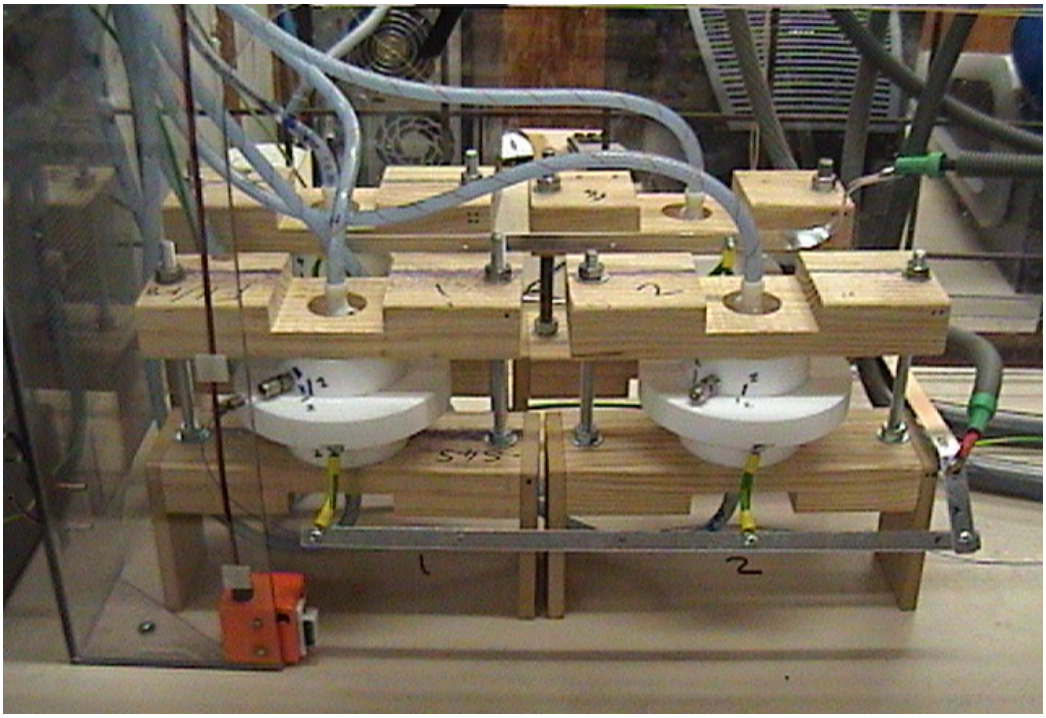

Fig. 6.8: The multi pass PEF chambers and the wooden clumps.

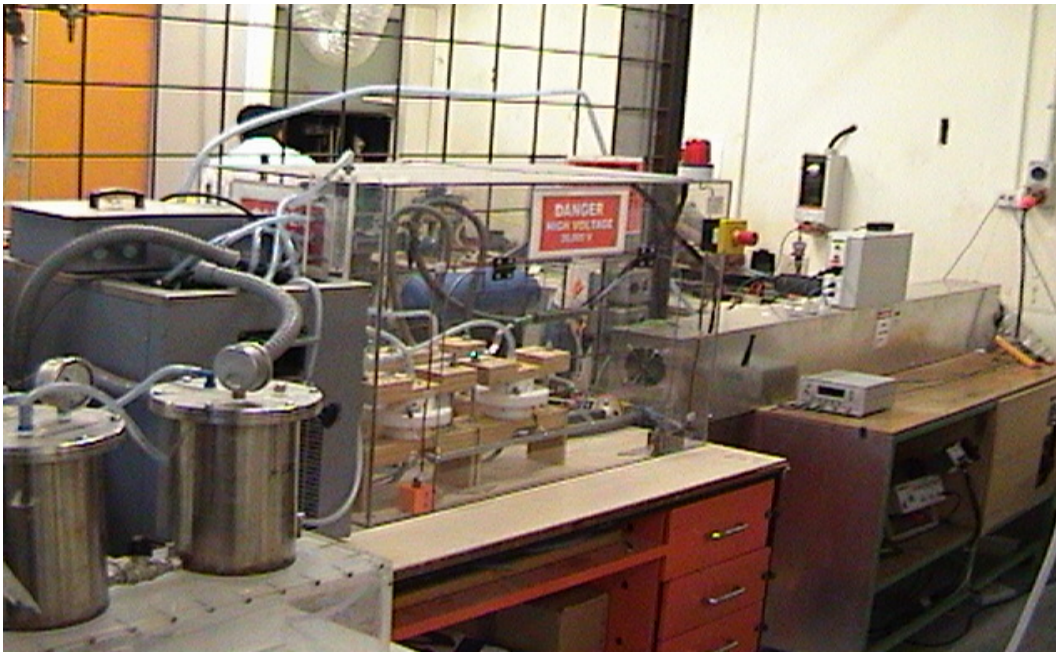

Fig. 6.9: PEF unit including the multi pass chambers.

6.1.3 Degassing Unit

The presence of gas bubbles in the liquid food caused the electric field magnitude to decrease significantly near the boundary of bubbles, thus threatening the uniformity of the PEF treatment across the chamber gap. To reduce the possibility of dielectric breakdown within the liquid food during processing, degassing of the liquid food was carried out by using the deaeration unit shown in (Fig. 6.10).

The degassing unit includes two stainless steel containers and a vacuum pump (specification of pump in Appendix A). One of the containers was used as a feed tank while the other was used for the sterile buffer that was pumped into the system before the liquid food as described earlier. Each tank was covered with stainless steel lid, where a pressure gauge was attached and a pipe connected each tank to the vacuum pump. The lids were bolted to the tanks with O rings to provide vacuum seal. . The volume of each tank was $3 lit$. The vacuum gauge was measuring 80 k Pascal during the application.

The liquid food was also pressurised during processing to prevent dielectric breakdown of gas bubbles entrapped in the liquid food flowing through the cavity of the pulsed electric field treatment chamber. The increase in pressure significantly reduce the volume/size of an entrapped air bubble, thus greatly minimize the risk of arcing, and also the under processing of the surrounding food. A backup pressure regulator was used to ensure the applied energy has remained within design limits without raising the temperature of the medium. The pressure was 50mmHg during the application.

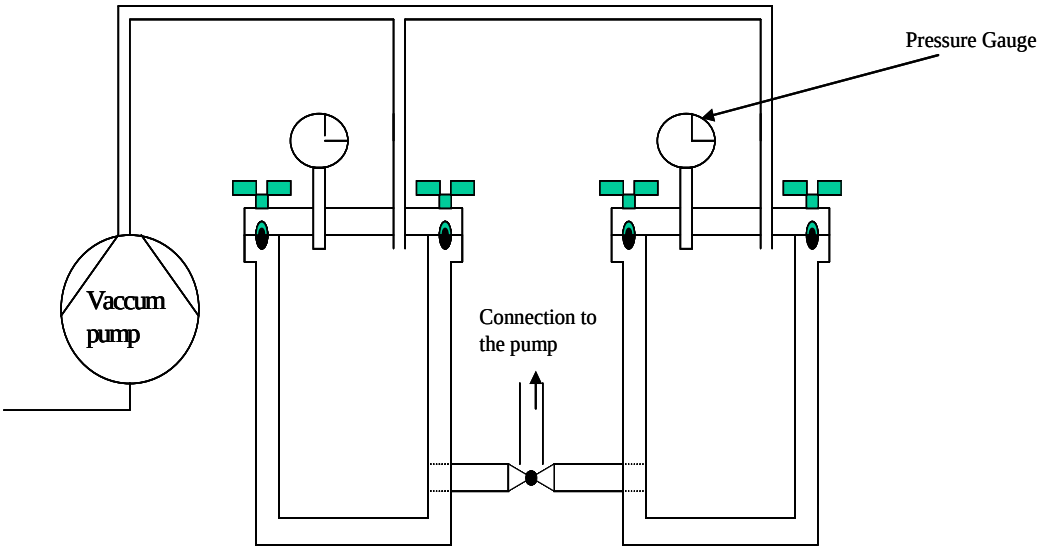

Fig. 6.10: Degassing unit, (The University of Auckland).

Gongora-Nieto (2002) studied the impact of air bubbles in a dielectric liquid when subjected to high field strengths, the dielectric breakdown threshold at a given pressure was more likely to be exceeded by bigger bubbles (>1mm) entrapped in a fluid processed in smaller treatment chamber gaps (3mm), than by smaller bubbles (<0.5mm) in bigger gaps (5mm). It was decided that in order to minimise the partial discharge and arcing phenomena, pressurization of the system should be implemented.

6.1.4 Temperature Control and Measurement

The maximum temperature increase during the PEF application when no cooling was employed was calculated using the basic thermodynamic equation (2.5).

Maximum thermal excursion was controlled below pre-selected values, by inter-cooling between the treatment chambers, to ensure the applied energy has remained within design limits without raising the temperature of the medium. The liquid food leaving each chamber passed through a coil immersed in a controlled refrigerated water bath, the water bath was provided by Grant instruments (Cambridge) Ltd (technical specification in Appendix A). The cooling coils dimensions were calculated to provide sufficient heat transfer area to ensure a final temperature equal to the bath temperature. The length and diameter of each cooling coil were $450cm$ and $0.5cm$ respectively.

The treatment temperature was kept less than the lethal temperature of the specific microorganism used in this work (will be discussed later in chapter 7), demonstrating that inactivation is not due to thermal effects induced by the high-voltage pulses. The product was cooled to refrigeration temperature (5-9ºC) exactly after the electric field treatment, to prevent cell wall repair of the micro organisms being treated by cooling to an inactive metabolic condition.

The liquid temperature was monitored during the PEF application using fibre optic temperature sensors developed by FISO technologies (Canada) (Fig. 6.11). The fibre optic temperature sensors has extremely fast response time of (less than 1 ms), very small size with a diameter of 150 microns and has up to $20Hz$ sampling rate (more specification are in Appendix A).

The fiberoptic sensors were attached to the inlet and outlet of each treatment chamber. A temperature sensing component placed on the tip of the

fiber optic's "free end". The other end was connected to a measuring system that collects the desired radiation and processes it into a temperature value.

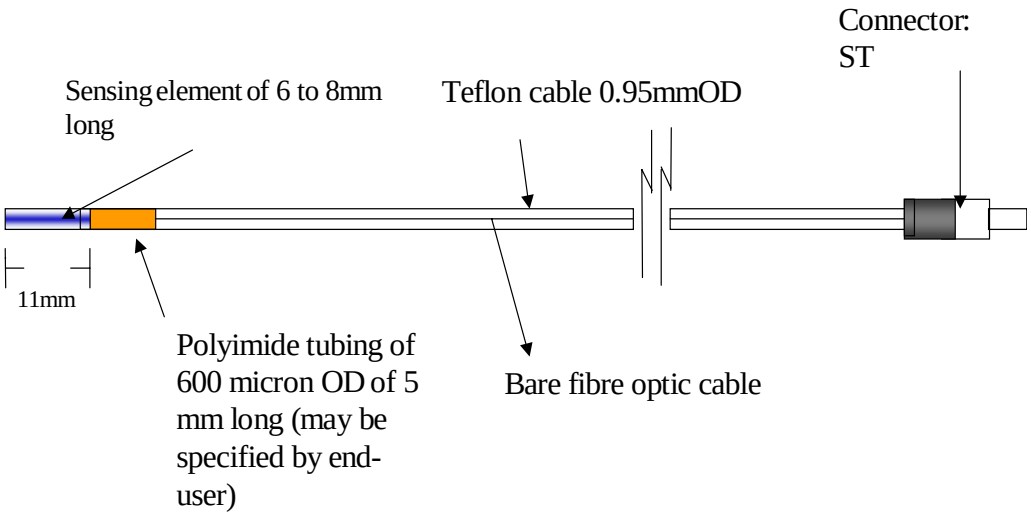

Fig. 6.11: Fibre optic temperature sensor.

Principle of Fibre Optic System

Fiber optic sensing system transmits light from a light source along an optical fiber to a fiber optic sensor. Fibre-optic gauges (Fig. 6.12) were designed around a Fabry-Pérot interferometer (FPI). A (FPI) consists of two mirrors facing each other, the space separating the mirrors called the cavity length. Light reflected in the FPI is wavelength-modulated in exact accordance with the cavity length.

The passive sensors were integrated with transducers that were affected by changes in the environment. These changes at the transducer cause changes in the characteristics of the light that is reflected from the sensor to the signal

processor. The modulated light is converted into a signal that is processed in the control system.

The UMI signal conditioner (Fig. 6.13) was designed to precisely convert the optical signal encoded by the transducer into engineering units. Data from the 4 sensors can be recorded by the UMI-4 unit and transferred to a computer through the standard RS-232 communication port. Data were then exported to Microsoft Excel (TM) and graphs created.

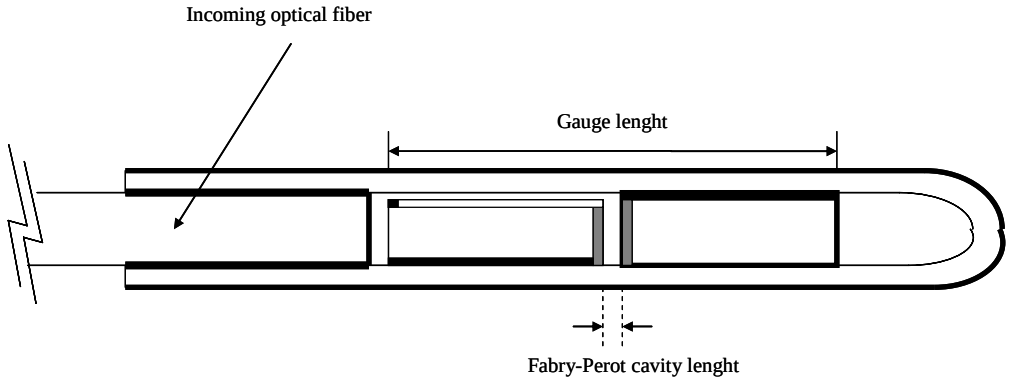

Fig. 6.12: FISO fibre optic gauge

Fig. 6.13: UMI signal conditioner

6.1.5 Process Safety

Safety of personal, products and the process equipments were considered in the design of the PEF unit. For personal safety, a safety cabinet with a suitable mechanical safety interlock was constructed as illustrated in (Fig. 6.14) to prevent inadvertent touching of the high voltage cables, electrode terminals and other live parts. Safety of the equipments was considered, by using the rogowski coil to measure the output current and a control system was employed to switch off the generator if the current increased to the upper pre selected limit.

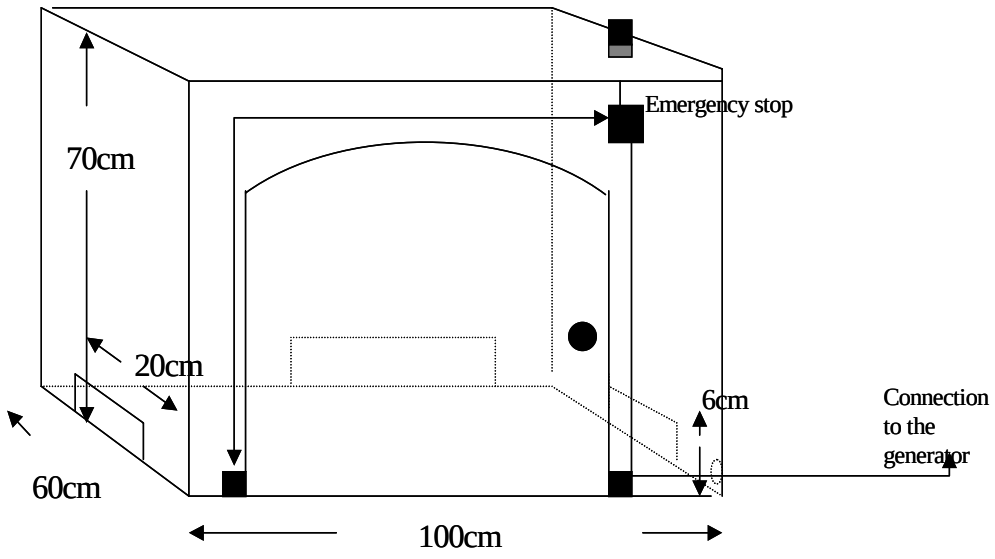

Fig. 6.14: Safety cabinet.

6.1.6 Aseptic Packaging

The electrically stressed fluid food after being subjected to the electric pulses was collected in sterilized bottles and was cooled immediately after

treatment to refrigerated temperature ($5^{o}C$) to diminish recovery and repair of microorganisms.

The control of storage temperature for the PEF treated food is important to provide long term microbiological viability (shelf life). The storage temperature depends on the type of product. Incubation at refrigeration temperatures after treatment can reduce the metabolic level of electrically treated microorganisms (Dunn & Pearlman, 1987).

The effectiveness of aseptic packaging has been demonstrated by the extended shelf-life of several PEF products (Qin, 1995).

6.1.7 Cleaning in Place

The PEF system was designed for cleaning in place. The cleaning procedure involved two stage cleaning cycle. First stage cleaning using a detergent solution of 2.0% w/w caustic soda in distilled water, the detergent circulation time was 15-20 minutes at 65-80ºC, followed by rinsing with distilled water at ambient temperature for 3 minutes.

In the secondary cleaning stage, the feed tank was filled with 3 litters of 1% w/w hypochlorite solution; the disinfectant circulation time was 10-15min at ambient temperature. When all hypochlorite has been expelled, the feed tank was filled with 3 litres of distilled water and pumped through the system and then expelled. The feed tank was filled again with 3 litre of distilled water. The water was allowed to circulate and then pumped to the drain.

6.2 Summery

In this work, an innovative PEF unit was designed and constructed to achieve high effectiveness of the PEF process. Many factors were considered. One of the main factors considered in the design was the proper design of the treatment chamber(s). The multi pass chambers described earlier were the proper chambers that were used in all the microbial testing. The design of the chambers provide uniform electric field and reduces the probability of dielectric breakdown in foods by using the proper electrodes to minimize electron emission and by removing the electrodes from the high field region and by using the inter cooling between the multi pass chambers.

Other factors considered were degassing prior to treatment to eliminate gas bubble formation and pressurizing the fluid food within the treatment chamber to prevent gas bubble. The electrical parameters including the pulse shape, rising and falling time, electric field intensity were also considered to increase the efficiency of the PEF process.

7. Chapter Seven: Experimental Studies on Microbial Inactivation Using Pulsed Electric Fields

Microbial tests were conducted to determine the effect of the pulsed electric fields treatment on microbial inactivation of the gram negative Escherichia coli ATCC 25922 suspended in simulated milk ultra filtrate (SMUF) of different concentrations. The effects of different process parameters and medium related parameters on the microbial inactivation rate were studied.

The morphological changes induced by PEF on E coli were investigated experimentally under transmission electron microscopy (TEM). TEM Pictures of untreated and treated E coli were presented and analysed in this chapter.

7.1 The Effect of Different Treatment Conditions on Microbial Inactivation

PEF inactivation depends on several factors. The evaluation of these factors is necessary to determine the efficiency of the PEF process. The PEF treatment was conducted at different process parameters (electric field intensity, treatment time, frequency, flow rate, treatment temperature and electrical energy applied) using media of different electrical conductivities. Error analysis of the experimental work is presented in Appendix D.

7.1.1 Materials and Methods

7.1.1.1 The PEF Unit

The PEF unit (consisting of four multi pass treatment chambers) described earlier in Chapter 6 was used for testing. SMUF at different concentrations (100%, 66.66% and 50% w/w) was used as the suspension medium. The use of different concentrations of SMUF was necessary to evaluate the effect of the electric field and the electric power consumed independently as will be described later in this Chapter.

7.1.1.2 Cultivation and Inactivation of Microorganisms

Escherichia coli ATCC 25922, a gram-negative facultative anaerobic microorganism, is often an indicator of focal contamination in food products. This microorganism usually found in animal products, in the intestinal tract of animals, in prepared foods handled by people and can be recovered from improperly sanitised working surfaces in processing plants (Banwart, 1989).

E coli culture was obtained from Fort Richard Lab Ltd in a nutrient agar slant. Cultures of E coli were sub-cultured to form a single colony and to obtain cultures in the stationary phase, the procedure by Pothakamury, Monsalvegonzalez, Barbosa-Ca`novas and Swanson (1995) was followed. Microbial cells were transferred to 200ml of Tryptic Soya Broth (TSB). The medium continuously agitated in a temperature controlled microprocessor shaker bath at 37^{o}C for 18hr to obtain cells at the early stationary phase. 20% glycerol

of the final volume was added to the medium. Every $4ml$ of the medium was kept in a test tube and stored at -20^{o}C. In all experiments, the initial concentration was in the range of 5×10^{6} to $2\times10^{7}\ cfu/ml$. Before each experiment, the frozen cultures were thawed and centrifuged at $5000 rev./\min$ at $4^{o}C$ for 10 minutes and washed twice with sterilised distilled water then suspended in $5ml$ SMUF for five minutes before using it. Samples of the treated suspension were analysed for microbial inactivation using the *Bac Trac* measuring technology, where the microbial detection was based on impedance splitting method as described in the following section.

7.1.1.3 Microbial Counts Using Impedance Analysis

Microbial metabolism utilises nutrient and produces lower molecular compounds, which alter the conductivity of the liquid nutrients. This change can be technically measured using two electrodes placed in the nutrient solution. If an electrical *AC* voltage is applied to the electrodes then the reduction of the solution impedance in the *AC* current field can be measured. The *BacTrac* measuring technology is based precisely on this principle, whereby the time lapse of the alteration of the ionic composition in the nutrient can be recorded. Despite the reduction of the measuring signal, positive measurement curves were produced as a result of relative representation (giving the relative signal change in relation to the starting value), which resemble typical microbial growth curve as illustrated in (Fig. 7.1). The typical *Bac Trac* measurement curve can be divided into 3 characteristic ranges:

Initial or adaptation phase.

Exponential phase.

Stationary phase.

During the adaptation phase, the microorganisms adapted their metabolism to the substrate present in the nutrient and begin exponential growth. The resulting ions cannot be measured technically. As soon as the exponentially proliferating microorganisms have reached a concentration of approximately $10^6 - 10^7\, CFU/ml$ of nutrient solution, the measuring curves reach their characteristic turning point and then rise in a linear form during the exponential phase. The point of inflection of the curve and the earlier exponential phase, have the most relevance in this measuring technology.

As a result of nutrient limitation or accumulation of toxic by-products, the microorganisms cause the metabolism and their exponential growth follows a transition to the stationary phase. The low molecular metabolites hardly increase at all and the measuring curve proceeds practically parallel to the time axis.

The typical measuring curve establishes more rapidly when more microorganisms are introduced to the nutrient solution under investigation. The formation of the measuring signal is thereby indirectly proportional to the bio burden load of the sample.

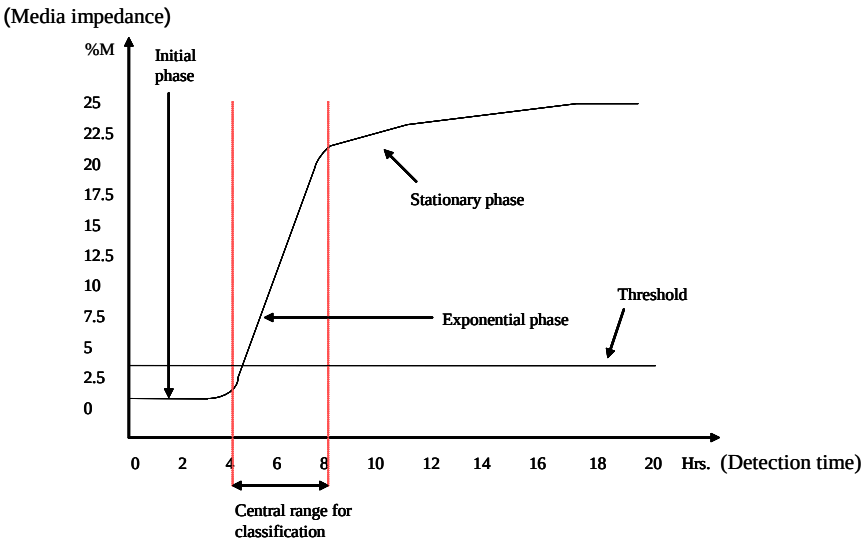

Fig. 7.1: Microbial growth curve and impedance signal.

