

APPLICATION OF PULSE ELECTRIC FIELD TECHNIQUES IN FOOD PROCESSING

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 **ETARERI GEORGE EMUAKPEJE:APPLICATION OF PULSE ELECTRIC FIELD TECHNIQUES IN FOOD PROCESSING**

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I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

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 **ABSTRACT**

The main aim of this MSc research project is to explore the nature and various principles of the pulse electric field technique in food processing and other uses which maybe found during the cause of my research.

 Pulse electric field technique works on the principle of the application of short burst of electricity and kills micro-organisms without causing any detriment to the quality of the food product that is meant to be treated.However it is a technique which seems very easy to set up, simple and easy to use in the laboratory involved in the processing of liquid and semi liquid food products,and nonetheless seems to be very promising.Furthermore, the accurate findings was also the low cost attributed to the use of this technique,other methods which includes the use of chemicals,enzymes,food additives often end up with the contamination of the food product and finally detrimental to human health,it was further gathered that the shelf life of products obtained from pulse electric field technique is comparable to other ways like pasteurization,sterilization and sometimes often better.

 Literature survey was carried out to understand this technique better and it was however concluded that the technique is a non thermal type of application,that uses short burst of electricity to deactivate micro-organisms by a phenomenon called electroporation,electroporation a phenomenon which might not be fully understood but by definition it may be said to be a process which involves the use of electricity to kill micro-organisms by widening the already existing pores or creating new pores of this micro-organisms leading to the leakage of the cytoplasmic content and finally cell lysis or death occurs. Literature survey was carried out on other uses of pulse electric field processing and it was investigated that the technique found its importance in the treatment of waste water with final important advantage of producing no environmental bye products.

 İn this Msc research project the effect of different process parameters on the cellular membrane permeabilization of different micro-organisms in different liquid foods was investigated.Literature experiment was carried out on apple juice,green pea soup,eggs and orange juice and it was found out that the main process parameters determining the effectiveness of the treatment where electric field strength,number of pulses and wave shape ,temperature,and time,while other parameters like conductivity,ionic strength,pH,particle nature of food,type of micro-organism,concentration of micro-organism,and growth stage of the micro-organism where also not to be ignored.

 Finally,major food borne micro-organisms like *bacillus* *spp* seem to be a bigger fish to fry by this technique,and as such,has left a lot of questions unanswered,which points in the direction of further hurdle technology to give more effectiveness of the technique.

Key words:pulse electric field,electroporation,permeabilization,process parameters,hurdle technology.

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 **DEDICATION.**

This MSc research project is dedicated to my mother,Mrs Patience Nekpen Osagie,brothers Mr Darius Ochuko Emuakpeje and Jefferey Osaze Osagie,sisters Mrs Onome Vera Uvwo and Lisa Osarumen Osagie also to my good friends of Cyprus Near East University,Orchun Ufuk,Ibrahim Sayman,Gulcem Nurray,Chimezie Stanley Ezewanfor,Yousef Kaseem,Ifeanyi Walter Okonkwo,Lawrence Aniedo,Kelvin Aderhoro,Chidera Onah Barbara and Steven Thyrone Jnr, others are Melvin Oduah,Stevenson Osose Okojie,Randy Iwunze and Paul Amaye.

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 İ will also like to acknowledge other important contributors which are the students of the Cyprus Near East University Department of Food Engineering whose class contribution and interactions has made my stay at the university as an international student more pleasurable,relaxing and profitable.

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 **LIST OF ABBREVIATIONS AND SYMBOLS**

**NAFDAC...**NigerianNationalAgencyforFoodDrugAdminiatrationandControl

**USDA....**UnitedStatesDepartmentofAgriculture

**EFSA....**EuropeanFoodSafetyAuthority

**PEF....**PulseElectricField

**E.NUMBER....**EuropeanNumber

**Em....**CellRelativePermitivity

**Δϕ....**TransmembranePotential

**SIP....**Sterilizeinplace

**CIP....**Cleaninplace

**HACCP....**HazardAnalysisCriticalControlPoint

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**CHAPTER 1**

 **INTRODUCTION**

* 1. **Introduction and literature review**

Pulse electric field techniques popularly known as (PEF),is a technique that is currently widely researched by many developed countries around the world to combat the problem of microbial food contamination,food contaminants maybe defined as the non intentional additives normally found in foods.Food contamination which is a normal discussion in everyday scientific journal is a main product of natural or artificial ways of life,the main natural occurring toxicants are mainly of plant,animal or microbial and environmental sources,artificial ways of contamination may arise from addition of food additives,nature and kind of food processing equipments and so on,furthermore,based on the processing of food products so many different types of food contaminants may be formed and subsequently identified and this usually includes nitrosoamines,polycyclic aromatic hydrocarbons,3MCPD,furan,benzene,ethyl cabamate,histamine and many others,however for the purpose of this Msc research project there will be more emphasis on microbial contaminants as opposed to chemical contaminants.

 So many countries around the world including Australia,New Zealand,the United States,Canada and the European Union countries are fighting so hard to see that microbial contamination becomes extinct but this has become more of a thorn in the flesh.Despite all efforts made by the rich and influential countries around the world,investing on suitable ways of removing microbial contamination, most ways seems abortive and frustrating.So many old techniques used in food to inactivate micro-organisms include pasteurization,sterilization,hurdle technology,addition of chemicals,addition of enzymes,refrigeration,vacuum-packing,picking,drying,freezing,salting,sugar,smoking,lye, jellying,jugging and irradiation,modified atmosphere,burial in the ground,controlled use of micro-organisms and bio preservation.Recent scientific surveys has described this ways of inactivating microbial spores as old fashioned,ineffective or obsolete and as such most different strains of microbial spores can now stand against this type of preservation techniques

 Food borne diseases or food borne illness or sometimes called food poisoning can be described as any illness caused from the ingestion of already contaminated food, normally attributed to pathogenic bacteria,viruses,as well as fungus as the case of poisonous mushrooms,most important food borne bacteria pathogens are *Campylobacter* *jejuni* which has been implicated in secondary gullain barre syndrome and periodontitis,*Clostridium* *perfringens* also called the cafeteria germ,*Salmonella* *spp* which maybe consumed as a result of poultry foods or eggs not properly cooked,*Eschericia* *coli* which can be enterohemorragic and causes hemolytic uremic syndrome*.*Subsequently different countries around the world both developed and developing involved in the export and import of food products most expecially the export nations are working so hard to get rid of microbial contamination from food products,recently around the world countries like New Zealand which are major exporters of processed food products have seen major outbreaks as a result of food contaminants and subsequently there is a need to counter this microbial contaminants and then make more profits,the most recent outbreak of 2013 was a result of *clostridium* *botulinum* an anearobic gram positive bacillus micro organism which is the causative agent of the disease called botulism,the anearobic bateria inhibits the production of acetylcholine within the nervous system and the symtops of this disease may include muscle weakness,respiratory failure,some strains are implicated in hypotension,constipation,nausea and vomitting,dryness in the mouth and throat as well as difficulty in talking,it was however found that the products that seems properly processed by a company called Fronterra,the world biggest diary exporter and a New Zealand annual multi billion dollar company involved mainly in the manufacturing and export of dairy products was however found to be contaminated by this micro-organism,it was however further investigated that the pipes that was seldomly used was connected in the processing stage of the diary product,and normal sterilization technique was found inadequate to destroy the micro-organisms,furthermore,New Zealand a country whose economy depends largely on food produced and subsequently exported was however suspended briefly from exporting food products to asia and asia pacific countries this as a result caused a lot of losses to the company as well as New Zealand as a country,additionally although the New Zealand ministry of primary industries has investigated that this problem was false,that the food product was never contaminated,the receiving countries insisted that their own result where different and that the organism where found in every sample of diary product imported from New Zealand,similarly more than 20 Belgian companies has been banned from exporting food products to Russia even the Spanish cucumber company remains in ruins as a result of microbial contamination,different countries around the world also has banns on Nigeria food products especially countries within the Sub-Saharan Africa also as a result of microbial contamination,this is sure a great concern for many establishments and relevant government ministries and parastatals to reduce the problem of microbial contamination and if possible eliminate it completely,in Nigeria for example agencies like the food and drug administration and control(NAFDAC),are working so hard to see how microbial contamination can be reduced similarly the european food safety commission(EFSA) and the united states department of agriculture(USDA) are licensed agencies in europe and the united states respectively, that works on food contamination and as such are desperately in need of new methods of reducing food contamination.