The instrument *BacTrac* 4300 shown in (Fig. 7.2) includes a built-in computer subsystem for incubator temperature control, impedance measurements, fault monitoring and bi-directional communication with the control PC. Impedance measurements are made at 1 *kHz* and each sample cell location is polled every 10 minutes. Incubator temperature set points are controlled by the PC and maintained by the 4300's on board computer.

Fig. 7.2: The instrument Bac Trac 4300.

7.1.1.4 Principles of the Bac Trac Measuring Technology

When micro organisms are introduced into liquid media, the result is proliferation of the microbes which decompose the higher molecular compounds and hence decreases the impedance of the solution media. The microbial metabolism usually leads to an increase in conductivity and capacitance and thereby to a drop in the impedance according to equation (8.1).

$$Z = \sqrt{R^2 + \left(\frac{1}{2\pi f C}\right)^2} \quad \text{.....(7.1)}$$

Where, Z is the impedance, C is the capacitance, R is the ohmic resistance and f is the frequency.

The instrument *Bac Trac* 4300 registered 2 specific impedance values for each single measurement. These values are displayed as M- value (media impedance) and E value (electrode impedance), graphs (displayed as relative changes compared to a starting value) and are based on the Impedance Splitting Method as the measurement principle.

The relative measurement offers the advantage that all measurement curves have an identical starting point, thereby eliminating product specific fluctuations in the starting conductivity.

The change in the M-value is therefore the relative change in the reduction of the media impedance (in percent) in relation to the starting value. The M- value is directly influenced by the ionic composition of the growth media

and sample used and therefore is not applicable in media of high conductivity since the signal changes due to microbial growth can hardly be registered.

Sterile measuring cells (glass or polystyrene) were filled immediately before use with the corresponding pre-prepared and sterilised impedance media under antiseptic conditions. The measuring cells have volume marking on the sides, and care was taken to insure strict constancy of the pre-determined volume, which was 9ml, since variations in volumes can result in altered impedance signals. Measuring cells were brought to room temperature before use.

Samples of $1ml$ solution were transferred into measuring cells filled with $9ml$ each nutrient using a micro-pipette with a sterile tip. The measuring cells were shacked gently and were placed into the *Bac Trac* incubator immediately after addition of the samples. After placing the samples in the incubator, the lids was turned approximately ¼ to ½ anti clockwise to allow an adequate oxygen supply during incubation. The time difference between inoculation and placing the measuring cell in the incubator did not exceed 15 minutes.

After the cells have been inserted they were recognised by the system automatically and the programme for the respective position was started according to the pre-set parameters. As the measurement cycle begins, a blue ring was superimposed onto the circle diagram whose circumference corresponds to the total duration of the warm-up phase plus the measurement period. As the measurement period for each position progresses, the circle segments turned dark blue, indicating the extent of progress. At the moment of detection (measurement single exceeds the set threshold value) the blue colour charged to indicate the

approximate impedance detection time. The impedance detection time detected responds in an indirectly proportional manner to the germ count in the sample.

The relationship between Colony Forming Unite (CFU) and *Bac Trac* Impedance Detection Time (t) for a calibrated *Bac Trac* application using a linear regression is:

Log CFU =-a * t + b……..(7.2)

For statistical reasons a calibration was produced using 30 samples of the suspension with wide range of E coli concentration. The microbial load in these samples was measured using the spread count method as the reference method against the impedance method. The impedance method saves time and is more accurate compared to the spread count method. The calibration curve is illustrated in Fig. (7.3).

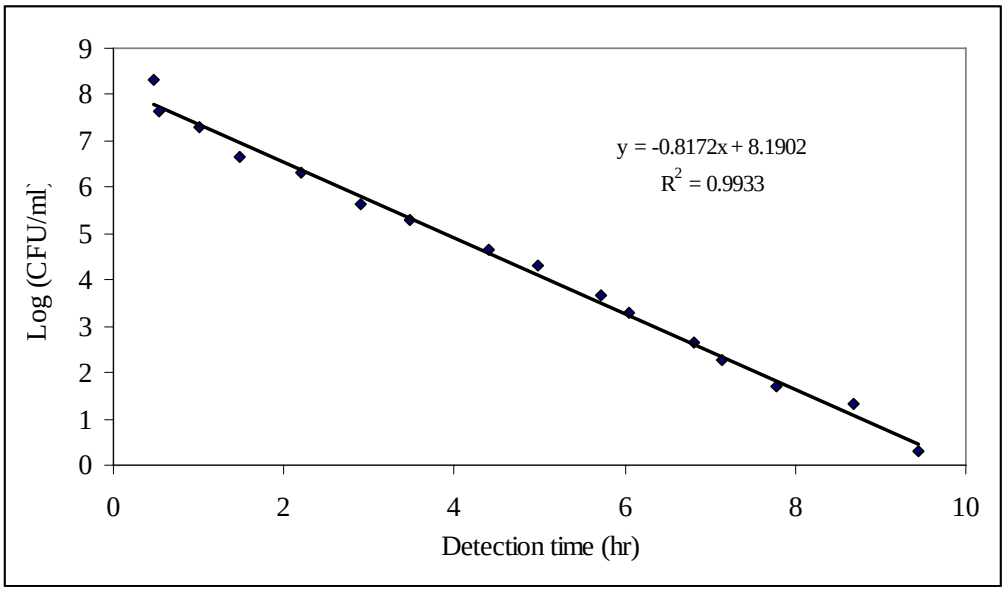

Fig. 7.3: Calibration of the Bac Trac application using SMUF (50% w/w) as the suspension medium incubated with E coli.

7.1.2 PEF Experimental Protocols

Pulsed electric fields treatment was applied to SMUF incubated with E coli. Two litres of SMUF were first deareated using the degassing unit explained earlier. The SMUF was pumped using a piston pump through a flow meter (rotameter) into the PEF multi pass treatment chambers. The SMUF was subjected to high voltage electric pulses at different electric field strengths (9.5-$43 kV/cm$). The treated suspension leaving each chamber passed through a cooling coil immersed in a controlled temperature refrigerated water bath to decrease its temperature to a pre selected temperature before entering the next treatment chamber. The treated suspension was also pressurised during processing to prevent dielectric breakdown of gas bubbles entrapped in the liquid food flowing through the cavity of the treatment chamber.

In order to avoid using large number of treatment chambers, the treated liquid was re-circulated more than once in some of the experiments to increase the treatment time and achieve the desired microbial inactivation. Samples of treated liquid food leaving the PEF chambers were then drawn for microbial analysis. Samples of the suspension media in the feed tank were also drawn at different times for microbial testing. Different experiments were conducted to investigate the effect of different treatment conditions as follows:

1. The effect of electric field intensity on the inactivation rate of Escherichia coli suspended in SMUF (100%, 66.66% and 50%w/w) was studied. SMUF (50%w/w) at flow rate of $43 ml/\text{sec}$ and inlet temperature of 25^{o}C was

subjected to different electric field intensities (16.2, 23.9, 28.5, 34.6 and $43 kV/cm$). Microbial load was measured before and after treatment. Similar measurements were conducted using SMUF (100%, 66.66% and 50%w/w) at flow rate of $2.5 ml/\sec$, with an inlet temperature of $10^{o}C$ and electric field intensity in the range of (28.6- $38.2 kV/cm$).

In the published literature, the effect of electric field intensity has been studied by changing the voltage applied. However, the use of higher voltages or shorter distance between the electrodes will allow higher current to pass through the solution. In order to accurately study the effect of electric field, it is necessary to maintain the power applied. This was achieved in this work by subjecting the suspension of SMUF (50%w/w) to electric field intensity of $28.6 kV/cm$ when the inlet temperature was 20^{o}C, and subjecting SMUF (50%w/w) to electric field intensity of $33.4 kV/cm$ when the inlet temperature was 10^{o}C. Accordingly, the input powers in these two experiments were very close (0.42 and $0.44 kJ/\sec$ respectively).

2. To study the effect of treatment time, samples of the treated suspension were drawn from the outlet of the last PEF chamber and from the feed tank at different times for microbial analysis. Treatment time, which is the pulsation time, was in the range of 0 -$1000 \mu s$.

3. The effect of flow rate and pulse frequency was investigated using SMUF (50% w/w) at 20^{o}C. At a constant frequency of $190 Hz$, and with an electric field intensity of $43 kV/cm$, the effect of flow rates $(2.5 - 8.33 l/\sec)$ was studied.

4. SMUF (50% w/w) at 20^{o}C and at flow rate of $2.5ml/\sec$ was subjected to electrical field intensity of $43kV/cm$ at different frequencies (*58-190Hz*).

5. The effect of initial temperature of the microbial suspension was investigated. SMUF (50% w/w) at flow rate of $5.83ml/\sec$ and different inlet temperatures (6-30^{0}C) was subjected to different electric field intensities (9.5-$43kV/cm$). Also SMUF (66.66% w/w) at flow rate of $2.5ml/\sec$ was subjected to electric field intensity of $33.4kV/cm$ at initial temperatures of $10^{o}C$ and $17^{o}C$. More experiments were carried out by subjecting SMUF of (50% w/w) to electric field intensity of $33.4kV/cm$ when the initial temperatures were 10, 20 *and* $25^{o}C$. Electric field intensities of 33.4 and $35.8kV/cm$ were also applied but for initial temperature of 10^{o}C only.

6. Thermal pasteurisation tests were also conducted using E coli suspended in SMUF (50% w/w) at different treatment temperatures and times to estimate the thermal decimal reduction time for E coli.

7.1.3 Results and Discussion

7.1.3.1 Effect of Pulsed Electric Field Intensity

In general, increasing the electric field intensity increases microbial inactivation, this observation was also reported by (Wouters & Smelt (1997), Casro (1993), Reina, Jin, Zhang & Yousef (1998), Yin, Zhang & Sastry (1997), Ravishankar, Fleischman & Balasubramaniam (2002), Aronsson, Lindgren, Johansson & Ronner (2001), Sensoy, Zhang & Sastry (1996) and many others).

The microbial inactivation shown in (Fig. 7.4) for one-through pass of the suspension medium through the four PEF chambers corresponds to a treatment time of $26.4\mu s$. Results show the strong effect of electric field intensity on microbial inactivation. The logarithmic inactivation is linearly proportional to the electric field, which has been also reported in the literature (Yin, Zhang & Sastry (1997), Reina, Jin, Zhang & Yousef (1998), Wouters & Smelt, 1997, Peleg, 1995, Dunn &Pearlman, 1987 and many others).

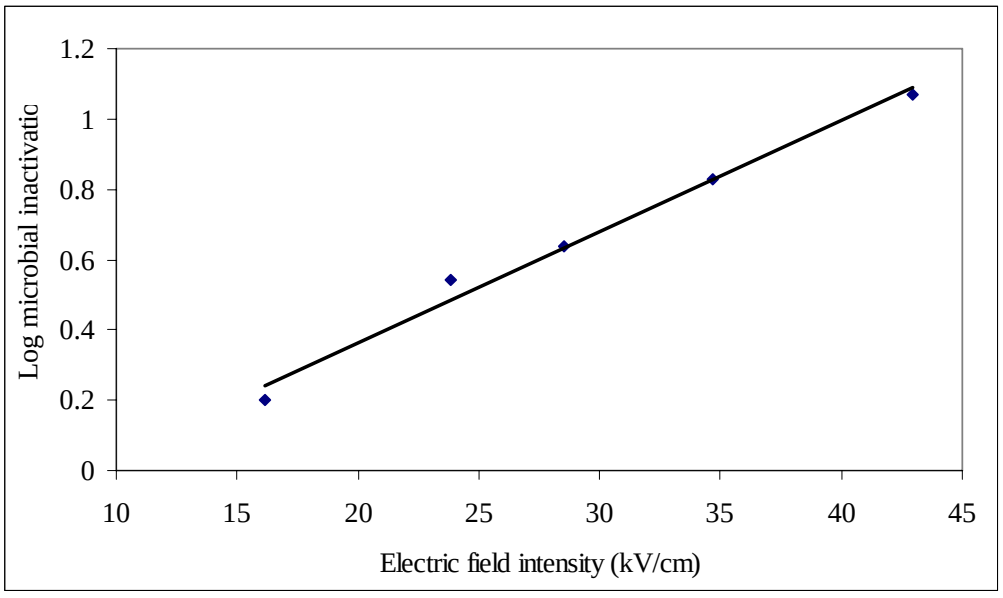

Fig. 7.4: Effect of electric field intensity on the inactivation rate of E coli suspended in SMUF (50% w/w) at flow rate of 5.8ml/sec, treatment time of 26.4μs and inlet temperature of 25^0C.

Using the recirculation mode (treatment time $800-900\mu s$), the inactivation rate of E coli suspended in SMUF (100%w/w, 66.66% w/w and 50%w/w) is shown in Fig. (7.5), (7.6) and (7.7). SMUF (100%w/w) subjected to electric field intensity of 28.6 and $33.4 kV/cm$ showed 5.1 and 6.3 log reduction. E coli suspended in SMUF (66.66%w/w) and subjected to electric field intensity of 33.4 and $38.2 kV/cm$ showed 5.3 and 6.9 log reduction, while the suspension of SMUF (50%w/w) subjected to 28.6, 33.4 and $35.8 kV/cm$ resulted on 4.6, 5.8 and 6.6 log reduction.

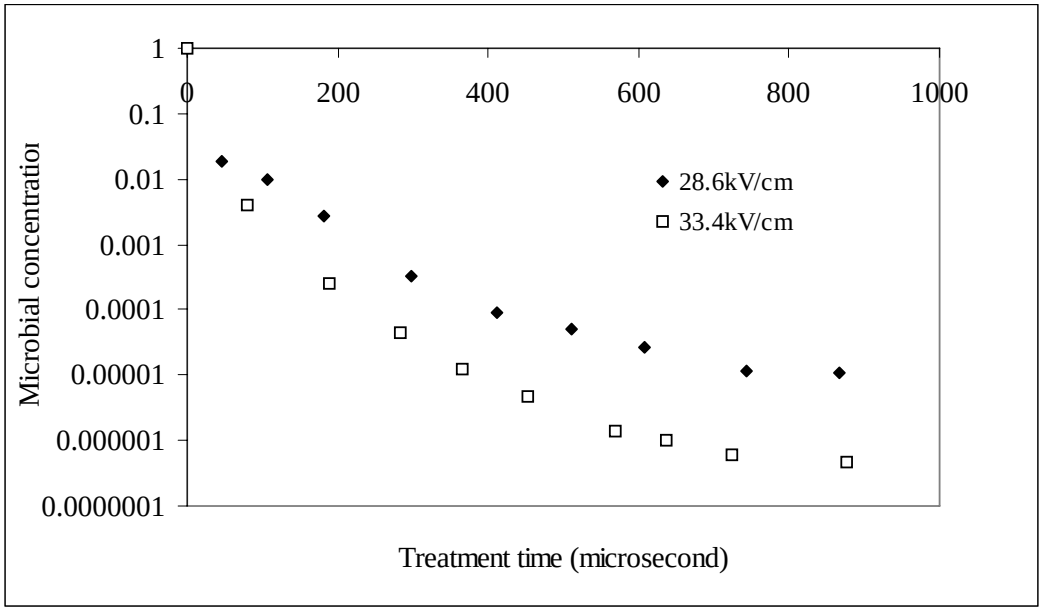

Fig. 7.5: Effect of electric field strength and treatment time on E coli deactivation suspended in SMUF (100% w/w) at 2.5ml/sec and 10 ^{o}C when subjected to electric field intensity of 28.6 and 33.4kV/cm and frequency of 190Hz.

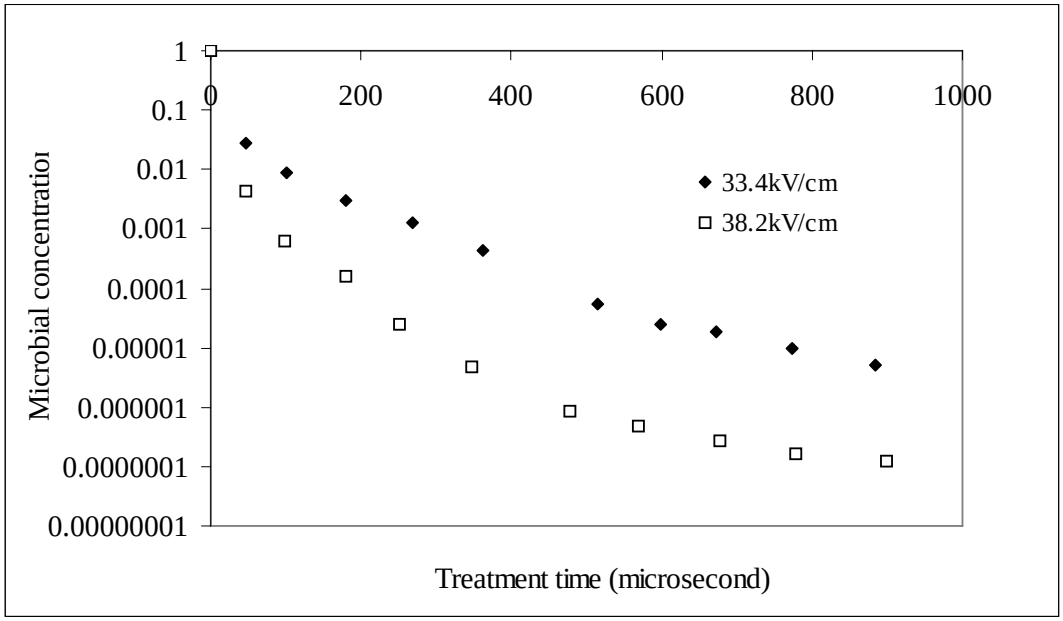

Fig. 7.6: Effect of electric field strength and treatment time on E coli deactivation in SMUF (66.66% w/w) at 2.5ml/sec and inlet temperature of 10°C when subjected to electric field intensity of 33.4 and 38.2 kV/cm and frequency of 190Hz .

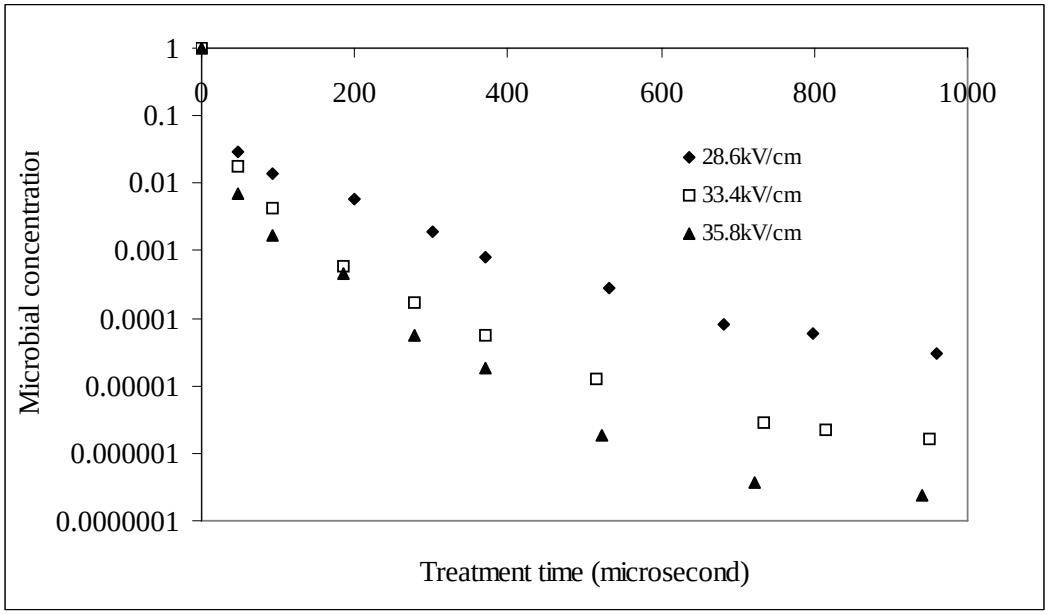

Fig. 7.7: Effect of electric field strength and treatment time on E coli deactivation in SMUF (50% w/w) at 2.5ml/sec and inlet temperature of 10^0Cwhen subjected to 28.6, 33.4 and 35.8 kV/cm and frequency of 190Hz.

The experimental results suggest that microbial inactivation was mainly due to the effect of electric field rather than the electrical power applied. This could be validated by two experiments with similar power input as shown in (Fig. 7.8). SMUF (50% w/w) at 20^{o}C inlet temperature was subjected to electric field intensity of $28.6 kV/cm$, while the same suspension at initial temperature of 10^{o}C was subjected to electric field intensity of $33.4 kV/cm$. The input powers in these two experiments were close (0.42 and 0.44 kJ/sec respectively). The results show that the microbial inactivation rate was higher at higher electric field intensity. However, the effect of electric field intensity being smaller than that observed previously in (Fig. 7.7). This is due to the temperature effect, since the operating temperature in the experiment with electric field of 33.4 kV/cm was lower than of 28.6 kV/cm, while in (Fig. 7.7) the inlet temperature was constant in all experiments. As the electric field increased, the operating temperature will increase due to the higher power absorbed and hence the effect shown maybe due to both electric field and temperature.

This point has not been well addressed in the literature. Our independent findings is in agreement with a recent publication found after writing this thesis (Sepulveda, Guerrero &. Barbosa-Cánovas, 2006). They found that the amount of electric current flowing through a suspension of *Listeria innocua* in McIllvaine buffer had no influence on the rate of inactivation.

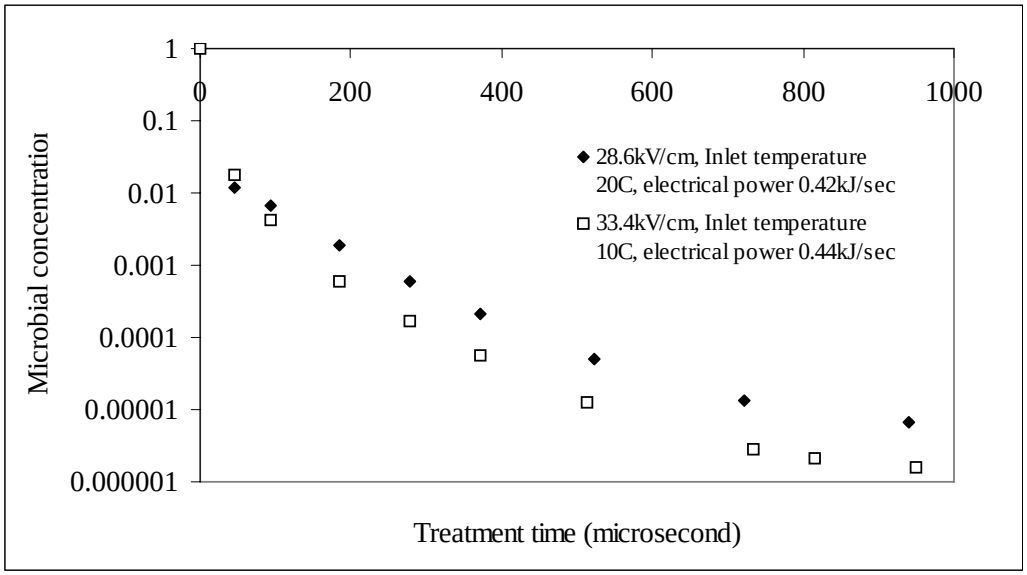

Fig. 7.8: Effect of electric field strength and treatment time on E coli deactivation in SMUF (50%w/w), when subjected to 28.6kV/cm and 33.4kV/cm when the initial temperatures were $20^{o}C$ and $10^{0}C$ respectively.

Tailing on the inactivation curves can be observed in all the measurements. This is in agreement with the observation of Alvarez, Vitro, Raso & Condon (2003), who reported that tailing could be due to protection resulting from the contents of dead cells, which shield the remaining survivors, or to the microbial aggregation during the treatment.

Contamination of the PEF unit may occur due to the recirculation of the microbial suspension; this could slightly affect the treatment. Analysis of the recirculation mode will be discussed in chapter 8.

7.1.3.2 Effect of Treatment Time

Treatment time was calculated from the product of the number of pulses and pulse width, which depends directly on the flow rate and pulse frequency when the treatment chamber volume is fixed. The number of pulses received by the circulated suspension was calculated from the number of cycles the suspension was circulated. Although the effect of treatment time was discussed in the previous section, more information will be presented in this section.

Number of Pulses

Increasing the number of pulses significantly increased the inactivation rate of microorganisms in all the experiments conducted in this work. The microbial killing was higher during the first 10-20 pulses and gradually decreased. This was also reported by Sensoy, Zhang & Sastry (1996) for the inactivation of Salmonella dublin in skim milk and by Dutreux, Notermans, Wijtzes, Gongora-Nieto, Barbosa-Ca`novas & Swanson (2000) for the inactivation of E.coli suspended in milk and phosphate buffer. The same effect has been also reported by others (Reina, Jin, Zhang &Yousef (1998), Zhang, Monsalve-Gonzalez, Barbosa-Ca`novas and Swanson (1994a), Hulsheger, H., Potel, J., and Niemann, E. G. (1981, 1983)).

Flow Rate

The number of pulses received by the liquid is inversely proportional to its flow rate when the pulse frequency and the treatment chamber volume are kept

constant. Results show that increasing the flow rate of the suspension medium decreases the microbial inactivation rate. The inactivation rate of E coli suspended in SMUF (50% w/w) at different flow rates (2.5, 3.33, 5, 6.66, 8.33 and $10 ml/s$) are shown in (Fig. 7.9). The electric field intensity was $43 kV/cm$, the frequency was *190Hz* and the inlet temperature was 20°C.

Frequency

The inactivation of E coli suspended in SMUF (50% w/w) at flow rate of $2.5 ml/\sec$ and subjected to electric field pulses at different frequencies (*58, 72, 100, 120, 145, 160 and 190Hz*) is shown in (Fig. 7.10). As expected, microbial inactivation increased with increasing the frequency. Results were analysed after one-pass of the suspension medium through the PEF unit.

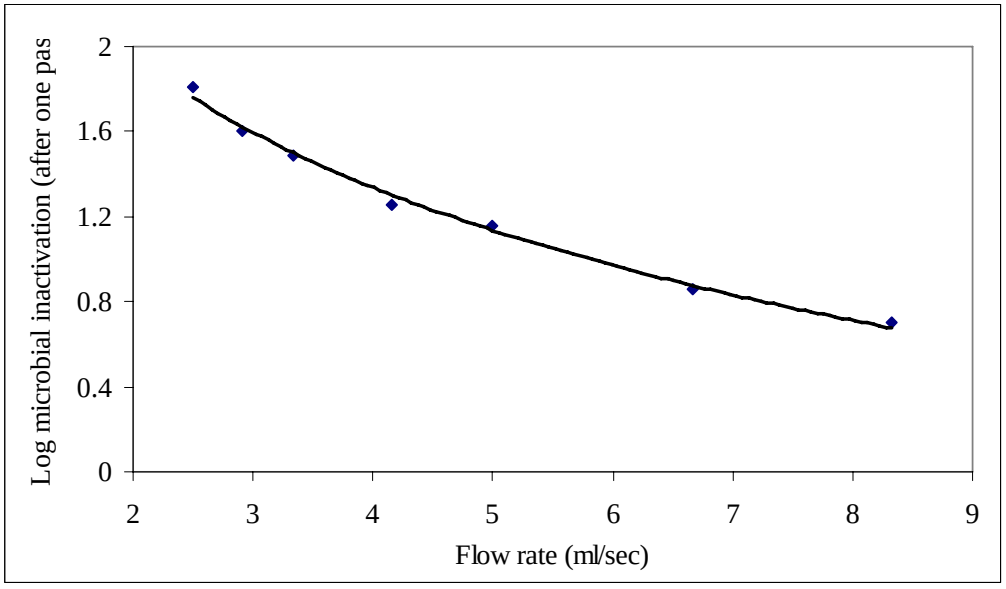

Fig. 7.9: The effect of flow rate of SMUF (50% w/w) incubated with E coli on the microbial inactivation at constant frequency, when the electric field intensity was 43kV/cm, frequency was 190Hz and 20°C inlet temperature.

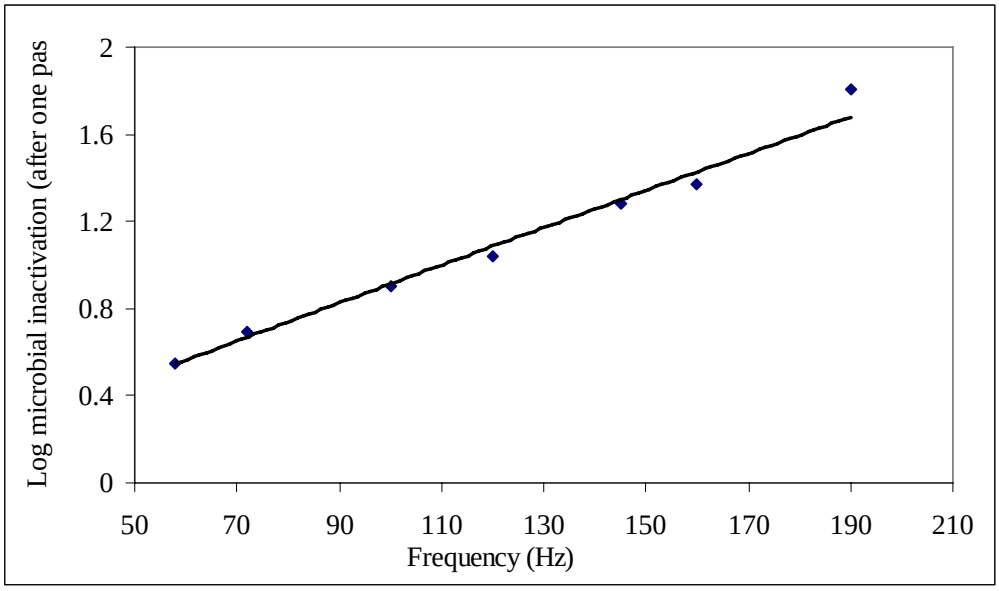

Fig. 7.10: The effect of frequency on the inactivation of E coli suspended in SMUF (50% w/w) at 2.5ml/sec, inlet temperature 20^0C and electric field intensity of 43kV/cm.

Treatment time maybe sustained the same by increasing or decreasing the flow rate or frequency. However for the same treatment time, our results show much better microbial inactivation at higher frequency and flow rate than at lower frequency and flow rate.

At the lower flow rate the suspension flow was transitioning to turbulent, while at the high flow rate, it was fully turbulent. At higher flow rate, fully developed flow can be achieved with high degree of mixing providing more uniform residence time distribution. Moreover, when the number of pulses applied is limited it is important to operate at high flow rate to insure that all the fluid elements receive the same number of pulses.

A comparison between the microbial inactivation of E coli at different frequencies and constant flow rate with those of different flow rate at constant frequency shown in (Fig. 7.11) confirm our conclusion.

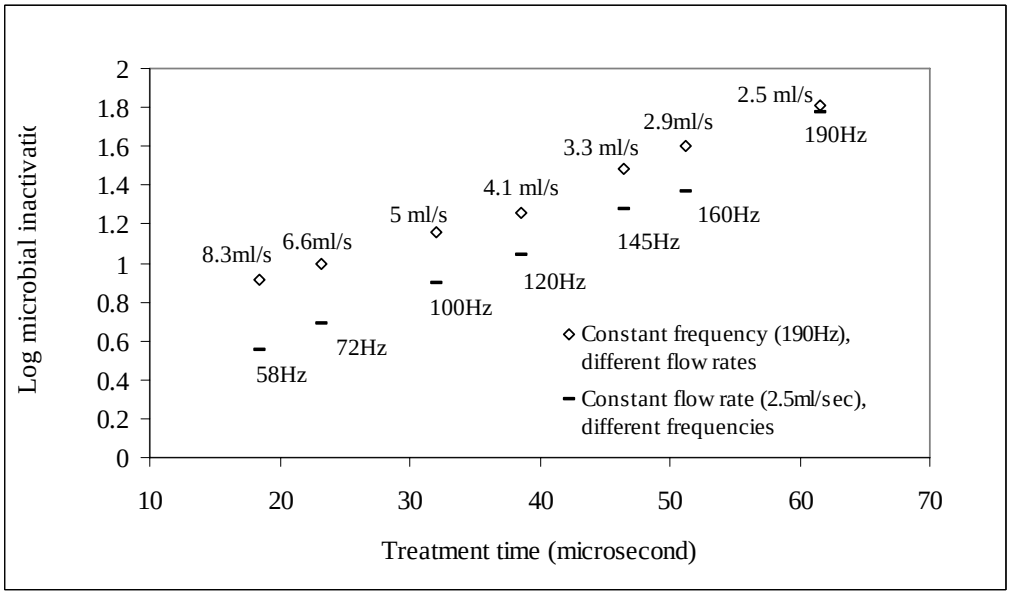

Fig. 7.11: Comparison between the effect of changing the pulse frequency when the flow rate was fixed and the effect of changing the flow rate at constant frequency on the microbial inactivation of E coli suspended in SMUF (50% w/w) at 20°C inlet temperature. The electric field intensity applied was 43kV/cm.

7.1.3.3 The Effect of Treatment Temperature

The experimental results suggest that treatment temperature influences significantly microbial inactivation due to its assistance in breaking down bacteria membrane. E coli suspended in SMUF (50% w/w) at flow rate of $5.8 ml/\sec$ and subjected to different electric field intensities (9.5, 16.7, 23.9 and

$43kV/cm$) showed more inactivation when the suspension inlet temperature was higher as shown in (Fig. 7.12).

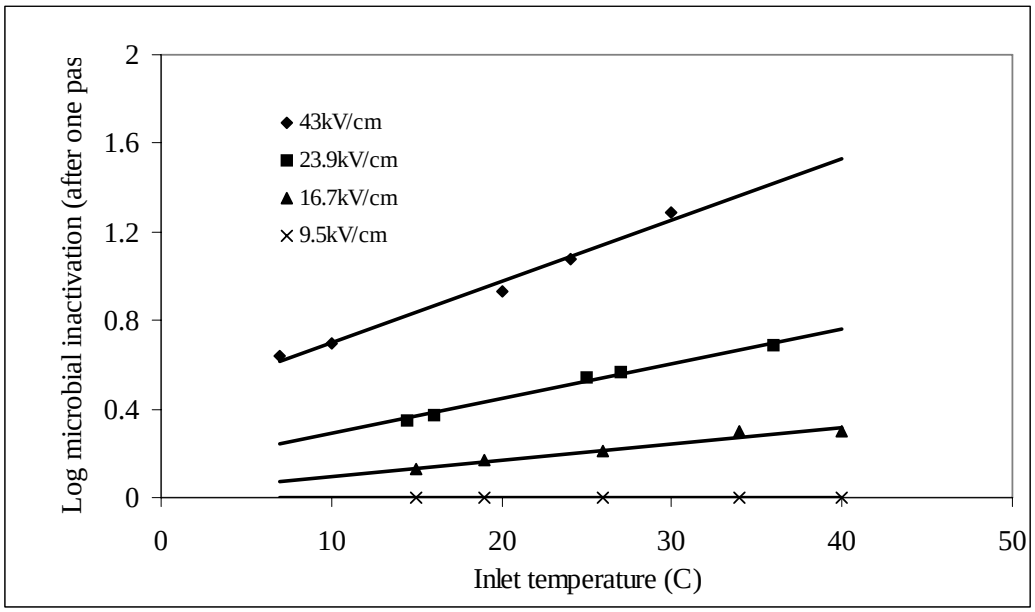

Fig. 7.12: The effect of the inlet temperature of the suspension on the microbial killing using different electric field intensities and frequency of 190Hz. SMUF (50% w/w) at flow rate of 5.8ml/sec.

Similar results were obtained when increasing the initial temperature of the microbial cell suspension (using SMUF 66.66% w/w) from 10 to 17^{o}C and subjected it to $33.4kV/cm$ for treatment time of $880\mu s$. The microbial inactivation increased from 5.3 to 6.6 log reduction as shown in (Fig. 7.13). Based on these results it is certain that lower electric field intensities be needed if

treatment temperature is slightly raised. Further analysis with regards the effect of temperature is presented in Chapter 8.

E coli suspended in SMUF (50% w/w) at flow rate of $2.5ml/\sec$ and subjected to electric field intensity of $28.6kV/cm$ for $1000\mu s$ showed 4.6, 5.3 and 5.8 log reduction when the initial temperature was 10^oC, 20^oC and 25^oC respectively as shown in (Fig. 7.14). The temperature of the suspension was kept below the lethal temperature of the microorganism studied (presented in Fig. 7.15), demonstrating that inactivation is not due to thermal effects induced by the current passing through the solution.

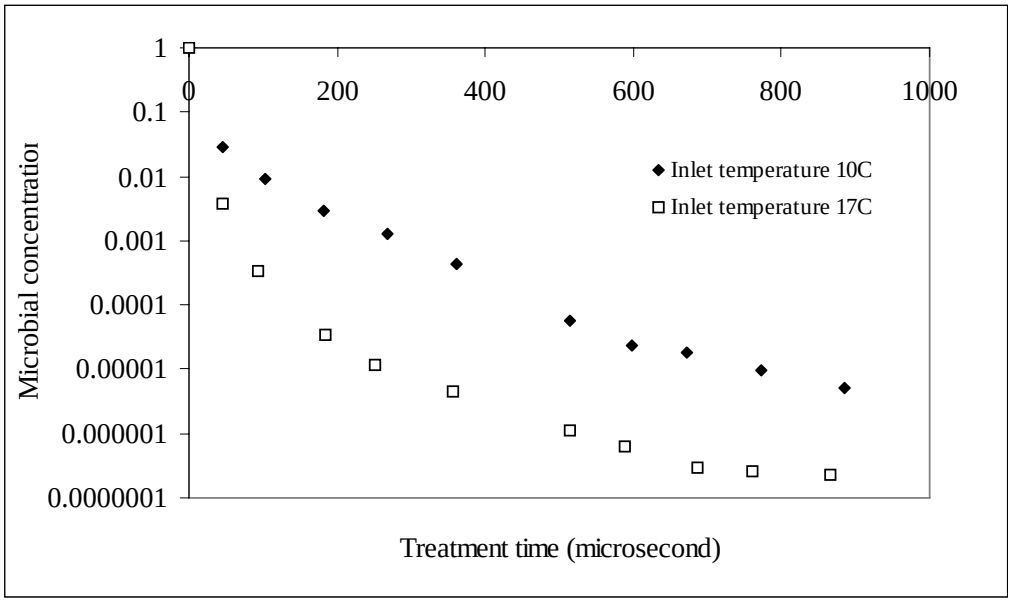

Fig. 7.13: Effect of treatment temperature on E coli deactivation in SMUF (66.66%w/w) at initial temperature of 10^oC and 17^oC when subjected to 33.4kV/cm.

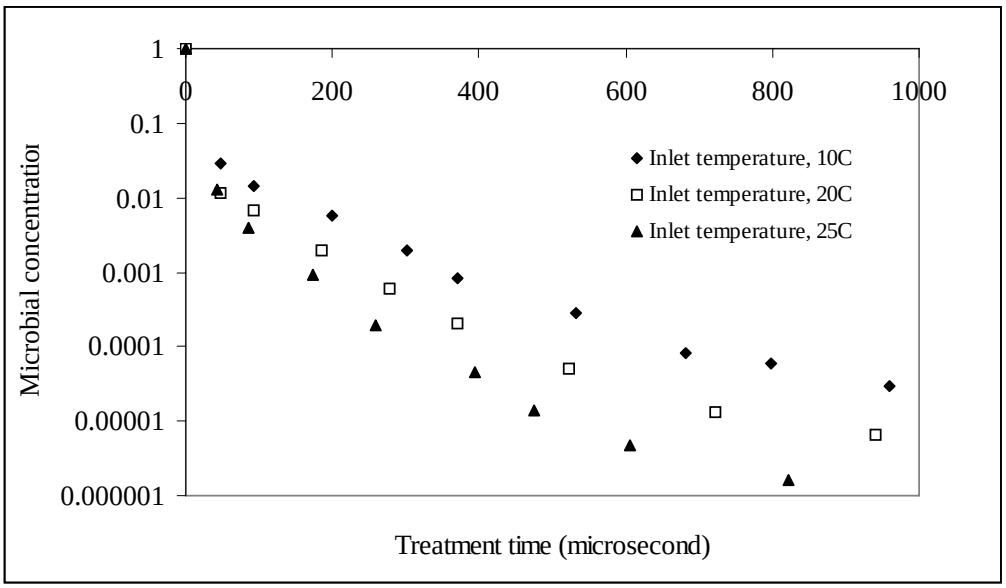

Fig. 7.14: Effect of initial temperature on E coli deactivation in SMUF (50%w/w) when subjected to electric field intensity of 28.6kV/cm.

Coster & Zimmermann (1975) and Stanley (1991) suggested that the increase in the rate of inactivation with increasing temperature maybe due to the decrease in the electric breakdown potential of the bacterial cell membrane. They reported that at low temperatures, the phospholips are closely packed into a rigid gel structure, while at high temperatures they are less ordered and hence, the temperature can affect the physical stability of the cell membrane. The associated reduction in bilayer thickness of the membrane may make the cell membrane more susceptible to breakdown at a relatively high temperature even when the electric field strength is small. These findings were also discussed in other studies (Aronsson & Ronner 2001 and Stanley 1991). This is in agreement with Dunn and Pearlman (1985), who recommend operating PEF units at moderate temperatures.

Thermal pasteurisation experiments conducted in this project on SMUF (50% w/w) incubated with E coli shows that the microorganism tested was not affected by temperatures below 45^{o}C. At higher temperatures, microbial inactivation is function of time and temperature as shown in (Fig. 7.15). The high temperatures deactivate microorganisms by causing lysis of the membrane or denaturation of critical enzymes.

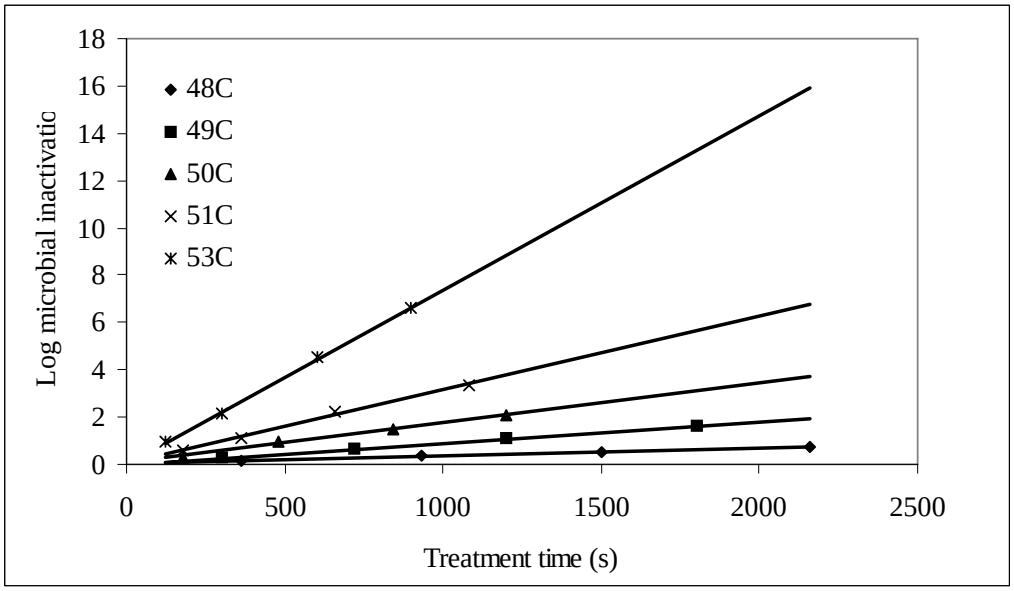

Fig. 7.15: The effect of the thermal pasteurisation treatment on E coli suspended in SMUF (50% w/w) at different times.

7.1.3.4 The Effect of Ionic Strength of the Suspension

The ionic strength plays an important role in microbial inactivation using PEF treatment. The reduced inactivation rate in high ionic strength solutions can be explained by the stability of the cell membrane when exposed to a medium with several ions. The experimental results shown in (Fig. 7.16) suggested that higher microbial inactivation can be achieved when using SMUF of the lower electrical conductivity. SMUF (50% w/w) and SMUF (66.66% w/w) at flow rate of $2.5 ml/\sec$ and inlet temperature of 10^{o}C were subjected to electric fields intensity of $33.4 kV/cm$. The logarithmic reductions achieved were 5.8 and 5.3 respectively, even though the outlet temperature of SMUF (66.6% w/w) was higher (28.3^{o}C) compared to outlet temperature of (22^{o}C) when using SMUF (50% w/w). However, SMUF (100%w/w) subjected to $33.4 kV/cm$ at the same inlet temperature (10^{o}C) shows more killing than the later experiments due to the excessive increase of processing temperature (39.5^{o}C).

Using liquids with low electrical conductivity, the difference in conductivity between the microbial cytoplasm and the medium increases, this increases the flow of ionic substances across the microbial membrane and makes it more sensitive to the PEF treatment. Furthermore, a low conductivity minimise ohmic heating of the suspension being treated and hence allows the use of higher electric field. Our results are in agreement with (Vega-Mercado, Pothakamury, Chang & Swanson, 1996, Dutreux, Notermans, Wijtzes, Gongora-Nieto, Barbosa-Ca`novas & Swanson, 2000a, Jayaram, Castle & Margaritis, 1993, Hulsheger, Potel & Niemann, 1981 and many others).

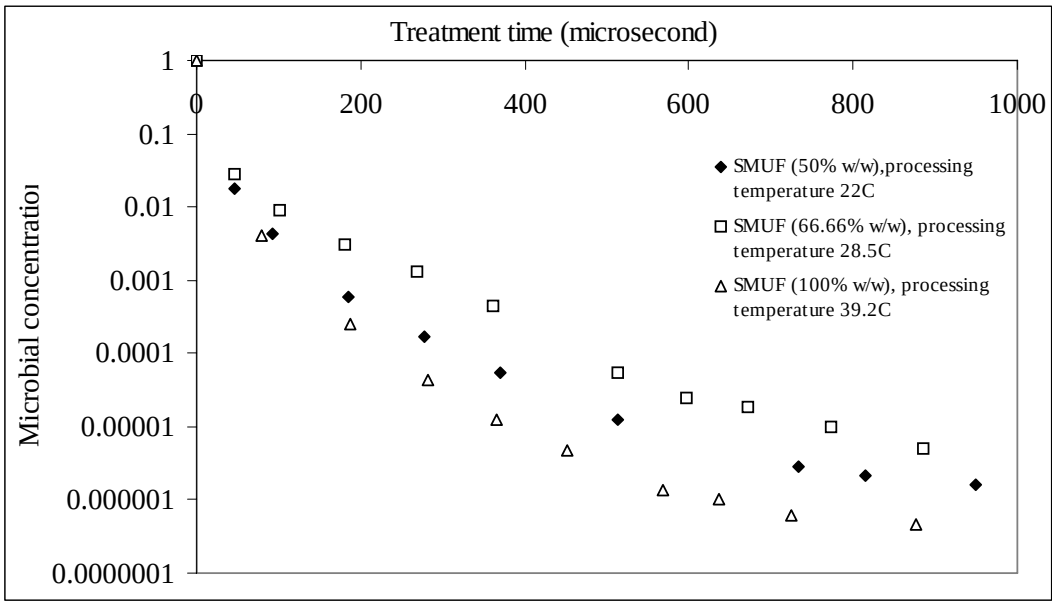

Fig. 7.16: The effect of the ionic strength of the microbial suspension on the inactivation rate of E coli suspended in SMUF of different concentrations. Electric field intensity of 33.4kV/cm and frequency of 190Hz.

7.1.3.5 Power Consumption during the PEF Application

The power consumption plays an important role on the efficiency of the PEF application. The average dissipated power in all the conducted microbial inactivation tests was calculated according to equation (5.2). The process parameters and medium specifications affects the power consumption and hence the efficiency of the PEF process. Increasing the feed temperature, the electric field intensity and the pulse frequency leads to an increase in the power consumption during the PEF application as shown in (Fig. 7.17). Equation (7.3) shows that the dissipated power is directly proportional to the square of electric field strength and to the conductivity of the solution, which explain the results

shown in (Fig. 7.17). Increasing the inlet temperature lead to higher solution conductivity and hence higher power dissipated.