 Nonetheless,addition of food additives to foods has been used by different food manufacturers over so many decades now and as such has seen so many disadvantages although they may be very easy to find and cheap to use the disadvantages seems to be more than the advatages in some rare cases.Food additives according to the European Commission maybe defined as "any substance which are not normally consumed or ingested as a food in itself and not normally or intentionally used as a characteristic ingredient of food whether or not it has a good nutritive value, the purposeful addition of which to food for a technological purpose in the processing, manufacture, treatment, preparation, packaging, handling,transport or storage of such food results, or may be reasonably thought to result, in it or its by-products becoming directly or indirectly a component of the related foods.A substantial and huge volume of many food additives maybe of natural origins or naturally occurring and some maybe even essential nutrients; it is the technical and skillful purpose that leads to these being classified as food additives and given an E number within the European Union countries,in Nigeria the E-number is replaced by the National Agency for Food and Drug Administration and Control(NAFDAC) number and stipulated within their own local lagal framework,although to be effective food control concerns could be national or international but usually contains same key components even though some differences may be found in different countries but to be effective they should be food legislation and regulation,policy and institutional frameworks,food monitoring and inspection,food service laboratories,dissemination and involvement of all stakeholders(Jane Omojukun et al 2011)additionally,the use of food additives which dates back in history despite modern day techniques is still seen as one of the best ways of preserving food products,food preservation was first identified when man saw the importance of resourcefulness,which started by salting and smoking of fish and meat,the Egyptians and the Romans where about the first set of people to identify the technique called preservation,the Egyptians used colourings and flavourings while the Romans used saltpetre(potassium nitrate),spices,colours for preservation and to improve the appearance of foods.Occasionally cooks normally use baking powder as a rising agent,thickners for sauces and gravies.

 Furthermore,for many years now the interest in food science and technology,food process engineering,chemical engineering has seen recent developments and the need to improve this food additives has become imperative,and therefore this food additives are now readily available in magarine as emulsifiers,in low calorie products as sweetners,and anti-oxidants which slow products from spoilage and rancidity,while maintaining freshness and taste.On the other hand recent development has also seen problems associated with this food additives and as such consumption of this food additives has been implicated in various forms of illnesses which may include hyperactivity,cancers,vomiting,stomach disorders to mention but a few,some of this food additives used in preservation of food products even though it is banned in some countries around the world some other countries still permit them sometimes in small amounts or including a label on food products to warn consumers so that they make a well and informed choice.

 Glycerol today is a common food additive used in the food industry,mostly as a humectant,sweetner,sometimes used as filler in preparation of low fat foods eg cookies, and most importantly to preserve foods,typically ,obtained from fat and oils,the food additive has not shown any main toxicity when used in humans but however this product can be confused with diethylene glycol which has the same physical attributes,different dubious food manufacturers around the world uses this particular product as a decoy as opposed to the main glycerine,the main difference between this two main products is toxicity,while diethylene glycol is toxic,glycerol is not toxic but sometimes very expensive to prepare.