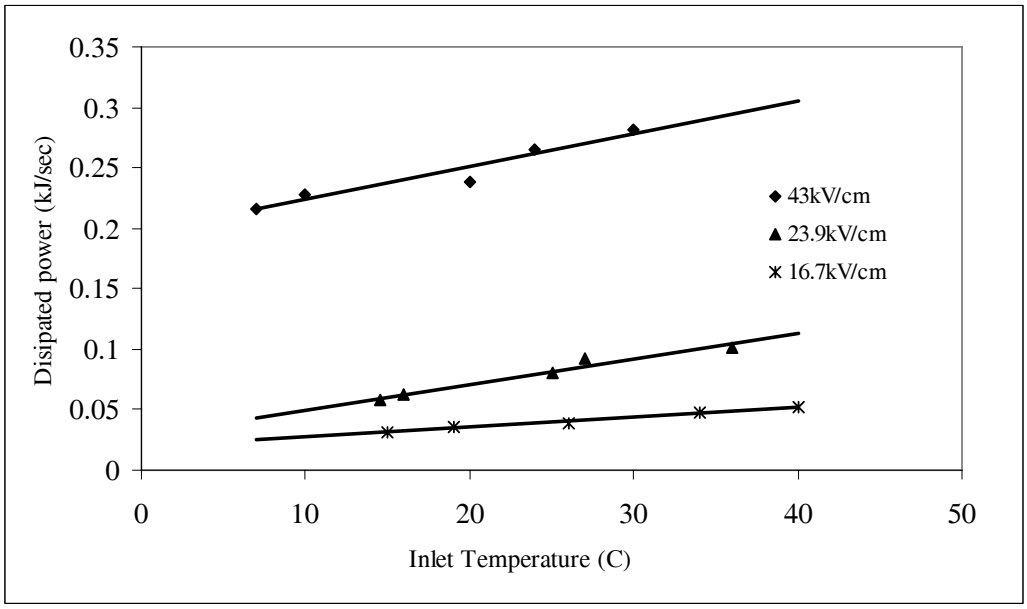

Fig. 7.17: The dissipated power at different inlet temperatures of the microbial suspension and electric field intensities. SMUF (50% w/w) at flow rate of 5.8ml/sec was used.

7.2 Experimental Study, Morphology of the Microbial Cells after PEF Treatment

The biological changes induced by the electric field pulses include electropermeabilisation, electrofusion, motility alteration and microorganism inactivation. Electropearmeabilisation is the increase in cell permeability when subjected to electric field and it is the key step in the process of electrofusion. Electrofusion is the connection of two separated membranes into one. Cell membranes must be in close contact for fusion to occur.

PEF treatment increases the transmembrane potential of cell membrane, which results in electroporation. The microbial inactivation mechanism depends on the treatment condition and the type of microorganism. The morphological changes of E coli suspended in SMUF (50% w/w) after the PEF treatment was investigated using the Transmission Electron Microscopy as discussed below.

7.2.1 Materials and Methods

7.2.1.1 PEF Treatment

E coli suspended in SMUF (50% w/w) at initial concentration of $6.3\times10^{6}\,CFU/ml$ and at flow rate of $2.5ml/s$ was subjected to square bipolar pulses of $1.7\mu s$ width in the PEF lab scale unit described earlier in Chapter 6. The electric field intensity applied was $28.6kV/cm$ and the processing temperature was 30^{o}C. Samples were harvested after 130 pulses for the TEM studies.

7.2.1.2 Transmission Electron Microscopy TEM

The technique and protocol followed was the standard preparation of biological samples for TEM (Hayat, 1989). The cells were fixed in 3% glutaraldehyde/0.1 M Sorensens phosphate buffer for three hours. The fixed cells were rinsed three times, each time for 10 min with 0.1M Sorensens phosphate buffer (pH 7.4) and then postfixed in 1% Osmium Tetroxide/0.1 M Sorensens phosphate buffer for one hour. The fixation was followed by rinsing operation.

The next phase was the dehydration accomplished with 30%, 50%, 70%, 90%, and 2*100% ethanol for 10 minutes in each solution then by 2*100% acetone for 10 minutes each time. The cells were infiltrated and embedded in 1:1 acetone: 812 epoxy resin for one hour (The resin is “EM bed-812” from Electron EM bed-812" from Electron Microscopy Sciences, Fort Washington, PA, USA) then in 100% epoxy resin overnight (16-24 hours). Samples were transferred to plastic moulds and cured at 60°C for 48 hours. The polymerized blocks were hand-trimmed and thin-sectioned using a microtome and a glass knife to prepare semi-thin sections (1- 5μ) with a glass knife and stained with Methylene Blue. Ultra thin sections were cut to approximately 70nm thick, and placed on a 200 mesh copper grids and stained with Uranyl acetate and Lead citrate.

7.2.2 Results and Discussion

The untreated E coli cells (the control) are shown in (Fig. 7.18). The cells exhibited uniform distribution of the cell membrane and show thick wall. E coli suspended in SMUF (50% w/w) and subjected to 130 pulses at electric field intensity of $28.6kV/cm$ shows 4.1 log reduction. The PEF treated E coli cells presented in (Fig. 7.19) shows change in the structure of the cells, an increase in the cell wall roughness, cytoplasmic clumping, cytoplasmic clumping, cells were condensed into relatively distinct areas of high and low electron density. Condensation of intracellular was also observed in E coli after PEF treatment by Aronsson, Lindgren, Johansson & Ronner (2001), although their electron

microscope examination of PEF-treated bacterial cells did not confirm membrane damage of E. coli subjected to 20 pulses at $23.1 kV/cm$.

Leakages of the intracullar material and cell shrinkage were also observed as shown in (Fig. 7.20). The induced cytoplasmic disruption and the rupture of the cell membrane and cell wall observed for E coli exposed to the selected electric field intensity supported the theory of dielectric breakdown (Zimmermann, Pilwat, Beckers & Riemann, 1976).

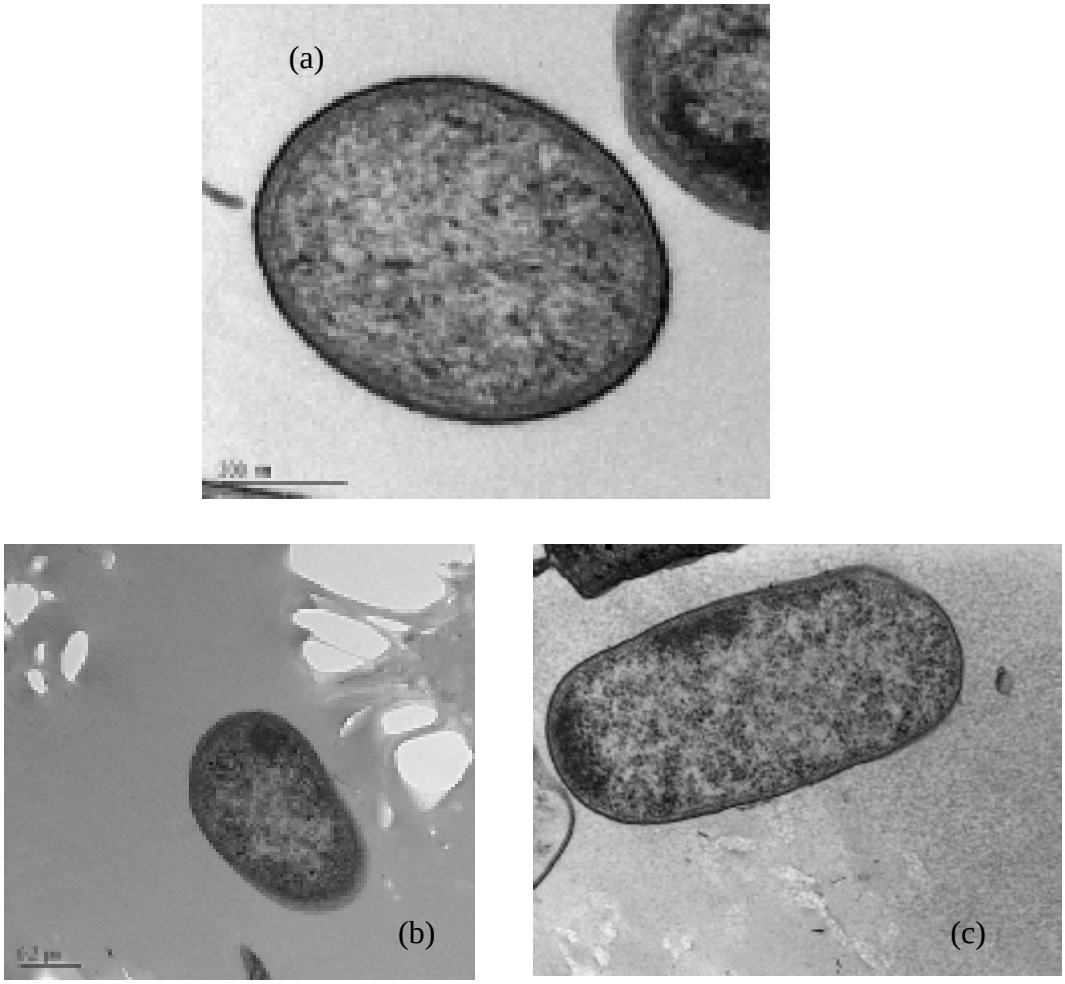

Fig. 7.18: a, b, & c, cross sections of the untreated E coli.

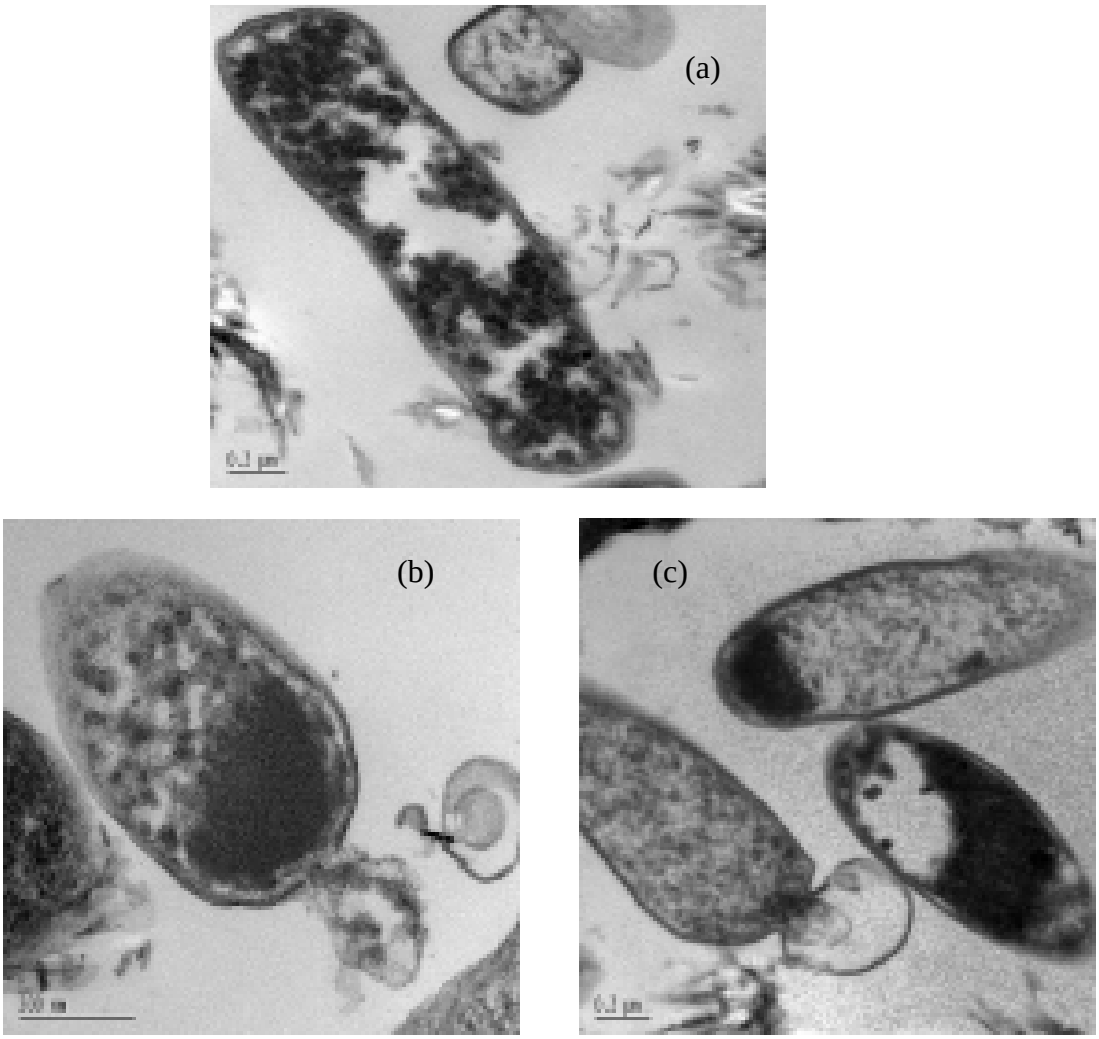

Fig. 7.19: a, b & c, Condensation of inter cellular of Escherichia coli ATCC 25922 suspended in SMUF (50% w/w) and exposure to 130.4 pulses at electric field intensity o f $28.6kV / cm$.

Similar results were found by Pothankamury (1995), the exposure E coli (ATCC 11229) cells to electric fields resulted in the cytoplasm drawing away from the outer membrane. Electric field treatment also caused the outer membrane to be created similar to the edge of a saw. It was also suggested that crenations observed in the PEF treated cells may be an indication of cell

shrinkage because untreated cells exhibited a more uniform distribution of cytoplasm, which was observed to be more granular and clumped than untreated cells.

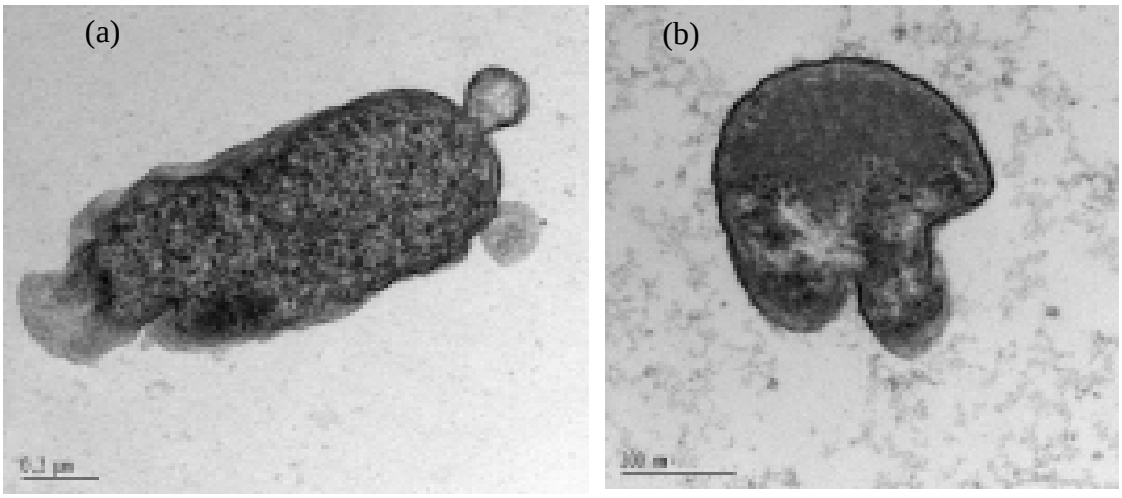

Fig. 7.20: a & b, Escherichia coli ATCC 25922 suspended in SMUF (50% w/w) and exposure to 130 pulses at electric field intensity of $28.6kV/cm$*. Electroporation, membrane damage and leakage of the cellular material.*

Dutreuxa, Notermans, , Wijtzesa, Góngora-Nietob, Barbosa-Ca`novas & Swanson (2000), also suggested that bacteria exposed to electric field pulses exhibit ruptured cell walls and leakage of the cytoplasmic contents and that the internal organization of the cells seemed to be damaged as well. This was demonstrated by the detachment of the cytoplasm from the cell membrane.

Electrofusion was observed in the E coli cells as presented in (Fig. 7.21). Cells were in close contacts and as these cells exposed to the electric field pulses, cell movement achieves close membrane contact, orientation and alignment.

Zimmermann, (1986) stated that the presence of electric field pulls the cells in the direction of the high field intensity, due to the generation of a dipole in cells because of the net force exerted. If the field strength is high enough to induce electropermeailisation, electrical breakdown occurs predominately in the contact zone, which leads to cell fusion.

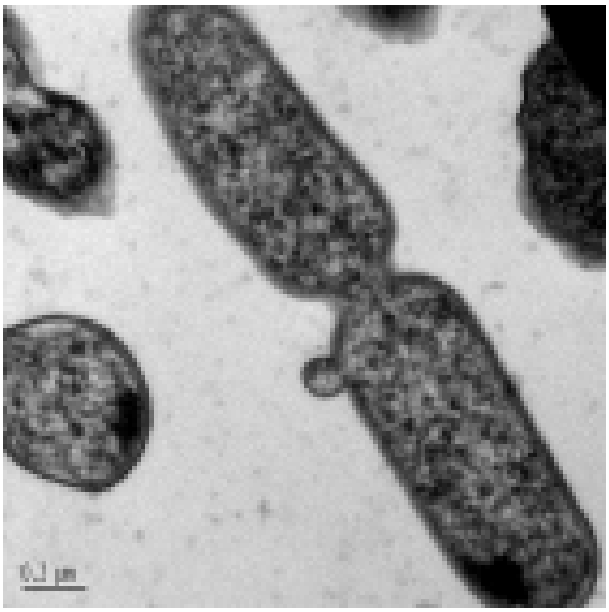

Fig. 7.21: Electrofusion of Escherichia coli ATCC 25922 suspended in SMUF (50% w/w) and exposure to 130.4 pulses at electric field intensity of $28.6 kV/cm$.

E coli inactivation was suggested to be due to irreversible cell membrane breakdown and leakage of cellular contents as presented in (Fig. 7.22). The molecular mechanism of the electroporation as discussed by some researchers

could be due to electroconformational changes in lipid or protein molecules, or due to osmotic imbalances between cells and their environment which leads to the pore formation.

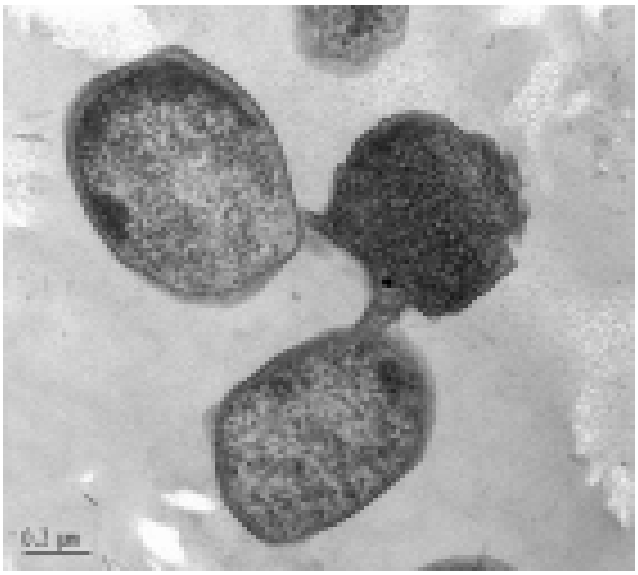

Fig. 7.22: Escherichia coli ATCC 25922 suspended in SMUF (50% w/w) and exposure to 130.4 pulses at electric field intensity of $28.6 kV/cm$.

7.3 Conclusions

In general increasing the intensity of the electric field, treatment time and processing temperature significantly enhances microbial inactivation of E coli. An accurate measurement of these parameters and the uniform distribution of electric field in the treatment chamber are necessary to obtain reliable inactivation kinetics. The effect of electric field on the degree of inactivation was very significant in all the solutions tested. The effect was initially high, and decreased

as the bacterial counts decreased. The effect of electric field was carefully studied in this work by operating at a condition of equal power when the electric field was varied. Such study which has not been conducted previously showed that the inactivation is due to the electric field rather than power or current; however the current density has also some effect of the microbial inactivation.

The inlet temperature of the microbial suspension plays an important role in the microbial inactivation. The increase in suspension temperature enhances the membrane breakdown during PEF treatment, which may depend on the microorganisms tested as well as media used. As the temperature increases, the dissipated power also increases. There is a dynamic relationship between electrical conductivity, energy input, and temperature increase, which suggest the need of accurate control of these parameters during this non-thermal pasteurisation.

Other processing factors that affect microbial inactivation using PEF treatment are flow rate and pulse frequency as these two parameters determine the treatment time. At higher flow rate and higher frequency, the microbial inactivation was higher due to the improved fluid residence time distribution as a result of mixing induced by turbulent.

PEF treatment can cause biological changes induced by the high potential developed within the microbial cells. The TEM study suggested that the inactivation of E coli was a consequence of cytological disruption of a large portion of the bacteria cellular organelles and also by the electroporation mechanisms. Electroporation (the permeabilization of the membranes of cells and

organelles) and electrofusion (the connection of two separate membranes into one) leads to microbial inactivation. PEF can cause the cell membrane to loss the property of semi permeability, and this leads to structural damage of cell membrane. Microbial inactivation mechanism depends on specific characteristics of microbial cell membranes, media, processing conditions and type of microorganisms.

8. Chapter Eight: Kinetic Analysis of Microbial Inactivation

This chapter presents the kinetic analysis of microbial inactivation due to PEF and thermal treatments of E coli suspended in SUMF. Comparison between measured (experimental) and predicted (theoretical) variation of Escherichia coli concentration with time after PEF treatment is discussed, taking into consideration the recirculation mode of the PEF treatment.

8.1 Kinetics of Microbial Inactivation

It is difficult to compare PEF inactivation rate of bacteria reported in the literature since these investigations were conducted at different conditions. Hence, it was decided to use the inactivation rate constant of E coli destruction for the purpose of comparing the different experimental results.

The values of the inactivation rate constant were calculated for different concentrations of E coli suspended in SMUF when subjected to PEF treatment at different operating conditions. The values of the rate constant were calculated from the one-pass (through the PEF unit) experiments. From the calculated rate constant at different temperatures and electric field, Arrhenius constants and activation energies were calculated to provide a generalized correlation for the

inactivation rate constant as a function of both electric field intensity and treatment temperature.

A model was developed to describe the recirculation mode of operation. The theoretical changes in the microbial concentration after PEF treatment (using the recirculation mode) were calculated based on the model and the correlation developed from the one-pass experiment and compared with the measured values.

8.1.1 Single Pass Operation

Assuming the concentration of microorganisms C_T in the feed tank is the same as that in the entrance of the PEF unit (Fig. 8.1), the rate of microorganisms destruction with respect to the concentration of microorganisms may be expressed based on first order kinetics as follows:

$$r = -kC \quad \text{.....(8.1)}$$

Where r is the rate of destruction [micro organism /(L/s)], C is the concentration of microorganisms (microorganism / L) and k is the inactivation rate constant (s^{-1}). The assumption of first order kinetics is acceptable (Esplugas, Pangan, Barbosa-canovas & Swanson, 2001) even though some deviation from first order kinetics has been reported in the literature (Alvarez, Vitro, Raso & Condon (2003).

For single pass operation, a mass balance of microorganisms around the PEF chamber can be made, assuming plug flow (Levenspiel, 1972):

$$\ln \frac{C}{C_T} = -k\tau$$

Where τ the residence time and is equal to v/q .

$$C = C_T e^{-kV/q} \quad \text{.....(8.2)}$$

Where, V (L) is the PEF chamber volume and q (L/s) is the volumetric flow of the treated fluid.

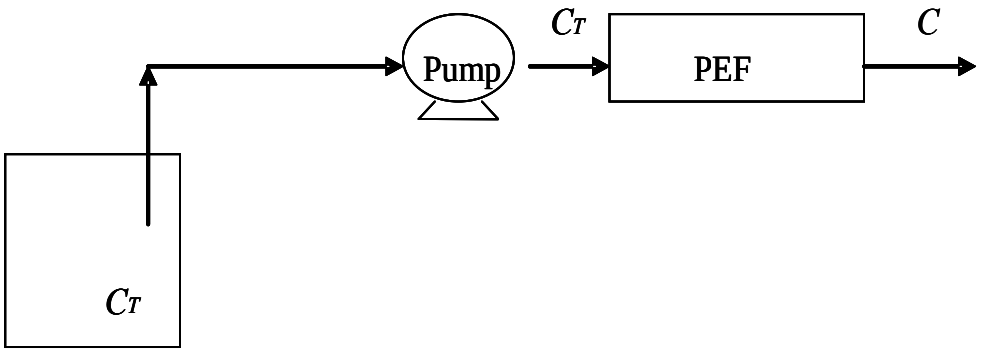

Fig. 8.1: The PEF Single pass application.

The values of inactivation rate constant were calculated according to equation (8.2) at different treatment conditions using the experimental measurements of microbial concentration before and after the PEF treatment.

Under the evaluated conditions, the influence of temperature on the inactivation rate constant (k) can be described by the Arrhenius equation as below.

$$k = k_0 \exp^{\frac{-E_a}{RT}} \quad \text{.....(8.3)}$$

Where $k_o\ (s^{-1})$ is the Arrhenius constant and (E_a) *(J/mol)* is the activation energy, which may be calculated from the slope and the intercept of the straight line of the plot of $\ln k$ *vs* $1/T$ (where T is the average treatment temperature). Arrhenius constant and the activation energy were calculated at different electric field intensities.

8.1.1.1 Results and Discussion

The values of the inactivation rate constant of the E coli suspended in SMUF of (50% w/w) and in SMUF of (66.6% w/w) at different electric field intensities and inlet temperatures after one pass through the PEF unit were calculated, according to equation (8.2) using the experimental results (presented in Chapter 7).

Plots of the rate constant against the average temperature of the microbial suspension at different electric field intensities are shown in (Fig. 8.2). Higher rate constants were obtained at higher electric field intensities and higher processing temperatures, suggesting that both the electric field intensity and the treatment temperature enhance PEF treatment.

The values of inactivation rate constant were also calculated for the thermal treatment presented in Chapter 7, and the results are plotted in (Fig. 8.3).

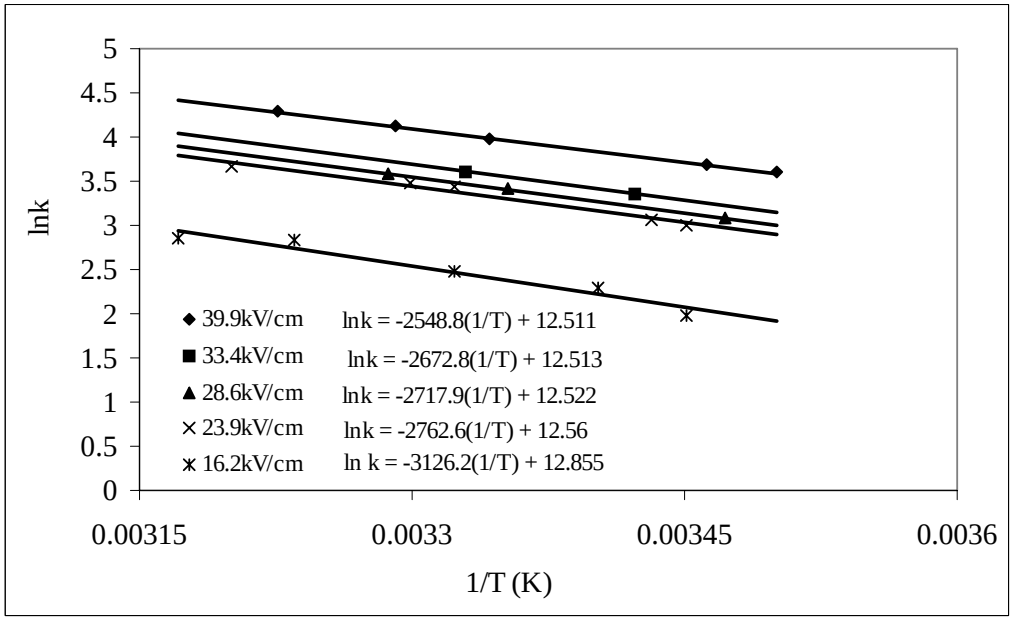

Fig. 8.2: The relation between the reaction constant and the average temperature for E coli suspended in SMUF (50% w/w) when subjected to electric field intensity of (43, 28.6, 23.9 and 16.2kV/cm) and in SMUF (66.6%w/w) when subjected to electric field intensity of (33.4kV/cm).

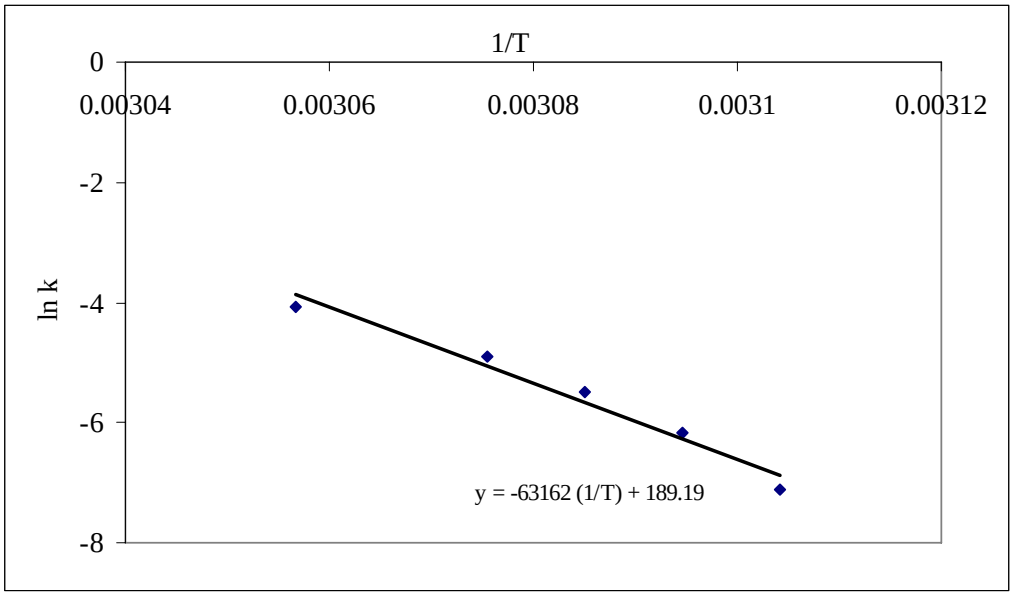

Fig. 8.3: The relation between the reaction constant and the average temperature according to the thermal treatment of E coli suspended in SMUF (50% w/w).

The values of Arrhenius constant and activation energy of microbial inactivation by PEF treatment were calculated from the intercept and the slope of the straight lines in Fig. (8.2). Lower processing temperatures were required when higher voltages were applied. The values of the activation energy were lower at higher electric field intensities as shown in (Fig. 8.4). The results also show that Arrhenius constant is also a function of the electric field intensity as shown in (Fig. 8.5).

The following correlation was developed for the inactivation rate constant as a function of electric field and temperature.

$$k = \left(aE^3 + bE^2 + cE + 1276747.2\right) \times EXP\left[-\left(dE^3 + eE^2 + fE + 53214.8\right)/RT\right]$$
.....(8.4)

Where, E is the electric field intensity, *a=-26.4, b=2707, c=-91006.6, d=-0.87, e=84.9 and f=2795.6.*

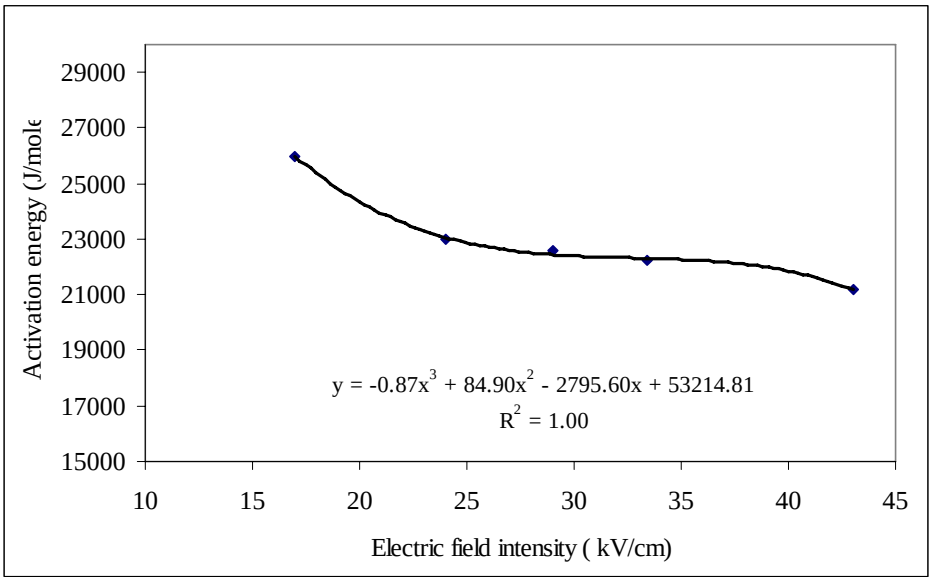

Fig. 8.4: Effect of the electric field intensity on the activation energy.

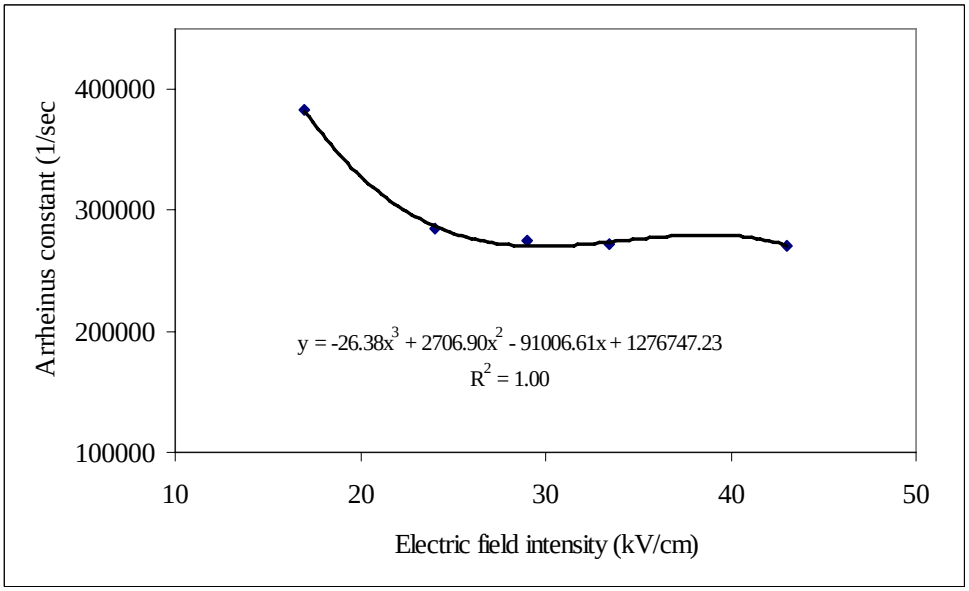

Fig. 8.5: Effect of the electric field intensity on Arrhenius constant.

In the area of food processing and preservation, first order kinetic is often described by the Thermal Death Time Model (TDT) (Bigelow, 1921), a concept that has ordinate from thermo bacteriology. In this model the decimal reduction time *D* (min) is defined as the time required for reducing initial count by 90%. Decimal reduction time is directly related to the rate constant as follows:

$$D = \frac{2.303}{k} \qquad (8.5)$$

D values for thermal and PEF treatment of E coli suspended in SMUF were calculated from our experimental results, and compared with the D values of E coli in skim milk based on high pressure processing (Liton, McClements & Patterson (2001) and Gervilla, Mor-Mur, Ferragut & Guamis (1999a)). As shown in Table (8.1), it is clear that the D values for PEF pasteurisation are significantly

lower than those of thermal and high pressure treatments. The D values calculated from our experimental results were calculated based on the residence time in the treatment chamber, and would be even smaller if calculated based on the treatment time.

Table (8.1): Microbiological inactivation kinetics using thermal, PEF and high pressure treatments.

Treatment method	*pressure (Mpa)*	*Electric field intensity (kV/cm)*	*Temp. (^{0}C)*	*D value (min)*	*Microorganism*	*Suspension*
Thermal pasteurisation	*0*	*0*	*48*	*47.5*	*E coli*	*SMUF (50%w/w)*
			49	*18.3*		
			50	*9.3*		
			51	*5.2*		
			53	*2.2*		
PEF treatment	*0*	*43*	*20*	*0.0008*	*E coli*	*SMUF (50%w/w)*
			20			
		23.9	*20*	*0.0014*		
		16.2		*0.0034*		
High pressure	*600*	*0*	*20*	*3.7-4.8*	*E coli*	*Skim milk*
	200		*25*	*9.5*	*E coli*	*Ewe's milk (6% fat)*
	250		*25*	*6.4*		
	300		*25*	*5.2*		

The values of the inactivation rate constant were calculated at different flow rates of the suspension medium as illustrated in (Fig. 8.6). Although the outlet temperature decreased when higher flow rate was used, our results higher microbial inactivation was achieved at higher flow rates and turbulent flow. The microbial inactivation was higher due to the better mixing caused by turbulent flow.

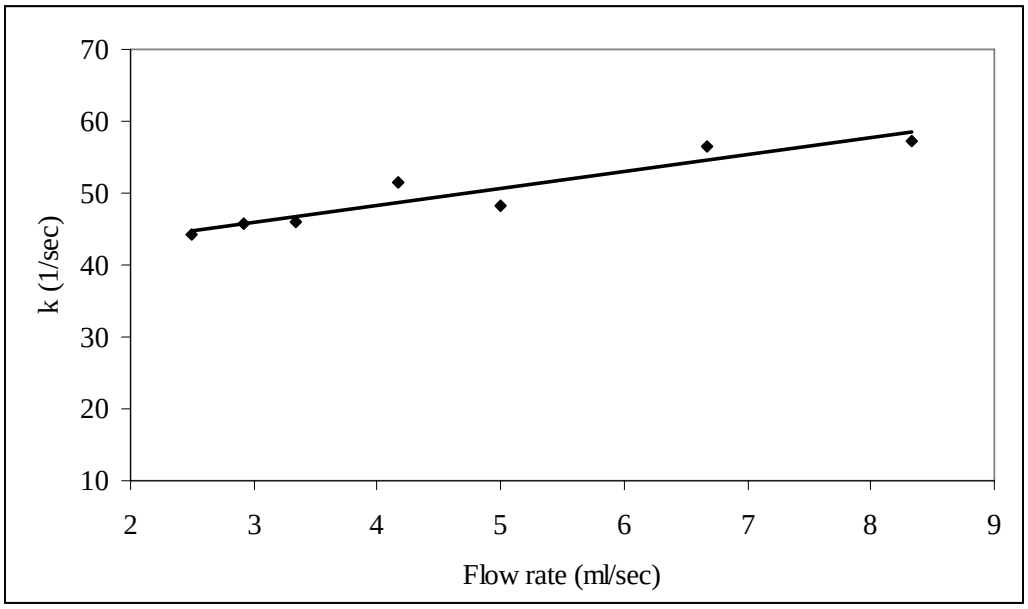

Fig. (8.6): The effect of the microbial suspension flow rate on the inactivation rate constant. Electric filed intensity was 43kV/cm, 190 Hz and 20°C inlet temperature.

8.1.2 Recirculation Operation

The liquid food was circulated during PEF treatment for specific time to achieve desirable microbial inactivation. This has been done to avoid building series of PEF units needed to achieve sufficient microbial inactivation. A systematic diagram of recirculation operation is illustrated in Fig. (8.7). Inactivation rate of E coli was measured during the PEF treatment at different times. Sample from the feed tank and from the outlet stream of the last PEF chamber were drawn at different time and tested for microbial load at different treatment times.

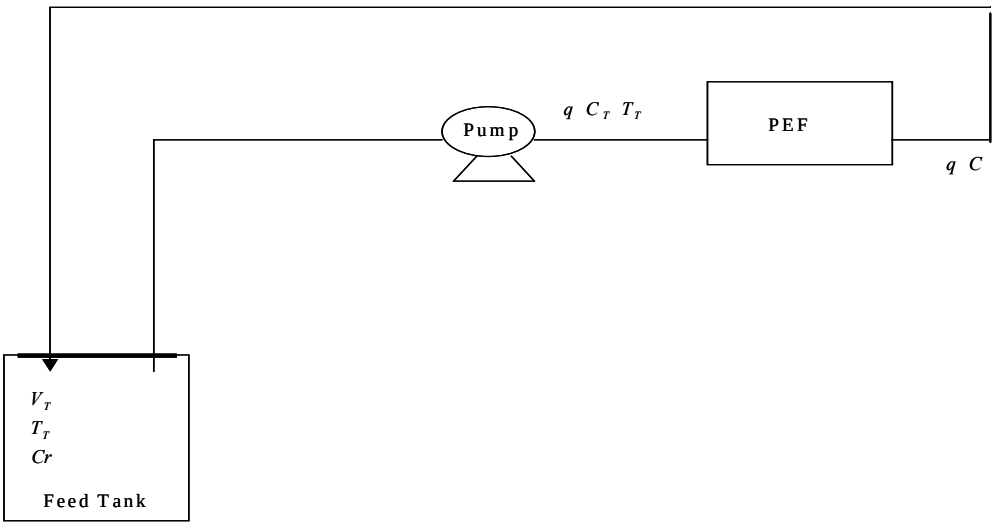

Fig. 8.7: The PEF recirculation application.

Microorganisms concentration in the feed tank and in the inlet of the PEF chamber are assumed equal and the rate of microorganisms inactivation (r) is assumed first order with respect to its concentration (C). Concentration of microorganisms in the inlet and outlet of the PEF chamber varies with time. Assuming perfect mixing and the absence of pockets of contamination within the recirculation loop, the mass balance around the recirculation PEF operation may be written as follows:

$$qC_r + k'C_T V_T = qC_T + V_T \frac{dC_T}{dt}$$

Where k' is the growth rate constant for E coli at the condition of tank. The temperature in the tank was kept below the growth temperature of the micro organism under study, thus the growth term (second term on the left hand side) was assumed negligible.

Substituting for C_r from equation (8.2):

$$q\left[C_T e^{(-kV_r/q)}\right] - qC_T = V_T \frac{dC_T}{dt}$$

This maybe arranged as follows:

$$\int_{C_{T0}}^{C_T} \frac{V_T dC_T}{qC_T\left[e^{(-kV_r/q)} - 1\right]} = \int_0^t dt$$

Integrating the equation gives:

$$\ln \frac{C_T}{C_{T0}} = \frac{q\left[e^{(-kV_r/q)} - 1\right] t}{V_T}$$

or

$$C_T = C_{T0} \exp\left\{-q/V_T\left(1 - \exp\left[-kV_r/q\right]\right) t\right\} \text{.....(8.6)}$$

Where V_r/q and V_T/q are the residence times in PEF and feed tank respectively and t is the total time (recirculation time).

According to equation (8.6) and the values of the inactivation rate constant calculated from equation (8.4), the variation of E coli concentration with time can be predicted theoretically at different process parameters.

8.1.2.1 Results and Discussion

1. Microbial concentration in the inlet and outlet of the PEF chamber

The change in the microbial count in the feed tank and in the stream leaving the PEF unit is shown in (Fig. 8.8), (Fig. 8.9) and (Fig. 8.10) when SMUF

(100%, 66.66% and 50% w/w) is subjected to electric field intensity of 28.6, 33.4 and 28.6kV/cm respectively. Results show that microbial inactivation in the stream leaving the PEF unit higher by at least 1 Log than the microbial inactivation in the feed tank. And while this stream entered the feed tank (recirculation), it was mixed with the liquid in the feed tank where the microbial load was higher. The recirculation of the suspension may cause some microbial contamination within the PEF system, which could slightly affect the treatment; the effect was not significant when compared to rate of killing (due to the first order kinetics). However, as bacterial count in the feed decreases, the degree of killing may become equal to the degree of contamination and hence the overall microbial reduction diminishes.

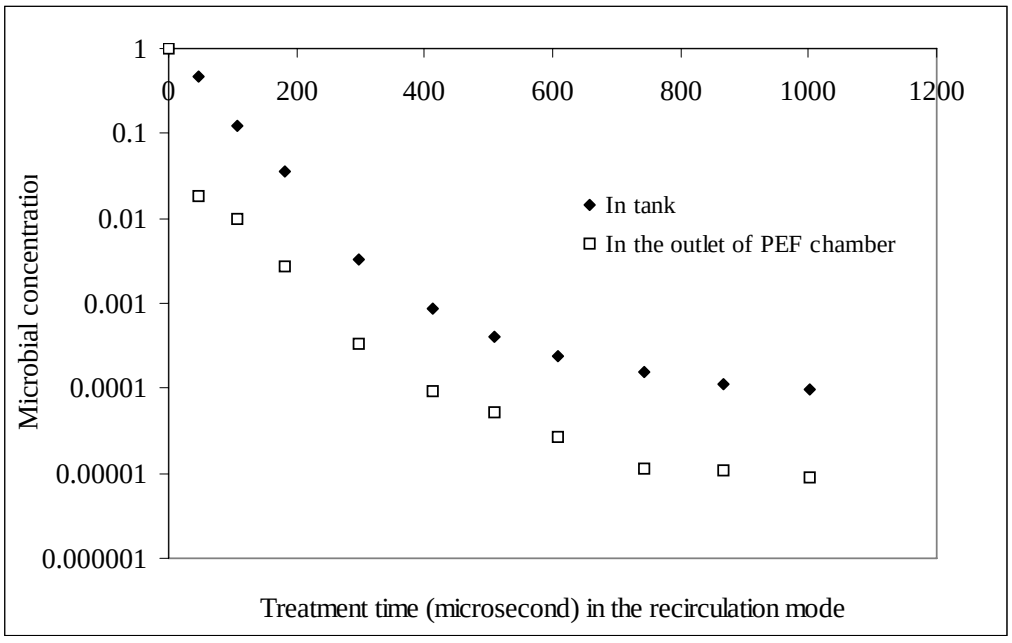

Fig. 8.8: The microbial load in the tank and in the outlet of PEF at different times, E coli suspended in SMUF (100% w/w) at flow rate of 2.5ml/sec and 10°C inlet temperature and subjected to electric field intensity of 28.6kV/cm.

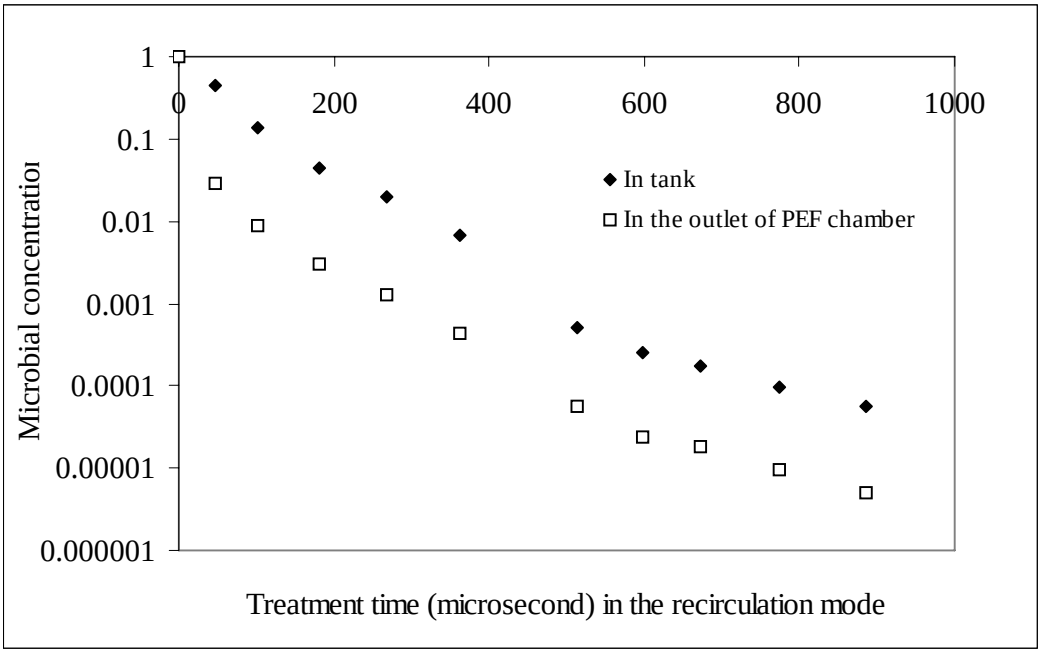

Fig. 8.9: The microbial load in the tank and in the outlet of PEF at different times, E coli suspended in SMUF (66.66% w/w) at flow rate of 2.5ml/sec and 10°C inlet temperature and subjected to electric field intensity of 33.4kV/cm.