 Recent outbreaks today in Nigeria has seen several episodes of this particular food additive mostly faked by food manufacturers to deceive consumers,making consumers believe they are using glycerol instead of this toxic food additive,the most recent outbreak of this food additive in Nigeria today was involved with a certain pharmaceutical company called Berewa pharmaceuticals that produces a teething mixture called “my pickin teething mixture”,my pickin which is a Nigerian vernacular or popularly known as pidgin english word means my baby in normal english,this mixture is made for babies to help them grow new set of teeth and finally put them to sleep while literally allow their first set of teeth to grow.Furthermore the mixture which was originally allowed to be sold in pharmaceutical shops around the country was implicated in the death of almost 84 Nigerian children and so many more hospitalized,the main symtops felt by the children at the time of occurrence in november 2008 was gastro intestinal symtops,nausea,vomiting,abdominal pain and diarrhea,others are mild hypotention or low blood pressure,coma and central nervous system depression as the first phase,the second phase may include acute kidney failure,pancreatitis,hypertention and so on,the last phase includes facial paralysis,dilated and non reactive pupils,coma and finally leading to death.Conversely several methods are possibly used to remove this additive from the body but they turn out to be so expensive causing more than $3000 dollars to treat a single patient as seen in the use of fomepizole a certain compound belonging to the azole family,but however each treatment seems to come with a minimal side and adverse effect.Other outbreaks includes episodes as seen in the United States in 1937 leading to the death of 105 people in 15 states,others are in Australia 1985 wine scandal leading to the bann of Australian wines in some part of the world,and the incidence in panama in 2006 where more than 116 death cases was recorded as a result of consumption of cough syrups,antihistamin tablets made at a Government hospital containing dyethylene glycol.

 Pasteurization,drying,salting,hurdle-technology,refrigeration and pickling are also important and common ways of preserving food stuffs but however this types of food preservation techniques also come with the lost of taste,flavour and sometimes nutritional value to food products,however increasing the temperature of normal food stuff in every day life for example cooking or warming of food already prepared although suitable for consumption,but definitely lacks the unique freshness of a newly prepared food and although not noticed labile vitamins and also important nutritional minerals are also lost in the process .Furthermore,likewise the use of pasteurization which is prominently used in the treatment of milk products as done in the New Zealand diary company Fronterra,which was in 2013 implicated in the outbreak of botulism caused by *clostridium* *botulinum*,pasteurization an age long technique which involves heating at 70 degree centigrade for 15 to 30 seconds will kill most strains of bacteria and micro-organisms present and cooled to 10 degrees centigrade to prevent new micro-organisms from growing, a simple technique formulated or discovered by a scientist called Louis Pasteur used in the laboratory especially food process science and technology companies involved in milk or diary production,a technique of which many recent research has shown many flaws which includes little loss of nutritional value,taste as a result of heating,colour as a result of heating,and also temperature resistant micro-organisms and their spores can still survive through it leading to many outbreaks of diseases around the world.

 Additionally, İt is because of all this draw backs in the use of additives in foods basically for preservation and deactivation of microbial spores,pasteurization,drying and salting that recent scientific research has been carried out in different government and private laboratories including top universities around the world to reduce microbial contamination and remove microbial spores from foods and therefore make food safer for human consumption that the top search for pulse electric field technique(PEF) in food processing was carried out and encouraged,although the technique dates back in history,but recent research for new ways of making food products safer has seen many promises in this technique and as such a lot of time and interest has been devoted into the technique to ensure its use commercially and wide acceptance around the world, by simple definition pulse electric field technique is a non thermal type of food sterilization that uses simple short burst of electricity to inactivate and kill micro-organism without causing any known detriment to the food quality such as taste,colour and flavour.

 Pulse electric field is generally believed to work on a principle called electroporation,electroporation a phenomenon not fully understood but to be effective it is said to widen the pores of bacteria cells or creating new ones leading to the leakage of the cytoplasmic content and finally the death of the cell.Cell membrane break down maybe reversible or irreversible depending on the nature of the cell and the mechanism of repair as well as the technique used to effect the breakdown, but for controlled or aided breakdown as seen in the case of food sterilization techniques it is mostly irreversible,therefore cell membrane breakdown may be defined as the irreversible breakdown or disintegration of a cell in order to cause cell lysis or death or maybe to increase the permeability of a cell as needed in various food industries,biotechnology,medicine and cellular molecular biology fields around the world.