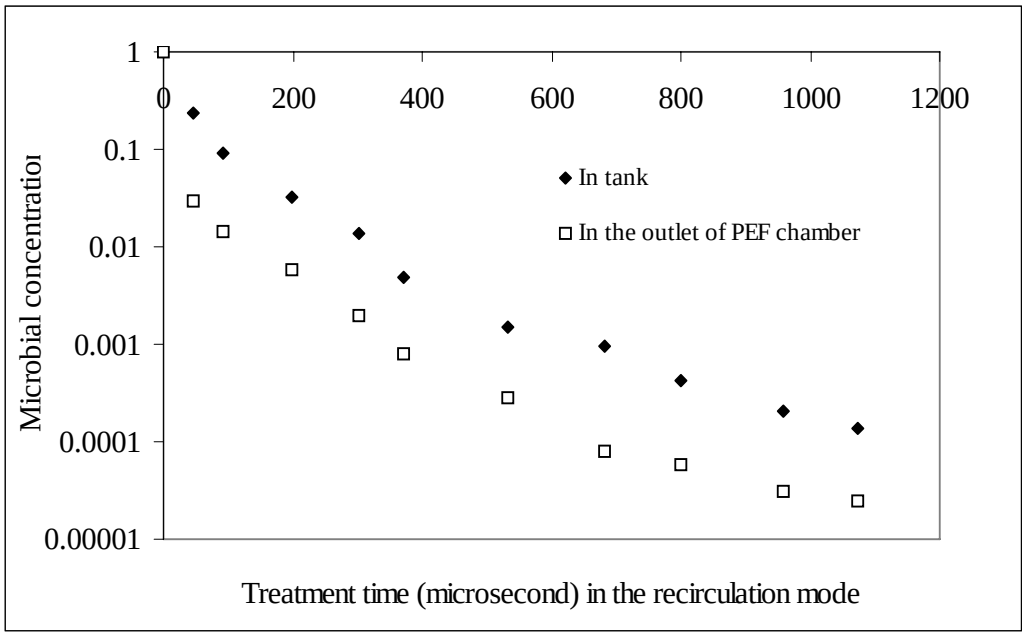

Fig. 8.10: The microbial load in the tank and in the outlet of PEF at different times, E coli suspended in SMUF (50% w/w) at flow rate of 2.5ml/sec and 10 °C inlet temperature and subjected to electric field intensity of 28.6kV/cm.

2. The inactivation rate constant using recirculation operation and comparison between theoretical and experimental results

The values of the inactivation rate constant of E coli suspended in SMUF (100, 66.66 and 50% w/w) at different process parameters were calculated according to the developed model presented in equation (8.4). Comparison between theoretical and experimental results of the change in E coli concentration after PEF treatment was obtained according to the estimated values of the inactivation rate constant and to equation (8.6).

Good agreement between the experimental results and values predicted by the model at high microbial inactivation. However, the experimental results deviate from the theoretical model at low microbial concentration as shown in (Fig. 8.11) to (Fig. 8.16) (the time in these figures is the total recirculation time). At low microbial concentration, the microbial growth can not be assumed negligible, which may have caused the tailing effect shown in these figures.

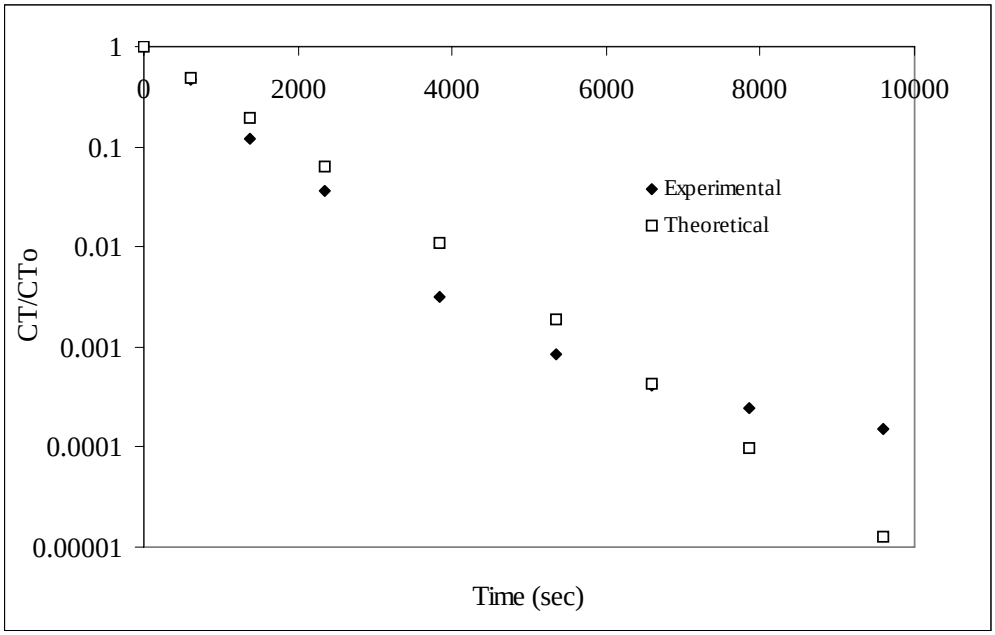

Fig. 8.11: Theoretical and experimental lines of the inactivation of E coli suspended in SMUF (100% w/w) at flow rate of 2.5ml/sec and inlet temperature of 10°C and subjected to square bipolar pulses at 28.6kV/cm.

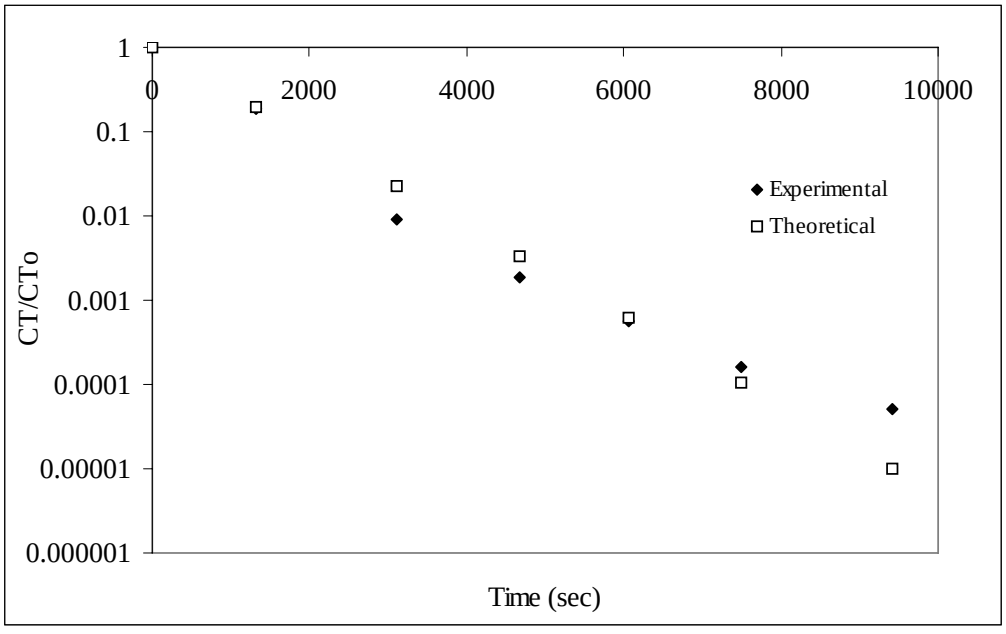

Fig. 8.12: Theoretical and experimental lines of the inactivation of E coli suspended in SMUF (100% w/w) at flow rate of 2.5ml/sec and inlet temperature of 10°C and subjected to square bipolar pulses at 33.4kV/cm.

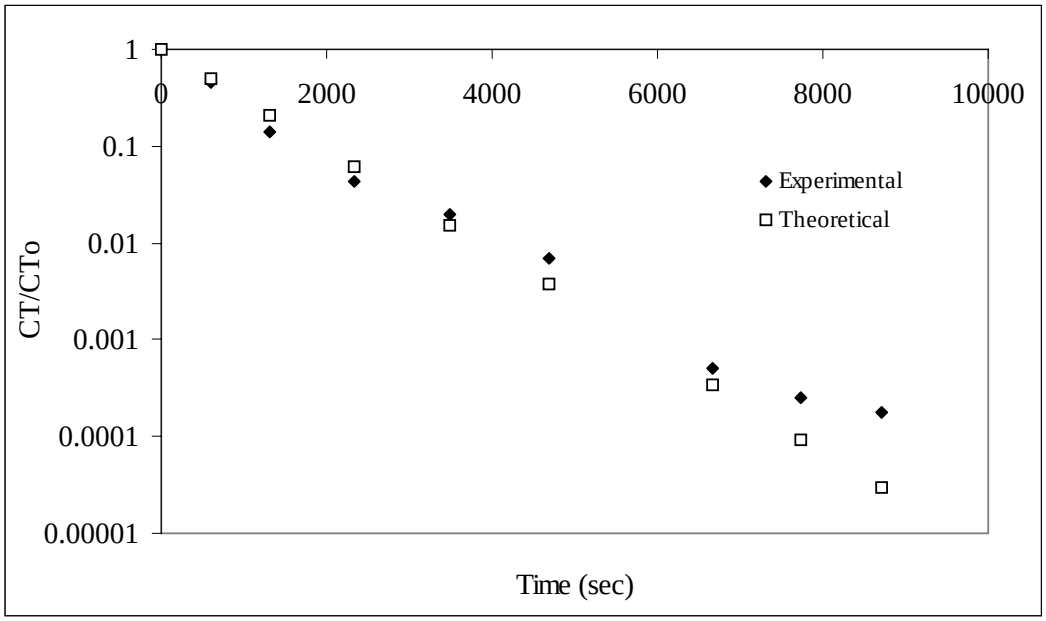

Fig. 8.13: Theoretical and experimental lines of the inactivation of E coli suspended in SMUF (66.6% w/w) at flow rate of 2.5ml/sec and inlet temperature of 10^{o}C and subjected to square bipolar pulses at 33.4kV/cm.

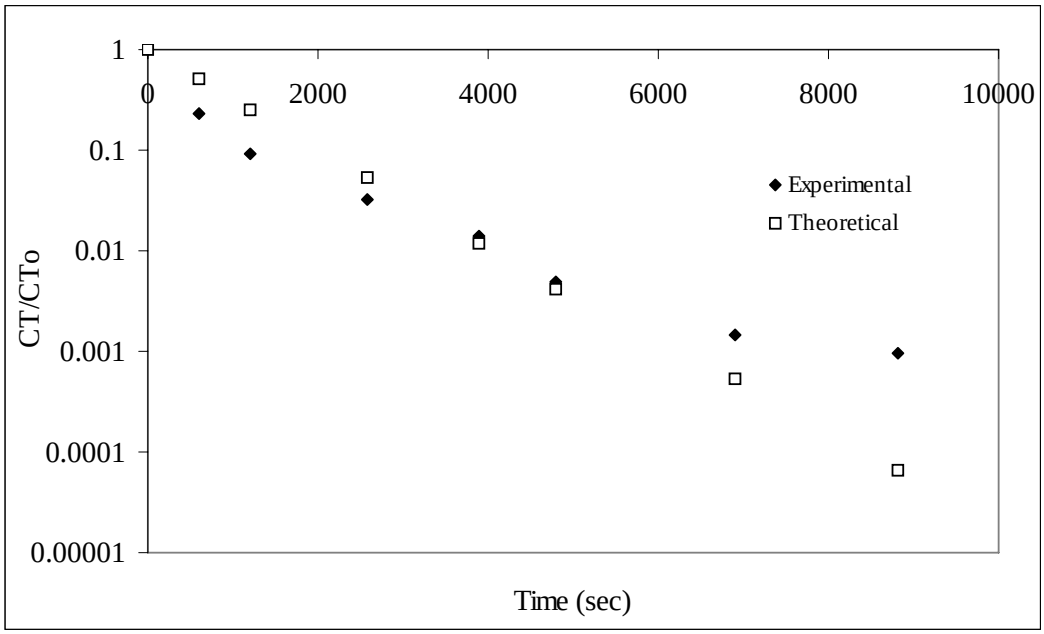

Fig. 8.14: Theoretical and experimental lines of the inactivation of E coli suspended in SMUF (50% w/w) at flow rate of 2.5ml/sec and inlet temperature of 10^{o9}C and subjected to square bipolar pulses at 28.6kVcm.

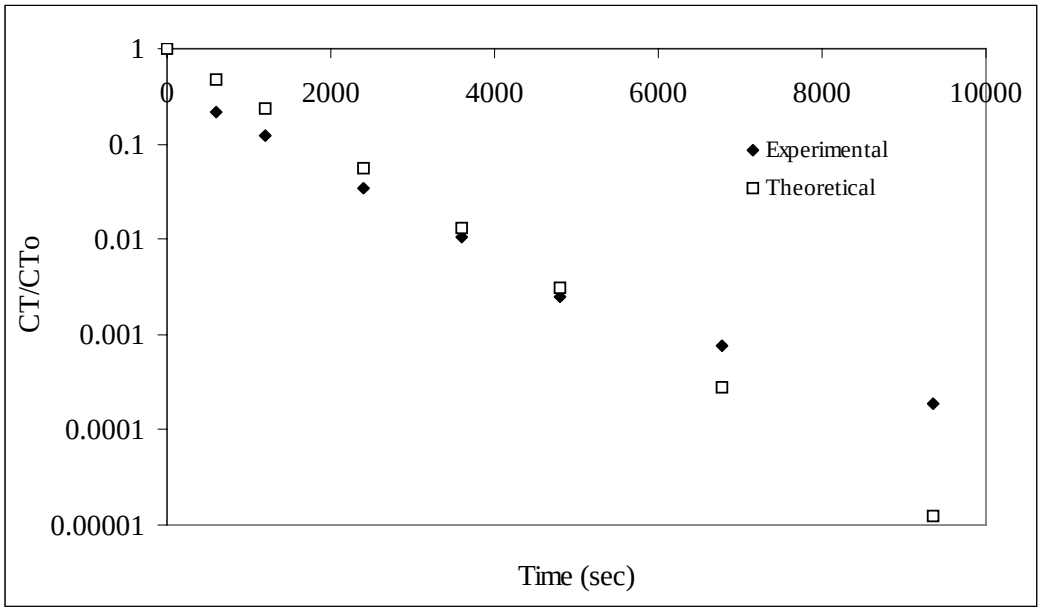

Fig. 8.15: Theoretical and experimental lines of the inactivation of E coli suspended in SMUF (50% w/w) at 20°C and subjected to square bipolar pulses at 28.6kVcm.

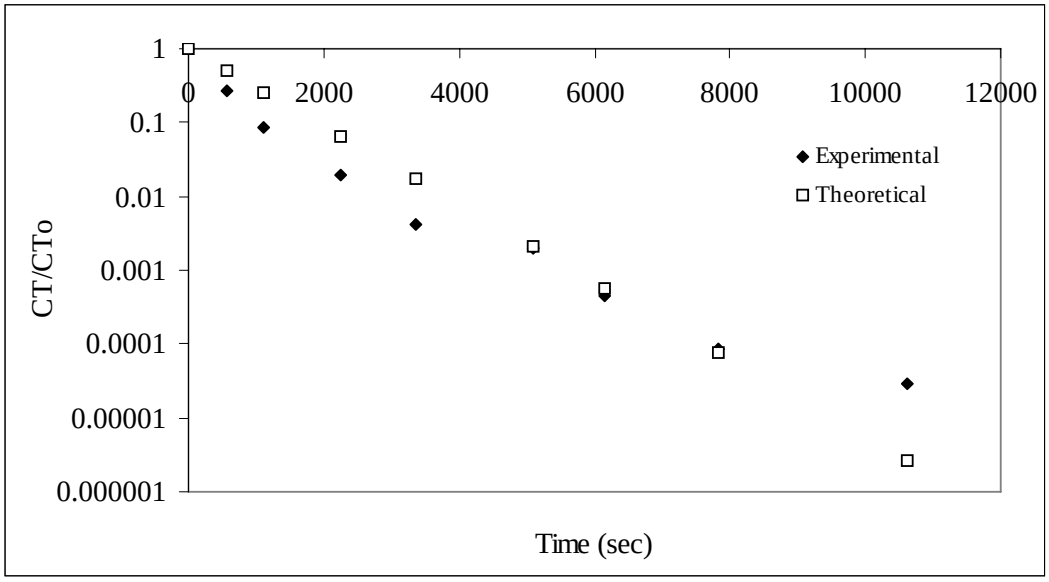

Fig. 8.16: Theoretical and experimental lines of the inactivation of E coli suspended in SMUF (50% w/w) at flow rate of 2.5ml/sec and inlet temperature of 25°C and subjected to square bipolar pulses at 28.6kVcm.

8.2 Conclusions

The inactivation rate constant of E coli suspended in SMUF of different concentrations and subjected to PEF treatment at different process parameters was found to increase with increasing the electric field intensity and treatment temperature. Both Arrhenius constant and activation energy were found to decrease with electric field intensity. A generalized correlation for the inactivation rate constant as a function of electric field intensity and treatment temperature was derived from the one-pass experiments.

A single model was used to describe the effect of solution recirculation based on mass balance of E coli. The kinetic model developed from the single pass measurements was used in the recirculation model. The experimental results and the values predicted by the recirculation model shows good agreement at high microbial concentration, with some deviation at low microbial concentrations.

Chapter Nine: Overall Discussions, Conclusions and Final Remarks

Non thermal inactivation of microorganisms in liquid food using PEF treatment has experienced considerable success. Applying pulsed electric field technology to the pasteurisation of milk poses a new challenge to the dairy industry. PEF technology provides consumers with microbiological safe, minimally processed, nutritious, fresh like foods and clearly provide enhanced inactivation at lower time/ temperature conditions than thermal processes.

The key elements to achieve high effectiveness of the PEF process include many factors. The main factors include the design of treatment chamber(s) (including the electrodes and insulator); design of the high voltage pulse generator (including the switches); cooling systems and data acquisition system and selecting proper process parameters and operating conditions.

The main conclusions derived from this work based on our experimental findings and the intensive literature review are summarised below.

Design of PEF Equipment

In this work, an innovative design of a PEF unit was presented. The unit includes multi-pass PEF treatment chambers, which were designed and constructed to provide uniform electric field distribution in the treatment zone leading to uniform treatment. The chambers were also designed to obtain high

electric field intensity in the treatment region, while maintaining minimum increase in liquid food temperature. Furthermore, using the intermediate cooling between chambers was necessary to maintain non thermal process.

Voltage loss outside the treatment region depends on the design and dimensions of the treatment chamber, which were determined based on our experimental investigations. The multi pass treatment chamber/s showed no problems of electrodes fouling, sparking or electrical breakdown during the treatment. The treatment chamber/s maintained the electric field below the dielectric strength of the fluid foods under the test conditions by removing the electrodes from the gap where the electric field intensity was concentrated. The electrodes surfaces used were smooth to minimize electron emission, and prevents sparking.

The high voltage pulse generator used in this work was designed using the modern Insulated Gate Bipolar Transistors *(IGBTs)* which considered the more convenient option for the PEF. The switches operates in pairs for opening and closing periodically to discharge the energy stored in the energy storage component, this results in the application of bipolar pulses across the treatment chamber. The bipolar pulses produced by this generator provides very fast pulse rise and fall times, so the power supplied was fully utilised for inactivate the microbes with less heating and hence increases the efficiency of the PEF treatment. Bipolar pulses are more lethal than monopolar pulses; the alternating changes in the movement of charged molecules cause a stress in the cell membrane and enhance its electric breakdown, and bipolar pulses offers

minimum energy utilization, reduced deposition of solids on the electrode surface, and decreased food electrolysis.

Degassing the liquid food was carried out prior to treatment to eliminate gas bubble formation. Liquid food was also pressurised to reduce electric breakdown when high electric fields are applied. The use of positive pressure also permits operation at temperatures above the atmospheric boiling point and prevent the liquid to evaporate locally.

Factors Affecting the PEF Process

The process parameters affecting microbial inactivation include the electric field intensity, frequency, treatment time, pulse wave shape and width and treatment temperature.

Microbial inactivation increased with the increase of electric field strength and/or pulse number. Processing temperature has also significant effect on microbial inactivation. Our investigations also showed that increasing the electric field intensity enhances microbial inactivation even when the dissipated power was kept constant, and this conforms the fact that microbial inactivation is due to electric field rather than electrical energy input.

Treatment time can be selected either by changing pulse frequency at constant flow rate or by changing the flow rate at constant frequency. Increasing the treatment time by any method increases microbial inactivation. However, at higher flow rates, the efficiency of the PEF process was higher. At higher flow

rates, better mixing of the treated liquid can be achieved, leading to more uniform inactivation.

Increasing the processing temperature of the microbial suspension was found to increase microbial inactivation, and this is due to the effect of heat on the cellular metabolism at temperatures greater than the growing temperature of the microorganism. In all our experiments the operating temperature was kept below the lethal temperature of the microorganism under investigation to insure that the inactivation was not due to thermal effects.

Treatment medium parameters can also affect microbial inactivation. At the same processing temperature, medium with the low electrical conductivity can increase inactivation. According to the literature, it was suggested that microbial inactivation is more difficult in foods that are rich in ionic species and that the addition of agents like ninsin enhances microbial inactivation. Other medium parameters which affect microbial inactivation are pH and water activity. Most microorganisms are more resistance to all kinds of treatment at their optimum pH for growth, and are more resistance at lower water activity.

Mechanism of Microbial Inactivation

There are several theories describes the mechanism of microbial inactivation using PEF technology. However, all the research done on this matter supports that membrane damage is the main factor causing microbial death. The exact membrane damage mechanism is not yet understood. Exposure of the microbial cells to electric field pulses was suggested to induce transmembrane potential of the microbial cell membrane, which leads to pores formation in the

cell membrane. Other researchers suggested that the pore formation is due to electroconformational changes in the lipid or protein molecules and others believes that it is a secondary effect of osmotic imbalances between the cell and the environment. It was also suggested that treatment conditions and kind of species and suspension affects microbial inactivation mechanisms. In this project, TEM micrographs obtained for E coli after PEF treatment clearly shows pores in the cell membrane and leakage of the intracellular material, leading to drawing of the cytoplasm away from the outer membrane.

Modelling of PEF

A generalized correlation for the inactivation rate constant as a function of electric field intensity and treatment temperature was derived from our experimental investigations.

Values of the inactivation rate constant calculated for the inactivation of E coli suspended in SMUF of different concentrations and subjected to PEF treatment (single-pass through) at different process parameters were used to estimate the constants of Arrhenius equation.

Kinetic analysis of the E coli inactivation using the PEF and thermal pasteurisation show that the values of Arrhenius constant and activation energy were increasing dramatically in absence of the electric field. According to literature, using high pressure treatment the activation energy was higher compared to PEF treatment.

Comparison between experimental and theoretical values of the variation of E coli concentration with time after treatment was discussed, taking into consideration the recirculation mode of the PEF treatment. Good agreement between the experimental results and the values predicted by the circulation model developed in this thesis. The experiments show tailing and hence significant deviation from the model at low microbial concentration. This model could be of interest for PEF design and applications.

PEF Future

PEF treatment technology shows a tremendous potential to replace thermal pasteurisation technology in some applications. Nevertheless, further research and further development of the technology is needed before it can be widely applicable in the food industry. More research is needed on different aspects dealing with quality, microbiological and nutritional characteristics of the food products as well as their processing conditions.

References

1) *Ade-Omowaye, B. I. O., and Angersbach, A. (2001). Impact of high intensity electric field pulses on cell permeabilisation and as pre-processing step in coconut processing. Innovative Food Science &Emerging Technologies, 1, 203-209.*

2) *Ade-Omowaye, B. I. O., Rastogi, N. K., Angersbach, A., and Knorr, D. (2003). Combined effects of pulsed electric field pre-treatment and partial osmotic dehydration on air drying behaviour of red bell pepper. Journal of Food Engineering, 89-92.*

3) *Alberts, J. E., and Wrolstad, R. E. (1979). Causative factors of color deterioration in strawberry preserves during processing and storage. Journal of Food Science 44(1), 75-78, 81.*

4) *Álvarez, Raso, J., Sala, F. J., and Condón, S. (2003). Inactivation of Yersinia enterocolitica by pulsed electric fields. Food Microbiology, 20(6), 691-700.*

5) *Alvarez, I., Pagan, R., Raso, J., and Condón, S. (2002). Environmental factors influencing the inactivation of Listeria monocytogenes by pulsed electric fields. Letters in Applied Microbiology, 36(6), 489-493.*

6) *Alvarez, I., Virto, J., Raso, J., and Condón, S. (2003). Comparing predicting models for the Escherichia coli inactivation by pulsed electric fields. Innovative Food Science & Emerging Technologies, 4, 195-202.*

7) *Ananta, E., Heinz, V., Schluter, O., and Knorr, D. (2001). Kinetic studies on high-pressure inactivation of Bacillus sterothermophilis spores suspended in food matrices. Innovative Food Science & Emerging Technologies, 2, 261-272.*

8) *Andrews, A. T., and Varley, J. (1994). "Biochemistry of milk products". The Royal Society of Chemistry. Cambrigde,UK.*

9) *Angersbach, A., Heinz, V., and Knorr, D. (2000). Effects of pulsed electric fields on cell membranes in real food systems. Innovative Food Science & Emerging Technologies, 1(2), 135-149.*

10) *Aronsson, K., Lindgren, M., Johansson, B. R., and Ronner, U. (2001a). Inactivation of microorganisms using pulsed electric fields: The influence of process parameters on Escherichia coli, Listeria innocua, Leuconostoc mesenteroides and Saccharomyces cerevisiae. Innovative Food Science &Emerging Technologies, 2, 41-54.*

11) *Aronsson, K., and Rönner, U. (2001). Influence of pH, water activity and temperature on the inactivation of Escherichia coli and Saccharomyces cerevisiae by pulsed electric fields. Innovative Food Science & Emerging Technologies, 2(2), 105-112.*

12) *Ayhan, Z., Yeom, H. W., Zhang, Q. H., and Min, D. B. (2001). Flavor, color, and vitamin C retention of pulsed electric field processed orange juice in different packaging materials. Journal of Agricultural and Food Chemistry, 49(2), 669-674.*

13) *Barbosa-Ca`novas, G. V., Gongora-Nieto, M. M., Pothakamury, U. R., and Swanson, B. G. (1999). "Preservation of foods with pulsed electric fields". Academic Press, USA.*

14) *Barbosa-Ca`novas, G. V., and Pothakamury, U. R. (1998). "Nonthermal preservation of foods". Marcel Dekker, Inc. New York.*

15) *Barbosa-Ca`novas, G. V., Qin, B.-L., Zhang, Q., G., O. R., Swanson, B. G., and Pedrow, P. D. (2000). Continuous flow electrical treatment of flowable food products. US Patent 6019031*

16) *Barbosa-Ca`novas, G. V., and Zhang, Q. H. (2001). "Pulsed electric fields in food processing", TECHNOMIC Publishing Company, Washington.*

17) *Barsotti, L., and Cheftel, J. C. (1999). Food processing by pulsed electric fields. 2 - Biological aspects. Sciences des Aliments, 19(1), 3-33.*

18) *Barsotti, L., Dumay, E., Mu, T. H., Diaz, M. D. F., and Cheftel, J. C. (2002). Effects of high voltage electric pulses on protein-based food constituents and structure. Trends in Food Science & Technology, 12, 136-144.*

19) *Barsotti, L., Merle, P., and Cheftel, J. C. (1999). Food processing by pulsed electric fields. I. Physical aspects. Food Reviews International, 15(2), 163-180.*

20) *Bartels, P. (2001). Definitions and guidelines for reporting on pulsed electrical field treatment. Workshop nonthermal processing, Konigin-Luise-Str. 22, D 14195 Berlin (Version 3.1).*

21) *Bartos, F. J. (2000). Medium voltage AC drives. Control Engineering Online, 20 (http://www.controleng.com/archives/2000/ct10201.00/000203.htm).*

22) *Bazhal, M. I., Lebovka, N. I., and E.Vorobiev. (2001). Pulsed electric field treatment of apple tissue during compression for juice extraction. Journal of Food Engineering, 50, 129-139.*

23) *Bendicho, S., Barbosa-Cánovas, G. V., and Martín, O. (2002). Review: Milk processing by high intensity pulsed electric fields. Trends in Food Science & Technology, 13(6-7), 195-204.*

24) *Bendicho, S., Espachs, A., Arantegui, J., and Martin, O. (2002a). Effect of High Intensity Pulsed Electric Fields and Heat Treatments on Vitamins of Milk. Journal of Dairy Research, 69(1), 113-123.*

25) *Bendicho, S., Estela, C., Fernandez-Molina, J., Barbosa-Ca`novas, G. V., and Martin, O. (2002b). Effect of high intensity pulsed electric field and thermal treatments on a Lipase from Pseudomonas fluorescens. Journal of Dairy Science, 85, 19-27.*

26) *Benz, R., and Zimmermann, U. (1980). Pulse-length dependence of the electric breakdown in lipid bilayer membranes. Biochemica et Biophysica Acta, 597, 637-642.*

27) *Bigelow, W. (1921). The logarithmic nature of thermal death time curves. Journal of Infectious Diseases, 29, 528-536.*

28) *Braddock, R. J. (1999). Single strength orange juices and concentate. In "Handbook of Citrus By-products and Processing Technology". Wiley, New York, 53-83.*

29) *Bruhn, R. E., Pedrow, P. D., and Olsen, R. G. (1998). Heat conduction in microbes exposed to pulsed electric fields. IEEE Transactions on Dielectrics and Electrical Insulation, Montreal, Quebec, Canada, 5, 878-885.*

30) *Bruhn, R. E., Pedrow, P. D., Olsen, R. G., and Swanson, B. G. (1996). Space charge near microbes during pulsed electric field pasteurization of liquid foods. IEEE International Symposium on Electrical Insulation, Montreal, Quebec, Canada, 858-861.*

31) *Buchholz, K. D., and Pawliszyn, J. (1994). Optimization of solid-phase microextraction conditions for determination of phenols. Analytical Chemistry, 66, 160-167.*

32) *Burhn, R., Pedrow, P. D., Olsen, R. G., Barbosa-Ca`novas, G. V., and Swanson, B. G. (1997). Electrical environmental surrounding microbes exposed to pulsed electric fields. IEEE Transactions on Dielectric and Electrical Insulation, 4, 806-812.*

33) *Bushnell, A. H., Clark, R. W., Dunn, J., and Lloyd, S. (1995). Prevention of electrochemical and electrophoretic effects in high-strength-electric-field pumpable-food-product treatment systems. US Patent5447733.*

34) *Bushnell, A. H., Clark, R. W., Dunn, J., and Samuel, L. (1996). Process for reducing levels of microorganisms in pumpable food products using a high pulsed voltage system. US Patent 5514391.*

35) *Bushnell, A. H., Dunn, J. E., and Pearlman, J. S. (1993). High pulsed voltage systems for extending the shelf life of pumpable food products. US Patent 5235905.*

36) *Busnell, A., Clark, R., Dunn, J., and w., L. S. (1996). Process for reducing levels of microorganisms in pumpable food products using a high pulsed voltage system. US Patent 5514391.*

37) *Butz, P., and Tauscher, B. (2002). Emerging technologies: chemical aspects. Food Research International, 35(2-3), 279-284.*

38) *Calderon-Miranda, M. L., Barbosa-Ca`novas, G. V., and Swanson, B. G. (1999). Inactivation of Listeria innocua in skim milk by pulsed electric fields and nisin. International Journal of Food Microbiology, 51, 19-30.*

39) *Calderon-Miranda, M. L., Barbosa-Ca`novas, G. V., and Swanson, B. G. (1999). Transmission electron microscopy of Listeria innocua treated by pulsed electric fields and nisin in skimmed milk. International Journal of Food Microbiology, 51, 31-38.*

40) *Calderon-Miranda, M. L., Raso, J., Gongora-Nieto, M., and Barbosa-Ca`novas, G. V. (1999). "Minimal Methods of Processing". 1456-1462.*

41) *Castro, A. J. (1994). Pulsed electric field modification of activity and denaturation of alkaline phosphatase. Ph.D., Washington State University, Pullman, WA.*

42) *Castro, A. J., Barbosa-Ca`novas, G. V., and Swanson, B. G. (1993). Microbial inactivation of foods by pulsed electric fields. Journal of Food Processing & Preservation, 17, 47-73.*

43) *Chang, D. C. (1989). Cell poration and cell fusion using an oscillating electric field. Biophysics Journal, 56, 641-652.*

44) *Chen, W., and Lee, R. (1994). Altered ion channel conductance and ionic selectivity induced by large imposed membrane potential pulse. Biophysics Journal, 67, 603-612.*

45) *Chernomordik, L. V., Sukharev, S. I., Popov, S. V., Pastushenko, V. F., Sokirko, A. V., Abidor, I. G., and Chizmadzhez, Y. A. (1987). The electrical breakdown of cell and lipid membranes: The similarity of phenonmenologies.*

46) *Corlett, D. A., and Brown, M. H. (1980). pH and acidity." Microbial ecology of foods". I. C. o. M. S. f. Foods., ed., Academic Press, New York.*

47) *Coster, H. G. L., and Zimmermann, U. (1975). The mechanism of electrical breakdown in the membranes of Valonia utricularis. Journal of Membrane Biology, 22, 73-90.*

48) *Damar, S., Bozoglu, F., Hizal, M., and Bayindirli, A. (2002). Inactivation and injury of Escherichia coli O15: H7 and Staphylococcus aureus by pulsed electric fields. World Journal of Microbiology & Biotechnology, 18(1), 1-6.*

49) *De HAAN, S. W. H., and Willcock, P. R. (2002). Comparison of the energy performance of pulse generation circuits for PEF. Innovative Food Science & Emerging Technologies, 3, 349-356.*

50) *Dejong, P., and Vanheesch, E. J. M. (1999). Pulsed electric field treatment system. US Patent 2001013467.*

51) *De-jong, P., and Van-heesch, E. J. M. (1998). Review - effect of pulsed electric fields on the quality of food products. Milchwissenschaft-Milk Science International, 53(1), 4-8.*

52) *Diamond, D. and Hanratty V. (1997). "Spreadsheet applications in chemistry using Microsoft excel". John Wiley & Sons, Inc.*

53) *Dimtrov, D. S. (1984). Electric field induced breakdown of lipid layers and cell membranes: A thin viscoelastic model. Journal of Membrane Biology, 78, 53-60.*

54) *Dimtrov, D. S., and Jain, R. K. (1984). Membrane stability. Biochemica et Biophysica Acta, 779, 437-468.*

55) *Doevenspeck, H. (1960). Verfahren and vorrichtung zur gewinnung der einzeknen phasen nus dispersen systemen. German Patent 1237541.*

56) *Dunn, J. (2001). Pulsed electric field processing: An overview. In "Pulsed Electric Fields in Food Processing". G. V. Barbosa-Ca`novas and Q. H. Zhang, eds., TECHNOMIC publication, USA.*

57) *Dunn, J. E., and Pearlman, J. S. (1987). Methods and apparatus for extending the shelf life of fluid food products. US Patent 4695472.*

58) *Durance, T. D. (1997). Improving canned food quality with variable retort temperature processes. Trends in Food Science & Technology, 8, 113-118.*

59) *Dutreux, N., Notermans, S., Gongora-Nieto, M. M., Barbosa-Ca`novas, G. V., and Swanson, B. G. (2000). Effects of combined exposure of Micrococcus Luteus to nisin and pulsed electric fields. International Journal of Food Microbiology, 60, 147-152.*

60) *Dutreux, N., Notermans, S., Wijtzes, T., Gongora-Nieto, M. M., Barbosa-Ca`novas, G. V., and Swanson, B. G. (2000). Pulsed electric fields inactivation of attached and free-living Escherichia coli and Listeria innocua under several conditions. International Journal of Food Microbiology, 54(1-2), 91-98.*

61) *Dutreux, N., Notermans, S., Wijtzes, T., Gongora-Nieto, M. M., Barbosa-Ca`novas, G. V., and Swanson, B. G. (2000a). Pulsed electric fields inactivation of attached and free-living Escherichia coli and Listeria innocua under several conditions. International Journal of Food Microbiology, 54(1-2), 91-98.*

62) *Efremov, N. M., Adamiak, B. Y., and Blochin, S. J. (2000). Experimental investigation of the action of pulsed electrical discharges in liquids on biological objects. IEEE Transactions on Plasma Science, 28, (1), 224-229.*

63) *Esplugas, S., Pangan, R., Barbosa-canovas, G. V., & Swanson, B. G. (2001). In "Pulsed electric fields in food processing", TECHNOMIC Publishing Company, Washington.*

64) *Evrendilek, G. A., Z. T. Jin, K. T. R., Qiu, X., Zhang, Q. H., and Richte, E. R. (2000). Microbial safety and shelf-life of apple juice and cider processed by bench and pilot scale PEF systems. Innovative Food Science and Emerging Technologies, 1(1), 77-86.*

65) *Evrendilek, G. A., and Zhang, Q. H. (2005). Effects of pulse polarity and pulse delaying time on pulsed electric fields-induced pasteurisation of E. coli O157:H7. Journal of Food Engineering, 68(2), 271-276.*

66) *Evrendilek, G. A., Zhang, Q. H., and Richter, E. R. (1999). Inactivation of Escherichia coli O157:H7 and Escherichia coli 8739 in apple juice by pulsed electric fields. Journal of Food Protection, 62(7), 793-796.*

67) *Evrendilek, G. A., Zhang, Q. H., and Richter, E. R. (2004). Application of pulsed electric fields to skim milk inoculated with Staphylococcus aureus. Biosystems Engineering, 87(2), 137-144.*

68) *Fiala, A., Wouters, P., Bosch, E. v. d., and L.M.Creyghton, Y. (2001). Coupled electrical-fluid model of pulsed electric field treatment in a model food system. Innovative Food Science & Emerging Technologies, 2, 229-238.*

69) *Fiala, A., Wouters, P. C., Bosch, E. v. d., and Creyghton, Y. L. M. (2001). Coupled electrical fluid model of pulsed electric field treatment in a model food system. Innovative Food Science & Emerging Technologies, 2, 229-238.*

70) *Fox, P. F. (1991). "Food Enzymology", Elsevier Applied Science, New York.*

71) *Fuleki, T., and Francis, F. J. (1968). Quantitative methods for anthocyanins. 3. Purification of cranberry anthocyanin. Journal of Food Science, 33, 266-274.*

72) *García, D., Gómez, N., Raso, J., and Pagán, R. (2005). Bacterial resistance after pulsed electric fields depending on the treatment medium pH. Innovative Food Science & Emerging Technologies. Article in press.*

73) *Gaskova, D., Sigler, K., Janderova, B., and Plasek, J. (1996). Effect of high voltage electric pulses on yeast cells: Factors influencing the killing efficiency. Bioelectrochem. Bioenergetics, 39, 195-202.*

74) *Gervilla, R., Mor-Mur,M., Ferragut, V., and Guamis, B. (1999a). Kinetics of destruction of Escherichia coli and Pseudomonas fluorescens inoculated in ewe's milk by high hydrostatic pressure. Journal of Dairy Science, 82, 1099-1107.*

75) *Giner, J., Gimeno, V., Espachs, A., Elez, P., Barbosa-Cánovas, G. V., and Martín, O. (2000). Inhibition of tomato (Licopersicon esculentum Mill.) pectin methylesterase by pulsed electric fields. Innovative Food Science and Emerging Technologies, 1(1), 57-67.*

76) *Góngora-Nietoa, M. M., Pedrow, P. D., Swanson, B. G., and Barbosa-Ca`novas, G. V. (2003). Impact of air bubbles in a dielectric liquid when subjected to high field strenghts. Innovative Food Science & Emerging Technologies, 4, 57-67.*

77) *Góngora-Nietoa, M. M., Younce, F., Hyde, G., Pedrow, P. D., Swanson, B. G., and Barbosa-Ca`novas, G. V. (2002). Metrology system for pulsed electric fields processing. Innovative Food Science & Emerging Technologies, 3, 337-348.*

78) *Góngora-Nietoa, M. M., Pedrowb, P. D., Swansonc, B. G., and Barbosa-Cánovas, G. V. (2004). Use of circuit analysis simulations in pulsed electric fields food processing. Journal of Food Engineering, 61(3), 413-420.*

79) *Grabtree, K. T., and Hinsdill, R. D. (1974). "Fundamental experiments in microbiology". Press of W. B. Saunders Company, USA.*

80) *Graham, R., (1993). "Data analysis for the chemical sciences". VCH publisher, Inc.*

81) *Grahl, T., and Markl, H. (1996). Killing of microorganisms by pulsed electric fields. Applied Microbiology and Biotechnology, 45, 148-157.*

82) *Grahl, T., Sitzmann, W., and Markl, H. (1992). Killing of microorganisms in fluid media by high voltage pulses. DECHEMA Biotechnol. Conference Series, 5(5B), 675-678.*

83) *Gudmundsson, M., and Hafsteinsson, H. (2001). Effect of electric pulses on microstructure of muscle foods and roes. Trends in Food Science & Technology, 12, 122-128.*

84) *Hamilton, W. A., and Sale, A. J. H. (1967). Effects of high electric fields on microorganisms II. Mechanism of action of the lethal effect. Biochemica et Biophysica Acta, 148, 789-800.*

85) *Hann, S. W. H. d. (2002). Comparison of the energy performance of pulse generation circuits for PEF. Innovative Food Science & Emerging Technologies, 3, 349-356.*

86) *Harrison, S. L., Barbosa-Ca`novas, G. V., and Swanson, B. G. (1997). Saccharomyces cerevisiae Structural Changes Induced by Pulsed Electric Field Treatment. Academic Press Limited, 30, 236-240.*

87) *Hayakawa, S., and Nakai, S. (1985). Relationships of hydrophobicity and net charge to the solubility of milk and soy proteins. Journal of Food Science, 50, 486-491.*

88) *Hayat, M. A. (1989). "Principles and techniques of electron microscopy". 3rd edition, CRC Press, Boca Raton, Florida.*

89) *Heinz, V., Alvarez, I., Angersbach, A., and Knorr, D. (2001). Preservation of liquid foods by high intensity pulsed electric fields-basic concepts for process design. Trends in Food Science & Technology, 12(3-4), 103-111.*

90) *Heinz, V., and Knorr, D. (2000). Effect of pH, ethanol addition and high hydrostatic pressure on the inactivation of Bacillus subtilis by pulsed electric fields. Innovative Food Science & Emerging Technologies, 1(2), 151-159.*

91) *Hero, D. H. S. W., Abraham, F. J., Franc, M. P. H., and Francis, V. D. B. H. (2001). Apparatus and method for preserving food products in a pulsed electric field. EP Patent 1123662.*

92) *Ho, S., and Mittal, G. (1996). Electroporation of cell membranes: A review. Critical Reviews in Biotechnology, 16(4), 349-362.*

93) *Ho, S., and Mittal, G. S. (2000). High voltage pulsed electrical field for liquid food pasteurization. Food Reviews International, 16(4), 395-434.*

94) *Ho, S. Y. (1997). Effects of high field electric pulses on the activity of selected enzymes. Journal of Food Engineering, 31, 69-84.*

95) *Ho, S. Y., Mittal, G. S., Cross, J. D., and Griffiths, M. W. (1995). Inactivation of Psedomonas Fluorescens by High Voltage Electric Pulses. Journal of Food Science, 60(6), 1337-1343.*

96) *Hofmann, G. A. (1989). Cells in electric fields, physical and practical electronic aspects of electro cell fusion and electroporation. In "Electroporation and electrofusion in cell biology". E. Neuman, A. E. Sowers, and C. A. Jordan, eds., Plenum Press, NY.*

97) *Holman, J. P. (1986). "Heat Transfer". McGraw-Hill, Inc., USA.*

98) *Hulsheger, H., and Niemann, E. G. (1980). Lethal effect of high voltage pulses on E. coli K12. Radiation and Environmental Biophysics, 18, 281-288.*

99) *Hulsheger, H., and Niemann, E. G. (1980). Killing of bacteria with electric pulses of high field strength. Radiation and Environmental Biophysics, 18, 281-288.*

100) *Hulsheger, H., Potel, J., and Niemann, E. G. (1983). Electric field effects on bacteria and yeast cells. Radiation and Environmental Biophysics, 22, 149-162.*

101) *Ingenieure, V. D., Verfahrenstechnik, V.-G., and (GVC), C. (1993). VDI Heat Atlas, VDI-Verlag GmbH, Dusseldorf, Germany.*

102)Iu, J., Mittal, G. S., and Griffiths, M. W. (2001). Reduction in levels of Escherichia Coli O157:H7 in apple cider by pulsed electric fields. Journal of Food Protection, 64(7), 964-969.

103)Jacob, H. E., Forster, W., and Berg, H. (1981). Micrological implication of electric field effects II. Inactivation of yeast cells and repair of their cell envelope. Zeitschrift Fur Allgemeine Mikrobiologie, 21(3), 225-233.

104)Jayaram, S., and Castle, G. S. P. (1992). Kinetics of sterilization of Lactobacillus brevis cells by the application of high voltage pulses. Biotechnology and Bioengineering, 40, 1412-1420.

105)Jayaram, S., Castle, G. S. P., and Margaritis, A. (1993). The effects of high field DC pulse and liquid medium conductivity on survivability of Lactobacillus brevis. Applied Microbiology and Biotechnology, 40, 117-122.

106)Jeantet, R., Baron, F., Nau, F., Roignant, M., and Brule, G. (1999). High intensity pulsed electric fields applied to egg white: Effect of Salmonella enteritidis inactivation and protein denaturation. Journal of Food Protection, 62(12), 1381-1386.

107)Jeyamkondan, S., Jayas, D. S., and Holley, R. A. (1999). Pulsed electric field processing of foods: A review. Journal of Food Protection, 62(9), 1088-1096.

108)Jia, M., Zhang, Q. H., and Min, D. B. (1999). Pulsed electric field Processing effects on flavor compounds and microorganisms of orange juice. Food Chemistry, 65, 445-451.

109)Jin, Z. T. (1999). Pulsed electric field inactivation of microorganisms and preservation of quality of cranberry juice. Journal of Food Processing Preservation, 23, 481-497.

110)Keith, W. D., Harris, L. J., Hudson, L., and Griffiths, M. W. (1997). Pulsed electric fields as a processing alternative for microbial reduction in spice. Food Research International, 30(3/4), 185-191.

111)Kekez, M. M., Savic, P., and Johnson, F. B. (1996). Contribution to the biophysics of the lethal effects of electric field on microorganisms. Biochemica et Biophysica Acta, 1278, 79-88.

112)Kinosita, K. J., and Tsong, T. Y. (1977). Voltage induced pore formation and hemolysis of human erythrocytes. Biochem. Biophys. Acta, 471, 227-242.

113)Knorr, D., and Angersbach, A. (1998). Impact of high-intensity electric field pulses on plant membrane permeabilization. Trends in Food Science & Technology, 9, 185-191.

114)Knorr, D., Angersbach, A., Eshtiaghi, M. N., Heinz, V., and Lee, D.-U. (2001). Processing concepts based on high intensity electric field pulses. Trends in Food Science & Technology, 12, 129-135.

115)Knorr, D., Geulen, M., Grahl, T., and Sitzman, W. (1994). Food application of high electric field pulses. Trans in Food Science &Technology, 5, 71-75.

116)Knorr, D., and Heinz, V. (2001). Development of nonthermal methods for microbial control. Disinfection, sterilization, and preservation. Block, S. S., ed., Lippincott Williams & Wilkins, Philadelphia, 853-877.

117)Krasuchi, Z. (1968). Breakdown of commercial liquid and liquid-solid dielectrics. In "High Voltage Technology". Alston, L. ed, Oxford University Press, London.

118)Lebovka, N. I., Bazhal, M. I., and E.Vorobiev. (2000). Simulation and experimental investigation of food material breakage using pulsed electric field treatment. Journal of Food Engineering, 44, 213-223.

119)Lebovka, N. I., Bazhal, M. I., and Vorobiev, E. (2001). Pulsed electric field breakage of cellular tissues: visualisation of percolative properties. Innovative Food Science & Emerging Technologies, 2, (2), 113-125.

120)Levenspiel, O. (1972). "Chemical reaction engineering", John Wiley & Sons, Inc.

121)Lewis, T. J. (1968). The electric conduction and strength of pure liquids. In "High voltage Technology". Alston, L. L. ed., Oxford University Press, London.

122)Lide, D. R. (1994). "Hand Book of Chemistry and Physics (CRC)". CRC Press, Inc., USA.

123)Lindgren, M., Aronsson, K., Galt, S., and Ohlsson, T. (2002). Simulation of the temperature increase in pulsed electric field (PEF) continuous flow treatment chambers. Innovative Food Science & Emerging Technologies, 3, 233-245.

124)Liton, M., McClements, J., and Patterson, M. (2001). Inactivation of pathogenic Escherichia coli in skim milk using high hydrostatic pressure. Innovative Food Science and Emerging Technologies, 2, 99-104.

125)Liu, X., Yousef, A. E., and Chismm, G. W. (1997). Combination of organic acids and pulsed electric fields. Journal of Food Safety, 16, 287-299.

126)Loey, A. V., Verachtert, B., and Hendrickx, M. (2002). Effects of high electric field pulses on enzymes. Trends in Food Science & Technology, 12, 94-102.