 Furthermore, mass transfer processes which usually involves unit of operations including solid-liquid extraction,drying,stripping usually includes the disintegration of microbial cell membrane are widely used in the food industry,definitely when a cell membrane of a micro-organism is broken down cell lysis or death takes place leading to the death of the entire cell as a result of leakage of the cytoplasmic content,in this way food preservation and prolonged shelf life will be achieved as the cause of food spoilage or food degradation is usually the presence of undesired food micro-organisms

 Similarly,cell membranes of various micro-organisms are important backbones or agent involved with the protection of a cell cytoplasmic contents,breaking this barrier and leaking of the cytoplasmic contents into a neighboring medium are important ways of effecting mass transport processes which are important unit of operations in the food industry.

 Constantly,research has been on for several years to investigate the use of PEF in the deactivation of vegetative micro-organisms and some useful conclusions has been drawn so far including the use in various liquid food stuffs as seen in apple juice,milk,liquid eggs and pea soup (Barbosa-Cánovas et al.,1993); Zhang et al., (1994); (Barbosa-Cánovas et al., 1995); (Qin et al., 1995),the technique has in recent years found its importance in different industries apart from the food industry,for example in the careful and controlled modification of plant based food tissue,also,in the recovery and production of commercial high value metabolites for example sugar and natural pigments i.e anthocyanins from food (Fincan et al 2002),also is the part played in intensifying diffusion (Serobiev et al 2005),others are acceleration of mass transport in drying processes (Ade Omowaye et al 2001),biosynthesis of microbial metabolite of commercial interest(knorr et al 2001),decontamination of waste water (Castro et al 1993),bio fouling prevention in cooling water (Schoenbach et al 2000),infusion of solutes in foods (Barsotti et al 1999),pretreatment of milk for cheese making (Sepulveda-Ahumada et al 2000)

 Furthermore, based on all this important literature conclusions some different scientist has shown contributions especially in the case of the ability of PEF techniques to inhibit some unwanted or undesirable enzymes in foods,while some reports shows that the technique has the ability and efficacy to do so others says it is highly of no significance to enzyme activity (Hamilton and Sale 1967)

 Dielectric breakdown theory which was proposed many years ago by (Zimmerman et al 1974),is one of the principles which this particular technique is based on, the theory considers the cell membrane as a capacitor filled with dielectric materials of low permittivity where  *Em*=2,where Em is the cell relative permittivity, the corresponding values are lower inside than outside the cell,leading to destabilization of the cell in a phenomenon called transmembrane potential as a result of the electrochemical gradient,usually cell membranes maintains an electrical and chemical gradient within and outside of the cell,usually to be very efficient there is accummulation of negative charges inside the cell as opposed to positive charges outside the cell,of a 0.1V transmembrane potential resulting in a resting potential (Guyton et al 1986),when the required electric field E is applied,the negative ions move up the cell surface leading to accummulation of free charges at both sides of the membrane (Zimmermann et al 1976) leading to an increase in the potential difference at the surface of the cell membrane,the transmembrane potential which is defined as the difference between the intra and extra cellular potentials is then increased as a result of the applied electric field E.

 Δϕ is the transmembrane potential, M is the point of interest on the membrane, F is the shape factor, E is the applied electric field strength, *c r* is the cell radius, ϑ is the angle between the direction of E and M, t is the time after the field is turned on;  *Em* is the relative permittivity of the membrane evaluated, where σe , σ i and σ m are the electrical conductivities of the external medium,the cytoplasm and the cell membrane different mathematical models may be derived to determine the threshold value, which is the critical transmembrane potential *c* Δϕ,the critical transmembrane potential maybe defined as the sum of the induced potential and the resting potential which when reached or exceeded the breakdown of the cell membrane occurs or it may also be defined as the sum of the induced potential and the resting potential below which a cell membrane breakdown will not occur,furthermore with an equation that corresponds to  *c*Δϕ=1.5*Ec rc* ,where the shape factor F equals 1.5 assuming the cell is spherical and Ec stands for critical electric field strength,the the transmembrane potential may be calculated and therefore an understanding of the rate and time needed to effect cell membrane breakdown may be achieved.The cell diagram below shows a cell under the influence of an applied electric field.