127)Mabrook, M. F., and Petty, M. C. (2003). Effect of composition on the electrical conductance of milk. Journal of Food Engineering, 60, (3), 321-325.

128)MacGregor, S. J. (2000). Inactivation of pathogenic and spoilage microorganisms in a test liquid using pulsed electric fields. IEEE Transactions on Plasma Science, New York, 28(1), 144.

129)Manas, P., Barsotti, L., and Cheftel, J. C. (2001). Microbial inactivation by pulsed electric fields in a batch treatment chamber: effects of some electrical parameters and food constituents. Innovative Food Science & Emerging Technologies, 2, 239-249.

130)Mackinney, G. and Chichester, C.O. (1952). Color deterioration in strawberry preserves. Canner, 114(12), 13.

131)Markakis, P. (1982). Anthocyanins as food additives. Ch. 9, In "Anthocyanins as food Colors". Markakis, P. ed., pp. 245-253, Academic press, New York.

132)Marquez, V. O. (1997). Destruction and inhibition of bacterial spores by high voltage pulsed electric field. Journal of Food Science, Chicago, 62(2), 399-403.

133)Marth, E. H., and Steele, J. L. (1998). "Applied Dairy Microbiology". Marcel Dekker, Inc, New York.

134)Martin, L., Aronsson, K., Galt, S., and Ohlsson, T. (2002). Simulation of the temperature increase in pulsed electric field (PEF) continuous flow treatment chambers. Innovative Food Science & Emerging Technologies, 3, 233-245.

135)Martin, O., Qin, B. L., Chang, F. J., Barbosa-Ca`novas, G. V., and Swanson, B. G. (1997). Inactivation of Escherichia coli in skim milk by high intensity pulsed electric fields. Journal of Food Process Engineering, 20(4), 317-336.

136)Martin, O., Zhang, Q., Castro, A. J., Barbosa-Ca`novas, G. V., and Swanson, B. G. (1994). Pulsed electric fields of high voltage to preserve foods. Microbiological and engineering aspects of the process. Spanish J. Food Science Technology, 34, 1-34.

137)Mastwijk, H. C., and Bartels, P. V. (2000). Integrated Modular Design of a Pulsed Electric Field Treatment Chamber. EP Patent 1000554.

138)Matsumoto Y., Satake, T., Shioji N., & Sakuma, A. (1991). Inactivation of microorganisms by pulsed electric field high voltage applications. Conf. Rec. of IEEE Industrial Applications Society Annual Meeting, 652-659.

139)Mazurek, B., and Lubicki, P. (1995). Effect of Short HV Pulses on Bacteria and Fungi. IEEE Transactions on Dielectric and Electrical Insulation, 2(No. 3), 418-424.

140)McDonald, C. J. (2000). Effects of pulsed electric fields on microorganisms in orange juice using electric field strengths of 30 and 50kV/cm. Journal of Food Science, Chicago, 65(6), 984.

141)Mermelstein, N. H. (1998). Interest in Pulsed Electric Field Processing Increases. Food Technology, 52(1).

142)Morren, J., Roodenburg, B., and Han, S. W. H. D. (2003). Electrochemical reactionsand electrode corrosion in pulsed electric field (PEF) treatment chambers. Innovative Food Science & Emerging Technologies, 4, (3), 285-295.

143)Morshuis, P. H. F., De Haan, S., Ferreira, J., and Van Den, B. (2001). Apparatus and method for preserving food products in a pulsed electric field. Patent number EP1123662.

144)Neumann, E., and Rosenheck, K. (1972). Permeability changes induced by electric impulses in vesicular membranes. Journal of Membrane Biology, 10, 279-290.

145)Ohshima, T., Hama, Y., and Sato, M. (2000). Releasing profiles of gene products from recombinant Eschericha coli in a high-voltage pulsed electric field. Biochemical Engineering Journal, 5, 149-155.

146)Ohshima, T., Okuyama, K., and Sato, M. (2002). Effect of culture temperature on high-voltage pulse sterilization of Escherichia coli. Journal of Electrostatics, 55(3-4), 227-235.

147)Ohshima, T., Sato, K., Terauchi, H., and Sato, M. (1997). Physical and chemical modifications of high voltage pulse sterilization. Journal of Electrostatics, 42, 159-166.

148)Oliveira, F. A. R., and Oliveira, J. C. (1999). "Processing foods, quality optimization and process assessment", CRC Press, USA.

149)Ortega-Rivas, E., Zarate-Rodriguez, E., and Barbosa-Ca`novas, G. V. (1998). Apple juice pasteurization using ultrafiltration and pulsed electric fields. Trans IChemE, 76(Part C), 193-198.

150)Pearce, L. E., Truong, H. T., Crawford, R. A., Yates, G. F., Cavaignac, S., and Lisle, G. W. (2001). Effect of turbulent-flow pasteurisation on survival of Mycobacterium avium Subp. Paratuberculosis Added to raw milk. Applied & Environmental Microbiology, 67(9), 3964-3969.

151)Peleg, 1995. A model of microbial survival after exposure to pulsed electric fields. Journal of Science Food Agriculture, 67, 93-99.

152)Perez, O. E., and Pilosof, A. M. R. (2004). Pulsed electric fields effects on the molecular structure and gelationof B-lactoglobulin concentrate and egg white. Food Research International, 37, 102-110.

153)Perry, J. H. (1963). "Chemical Engineers Hand Book", McGraw-Hill, Inc., USA.

154)Picart, L., Dumay, E., and Cheftel, J. C. (2002). Inactivation of Listeria innocua in dairy fluids by pulsed electric fields: influence of electric parameters and food composition. Innovative Food Science & Emerging Technologies, 3, 357-369.

155)Picart, L., Dumay, E., and Cheftel, J. C. (2002). Inactivation of Listeria innocua in dairy fluids by pulsed electric fields: influence of electric parameters and food composition. Innovative Food Science & Emerging Technologies, 3, 357-369.

156)Pol, I. E., Mastwijk, H. C., Slump, R. A., Popa, M. E., and Smid, E. J. (2001). Influence of food matrix on inactivation of Bacillus cereus by combinations of nisin, pulsed electric field treatment, and carvacrol. Journal of Food Protection, 64(7), 1012-1018.

157)Pothakamury, U. R., Barbosa-Ca`novas, G. V., Swanson, B. G., and Spence, K. D. (1997). Ultrastructural changes in staphylococcus aureus treated with pulsed electric fields. Food Science & Technology International, 3(2), 113-121.

158)Pothakamury, U. R., Barbosa-Ca`novas, G. V., Swanson, B. G., and Spence, K. D. (1997). Ultra structural changes in staphylococcus aureus treated with pulsed electric fields. Food Science & Technology International, 3(2), 113-121.

159)Pothakamury, U. R., Monsalvegonzalez, A., Barbosa-Ca`novas, G. V., and Swanson, B. G. (1995). High voltage pulsed electric field inactivation of

bacillus subtilis and lactobacillus delbrueckii. Revista Espanola de Ciencia y Tecnologia de Alimentos, 35(1), 101-107.

160)Pothakamury, U. R., Monsalvegonzalez, A., Barbosa-Ca`novas, G. V., and Swanson, B. G. (1995). Inactivation of Escherichia Coli and Staphylococcus Aureus in Model Foods by Pulsed Electric Field Technology. Food Research International, 28(2), 167-171.

161)Pothakamury, U. R., Vega, H., Zhang, Q. H., Barbosa-Ca`novas, G. V., and Swanson, B. G. (1996). Effect of growth stage and processing temperature on the inactivation of e-coli by pulsed electric fields. Journal of Food Protection, 59(11), 1167-1171.

162)Pothakamury, U. R., Vega Mercado, H., Zhang, Q. H., Barbosa-Ca`novas, G. V., and Swanson, B. G. (1996). Effect of growth stage and processing temperature on the inactivation of e-coli by pulsed electric fields. Journal of Food Protection, 59(11), 1167-1171.

163)Price, N. C., and Stevens, L. (1991). Enzymes: structure and function. In "Food enzymology", Elsevier Applied Science, New York, 1-25.

164)Qin, B., Zhang, Q., Barbosa-Ca`novas, G. V., Swanson, B. G., and Pedrow, P. D. (1995). Pulsed electric field treatment chamber design for liquid food pasteurization using a finite element method. Transactions of the ASAE, 38(2), 557-565.

165)Qin, B., Zhang, Q., Barbosa-Ca`novas, G. V., Swanson, B. G., and Pedrow, P. D. (1995a). Pulsed electric field treatment chamber design for liquid food

pasteurization using a finite element method. Transactions of the ASAE, 38(2), 557-565.

166)Qin, B.-L. (1995). Food pasteurization using high-intensity pulsed electric fields. Food Technology, 49, 55.

167)Qin, B.-L. (1998). Inactivating microorganisms using a pulsed electric field continuous treatment system. IEEE Transactions on Industry Applications [H.W. Wilson - AST], 34, 43.

168)Qin, B.-L., Pothakamury, U. R., Barbosa-Ca`novas, G. V., and Swanson, B. G. (1996). Nonthermal pasteurisation of liquid foods using high-intensity pulsed electric fields. Critical Reviews in Food Science and Nutrition, 36(6), 603-627.

169)Qin, B.-L., Zhang, Q., Barbosa-Ca`novas, G. V., Swanson, B. G., and Pedrow, P. D. (1994). Inactivation of microorganisms by pulsed electric fields of different voltage waveforms. IEEE Transactions on Dielectric and Electrical Insulation, 1(No. 6), 1047-1057.

170)Qiu, X., Sharma, S., Tuhela, L., Jia, M., and Zhang, Q. H. (1998). An integrated pef pilot plant for continuous nonthermal pasteurization of fresh orange juice. Transactions of the ASAE, 41(4), 1069-1074.

171)Qiu, X., and Zhang, Q. H. (2001). High Voltage Pulse Generator. US Patent 6214297

172)Raso, J., Alvarez, I., Condón, S., and Trepat, F. J. S. (2000). Predicting inactivation of Salmonella senftenberg by pulsed electric fields. Innovative Food Science and Emerging Technologies, 1(1), 21-29.

173)Raso, J., Calderon, M. L., Gongora, M., Barbosa-Ca`novas, G. V., and Swanson, B. G. (1998). Inactivation of Zygosaccharomyces Bailii in fruit juices by heat, high hydrostatic pressure and pulsed electric fields. Journal of Food Science, 63(6), 1042-1044.

174)Ravishankar, S., Fleischman, G., and Balasubramaniam, V. (2002). The inactivation of Escherichia coli O157: H7during pulsed electric field (PEF) treatment in a static chamber. Food Microbiology, 19(4), 351-361.

175)Reina, L. D., Jin, Z. T., Zhang, Q. H., and Yousef, A. E. (1998). Inactivation of listeria monocytogenes in milk by pulsed electric field. Journal of Food Protection, 61(9), 1203-1206.

176)Robbins, J. A. (2001). Process and apparatus for reduction of microorganisms in a conductive medium using low voltage pulsed electrical energy. US Patent 6331321.

177)Rodrigo, D. (2001). Study of inactivation of Lactobacillus plantarum in orange-carrot juice by means of pulsed electric fields: comparison of inactivation kinetics models. Journal of Food Protection, 64(2), 259-263.

178)Ross, A. I. V., Griffiths, M. W., Mittal, G. S., and Deeth, H. C. (2003). Review combining nonthermal technologies to control foodborne microorganisms. International Journal of Food Microbiology, 89, 125-138.

179)Rowan, N. J., MacGregor, S. J., Anderson, J. G., Cameron, D., and Farish, O. (2001). Inactivation of Mycobacterium paratuberculosis by pulsed electric fields. Applied & Environmental Microbiology, 67(6), 2833-2836.

180)Rowan, N. J., MacGregor, S. J., Anderson, J. G., Fouracre, R. A., and Farish, O. (2000). Pulsed Electric Field Inactivation of Diarrhoeagenic Bacillus Cereus Through Irreversible Electroporation. Letters in Applied Microbiology, 31, 110-114.

181)Ruhlman, K. T., Jin, Z. T., and Zhang, Q. H. (2001). Physical properties of liquid foods for pulsed electric field treatment. In "Pulsed Electric Fields in Food Processing". Barbosa-Ca`novas G. V. and Zhang, Q. H. eds., Technomic Publishing Company, Inc., USA.

182)Sale, A. J. H., and Hamilton, W. A. (1967). Effects of High Electric Fields on Microorganisms 1. Killing of Bacteria and Yeasts. Biochemica et Biophysica Acta, 148, 781-788.

183)Sale, A. J. H., and Hamilton, W. A. (1968). Effects of High Electric Fields on Microorganisms III. Lysis of Erythrocytes and Protoplasts. Biochemica et Biophysica Acta, 163, 37-43.

184)Schoenbach, K. H., Peterkin, F. E., Alden III, R. W., and Beebe, S. J. (1997). The effect of pulsed electric fields on biological cells: Experiments and applications. IEEE Transactions on Plasma Science, 25, 284-292.

185)*Schwarz, G. (1978). On the physicochemical basis of voltage-dependent molecular gating mechanisms in biological membranes. Journal of Membrane Biology, 43, 127-148.*

186)*Sensoy, I., Zhang, Q. H., and Sastry, S. K. (1997). Inactivation kinetics of Salmonella dublin by pulsed electric field. Journal of Food Process Engineering, 20(5), 367-381.*

187)*Sepulveda-Ahumada, D. R., Oritega-Rivas, E., and Barbosa-Ca`novas, G. V. (2000). Quality aspects of cheddar cheese obtained with milk pasteurized by pulsed electric fields. Trans IChemE, 78(Part C), 65-71.*

188)*Shah, R. K., and London, A. L. (1978). "Laminar Flow Forced Convection in Ducts". Academic Press, Inc., New York.*

189)*Sharma, S. K., Zhang, Q. H., and Chism, G. W. (1997). Development of a Protein Fortified Fruit Beverage and its Quality When Processed with Pulsed Electric Field Treatment. Journal of Food Quality, 21, 459-473.*

190)*Shaw, P. E. (1986). "The flavor of nonalcoholic fruit beverages. Food flavours. Part B. The flavour of beverages". Morton, E. D. and Macleod, A. J. eds., Elsevier, Amsterdam, The Netherlands, 337-368.*

191)*Simpson, R. K., Whittington, R., Earnshaw, R. G., and Russell, N. J. (1999). Pulsed High Electric causes 'all or nothing' membrane damage in Listeria monocytogenes and Salmonella typhimurium, but membrane H^+ -ATPase is not a primary target. International Journal of Food Microbiology, 48, 1-10.*

192)*Sitzmann, W. (1995). "High-voltage pulse techniques for food preservation". London.*

193)*Smith, K., Mittal, G., and Griffiths, M. (2002). Pasteurization of milk using pulsed electrical field and antimicrobials. Journal of Food Science, 67(6), 2304-2308.*

194)*Sowers, A. E. (1984). Characterization of electric field-induced fusion in Erythrocyte ghost membranes. The Journal of Cell Biology, 99, 1989-1996.*

195)*Sowers, A. E. (1986). A Long-lived fusogenic state is induced in Erythrocyte ghosts by electric pulses. The Journal of Cell Biology, 102, 1358-1362.*

196)*Spilimbergo, S., Bertuccoa, A., Lauro, F. M., and Bertolonib, G. (2003). Inactivation of Bacillus subtilis spores by supercritical CO2 treatment. Innovative Food Science & Emerging Technologies, 4(2), 161-165.*

197)*Stanley, D. W. (1991). Biological membrane deterioration and associated quality losses in food tissues. In "Critical reviews in food science and nutrition". Clydesdale F. M., ed., CRC Press, New York.*

198)*Sepulveda, D., R., Guerrero, J., A., and Barbosa-Cánovas, G.,V. (2006). Influence of electric current density on the bactericidal effectiveness of pulsed electric field treatments. Journal of Food Engineering, 76, (4), 656-663*

199)*Takayuki, O., Kanako, O., and Masayuki, S. (2002). Effect of culture temperature on high-voltage pulse sterilization of Escerichia coli. Journal of Electrostatics, 55, 227-235.*

200)Terebiznik, M., Jagus, R., Cerrutti, P., Huergo, M. d., and Pilosof, A. (2002). Inactivation of Escherichia coli by a combination of nisin pulsed electric fields, and water activity reduction by sodium chloride. Journal of Food Protection, 65(8), 1253-1258.

201)Terebiznik, M. R., Jagus, R. J., Cerrutti, P., de Huergo, M. S., and Pilosof, A. M. R. (2000). Combined effect of nisin and pulsed electric fields on the inactivation of Escherichia coli. Journal of Food Protection, 63(6), 741-746.

202)Tomlins, R. I. and Ordal, Z. J. (1976). Thermal injury and inactivation in vegetative bacteria. In "Inhibition and Inactivation of Vegetative Microbes". Skinner, F. A. & Hugo, W. B., eds., Academic Press, London, 153-90.

203)214. Tsong, T. Y. (1990). On electroporation of cell membranes and some related phenomena. Bioelectrochem. Bioenergetics, 24, 271-295.

204)Vega-Mercado, H., Gongora-Nieto, M. M., Barbosa-Ca`novas, G. V., and Swanson, B. G. (1999). Nonthermal Preservation of Liquid Foods Using Pulsed Electric Fields. In "Handbook of Food Preservation". Rahman M. S., ed., A Series of Monographs, Textbooks, and Reference Books.

205)Vega-Mercado, H., Luedecke, O., Hyde, G., and Barbosa-Ca`novas, G. V. (1996). HACCP and HAZOP for a Pulsed Electric Field Processing Operation. Dairy, Food and Enviromental Sanitation, 16(9), 554-560.

206)Vega-Mercado, H., Martinbelloso, O., Qin, B. L., Chang, F. J., Gongoranieto, M. M., Barbosa-Ca`novas, G. V., and Swanson, B. G. (1997). Non-thermal

food preservation - pulsed electric fields. Trends in Food Science & Technology, 8(5), 151-157.

207)Vega-Mercado, H., Pothakamury, Y. R., Chang, F.-J., Barbosa-Ca`novas, G. V., and Swanson, B. G. (1996). Inactivation of Escherichia coli by combining pH, ionic strength and pulsed electric fields hurdles. Food Research International, 29(2), 117-121.

208)Vega-Mercado, H., Powers, J. R., Barbosa-Ca`novas, G. V., and Swanson, B. G. (1995). Plasmin inactivation with pulsed electric fields. Journal of Food Science, 60, (5), 1143-1146.

209)Vega-Mercado, H., Powers, J. R., Barbosa-Ca`novas, G. V., and Swanson, B. G. (2001). Effect of added calcium and EDTA on the inactivation of a Protease from Pseudomonas fluorescens M3/6 when exposed to pulsed electric fields. In "Pulsed electric fields in food processing". Barbosa-Ca`novas, G. V. ed., TECHNOMIC publication, USA.

210)Villamiel, M., Vazquez, A., Morais, F., and Corzo, N. (2000). Chemical changes during high-temperature short-time treatment of milk in an industrial-scale plant. Milchwissenschaft-Milk Science International, 55(6), 320-322.

211)Walstra, P., Geurts, T., Noomem, A., Jellema, A., and van Boekel, M. (1999). Principles of milk-properties and processes. In "Dairy technology". New York: Basel Marcel Dekker, Inc.

212)Wightman, J. D., and Wrolstad, R. E. (1995). Anthocyanin analysis as a measure of glycosidase activity in enzymes for juice processing. Journal of Food Science, 60(4), 862-867.

213)Wouters, P. C., Alvarez, I., and Raso, J. (2001). Critical factors determining inactivation kinetics by pulsed electric field food processing. Trends in Food Science & Technology, 12.

214)Wouters, P. C., Dutreux, N., Smelt, J. P. P., and Lelieveld, H. M. (1999). Effects of pulsed electric fields on inactivation kinetics of Listeria innocua. Applied and Environmental Microbiology, 62(12), 5364-5371.

215)Wouters, P. C., and Smelt, J. P. P. M. (1997). Inactivation of microorganisms with pulsed electric fields: potential for food preservation. Food Biotechnology, 11(3), 193-229.

216)Yang, R. J., Li, S. Q., and Zhang, Q. H. (2004). Effects of pulsed electric fields on the activity of enzymes in aqueous solution. Food Chemistry and Toxicology, 69(4), 241-248.

217)Ye, A., Singh, H., Taylor, M., and Anema, S. (2002). Characterization of protein components of natural and heat-treated milk fat globule membranes. International Dairy Journal, 12(4), 393-402.

218)Yeom, H. W. (2000). Effects of pulsed electric fields on the activities of microorganisms and pectin methyl esterase in orange juice. Journal of Food Science, Chicago, 65, 1359.

219)Yeom, H. W., Streaker, C. B., Zhang, Q. H., and Min, D. B. (2000). Pulsed electric fields on the quality of orange juice and comparison with heat pasteurization. Journal of Agricultural and Food Chemistry, 48(10), 4597-4605.

220)Yeom, H. W., Zhang, Q. H., and Dunne, C. P. (1999). Inactivation of Papain by pulsed electric fields in a continuous system. Food Chemistry, 67, 53-59.

221)Yin, Y., Zhang, Q. H., and Sastry, S. K. (1997). High voltage pulsed electric field treatment chambers for the preservation of liquid food products. US Patent 5690978.

222)Zapata, J., Martinez, O., Salazar, B. C., Moreno, G., and F.Gallego. (2000). Inhibition of Escherichia coli growth by application of high intensity pulsed electric fields [Spanish]. Interciencia, 25(8), 391-397.

223)Zhang, Q., Barbosa-Ca`novas, G. V., and Swanson, B. G. (1995). Engineering aspects of pulsed electric field pasteurisation. Journal of Food Engineering, 25, 261-281.

224)Zhang, Q., Chang, F.-J., Barbosa-Ca`novas, G. V., and Swanson, B. G. (1994). Inactivation of microorganisms in a semisolid model food using high voltage pulsed electric fields. 27, 538-543.

225)Zhang, Q., Everndilek G. A. (2005). Effects of pulse polarity and pulse delaying time on pulsed electric fields-induced pasteurisation of E coli O157:H7. Journal of Food Engineering, 68, 2, 271-276.

226)Zhang, Q., and Monsalve-Gonzalez, A., Barbosa-Ca`novas, G. V., and Swanson, B. G. (1994a). Inactivation of E. coli and S. cerevisiae by pulsed electric fields under controlled temperature conditions. Transactions of the ASAE, 37, 581-587.

227)Zhang, Q., Monsalve-Gonzalez, A., Barbosa-Ca`novas, G. V., and Swanson, B. G. (1994b). Inactivation of Saccharomyces cerevisiae by square wave and exponential-decay pulsed electric fields. Journal of Food Process Engineering, 17, 469-478.

228)Zhang, Q. H., Qin, B. L., Barbosa-Ca`novas, G. V., and Swanson, B. G. (1995). Inactivation of e-coli for food pasteurization by high-strength pulsed electric fields. Journal of Food Processing & Preservation, 19(2), 103-118.

229)Zhang, Q. H., Qin, B. L., Barbosa-Ca`novas, G. V., and Swanson, B. G. (1995a). Inactivation of e-coli for food pasteurization by high-strength pulsed electric fields. Journal of Food Processing & Preservation, 19(2), 103-118.

230)Zimmermann, U. (1982). Electric Field-Mediated Fusion and Related Electrical Phenomena. Biochemica et Biophysica Acta, 694, 229-277.

231)Zimmermann, U. (1986). Electrical breakdown, electropermeabilisation and electrofusion. Rev. Phys. Biochem. Pharmacol., 105, 176-256.

232)Zimmermann, U., Pilwat, G., Beckers, F., and Riemann, F. (1976). Effect of external electrical fields on cell membranes. Biochemistry and Bioenergetics, 3, 58-83.

233)Zimmermann, U., Pilwat, G., and Riemann, F. (1974). Dielectric breakdown of cell membranes. Biophysical Journal, 14, 881-899.

234)Zimmermann, U., Pilwat, G., and Riemann, F. (1975). Preparation of erythrocyte ghosts by dielectric breakdown of the cell membrane. Biochemica et Biophysica Acta, 375, 209-219.

235)Zimmermann, U., Vienken, J., and Pilwat, G. (1980). Development of a drug carrier system: Electric field induced effects in cell membranes. Bioelectrochem. Bioenergetics, 7, 553-574.

236)Zuckerman, H., Krasik, Y. E., and Felsteiner, J. (2002). Inactivation of microorganisms using pulsed high-current underwater discharges. Innovative Food Science & Emerging Technologies, 3, 329-336.