 **Figure** **1**.**1.**Showing the schematic diagram of a cell exposed to electric field (Zimmermann 1986)

 Electroporation which maybe reversible or irreversible depending on the duration and number of pulses applied and the electric field strength (Zimmermann and Benz 1980) but to be effective and based on food industry application as it applies to this project the irreversible applications will be discussed in further details,invivo electroporation which has been used for many years has been used to increase the chemotherapy efficiency in cancer treatment and therefore called electrochemotherapy or popularly shortened as ECT (Miklavcic et al 1998). İt was however first shown by (Okino and Mohri 1987), that the phenomenon of electroporation can be used invivo to increase the concentration of the anti cancer agent in solid tumours 

**Figure** **1**.**2.**Reversible and irreversible breakdown diagram by (Zimmerman et al 1986)



**Figure** **1**.**3**.Showing the inactivation of a cell membrane by electroporation (Vega-Marcado 1996)

 **CHAPTER 2**

 **COST**

 **2.1.PULSE ELECTRIC FIELD A COST EFFECTIVE TECHNIQUE.**

Most developed countries around the world especially those whose economy solely rely on food produced and those exported are looking for cheaper ways to improve food product development,examples of such countries are New Zealand,Denmark and some few states in the United States of America,after series of food borne disease outbreaks,although much money is spent on other types of sterilization of food products,the effectiveness based on cost is still very high,in the United States today, the first commercial scale continuous PEF system was installed at the Ohio State University department of Food Science and Technology. This technique is part of a pioneer food treatment system assembled by an independent organization sponsored, University directed industry consortium.

 Additionally,according to the University of Ohio,the Diversified Technologies Incorporated,Bed ford,United States,is in charge of building commercial PEF systems whose processing volumes ranges from about 500 to 2,000 liters per hour,furthermore with the Ohio State University in charge of supplying the PEF treatment chambers with process using simple electricity,the facility meets electrical safety standards and no harmful environmental by-products are produced.An integrated PEF system may consists of a high voltage pulse generator,fluid handling unit, PEF treatment chambers, and usually a packaging machine.The fluid handling unit is in charge of delivering stable uniform flow with sterilize-in-place (SIP) and clean-in-place (CIP) functions,and the pulse generator in charge of supplying high voltage electrical field intensities into foods flowing through PEF treatment chambers,the treated foods are then packaged continuously.

 Furthermore,a PEF treatment chamber consists of at least two electrodes and insulation that forms a volume, for example the PEF treatment zone, where the foods receive electrical pulses,also the electrodes are made of inert materials,such as titanium,PEF is an energy efficient process compared to thermal pasteurization,sterilization that also uses power for full activity,a more interesting information is that,the PEF processing would add only more or less 0.07USD per litres to the average and final food costs,as opposed to other thermal methods which are more wasteful and definitely very costly,a commercial-scale PEF system can process approximately 5,000 liters of liquid foods per hour and this equipment can be ranked in scales.Generation of a high electrical field intensity having sufficient peak power (typically megawatts)is the limitation in processing large quantities of fluid economically.The discovery of solid state power electric systems,which can be literally sized by combining switch like modules in series and parallel,removes this limitation,currently different regulatory laws are coming up,but will likely include the development of Hazard Analysis Critical Control Point (HACCP),plans for most liquid foods for example juices,liquid soups,liquid eggs and beverages,has been a topic of modern research which is on by the United States Department of Agriculture in the form of a project which will majorly discus and address all these very points.An industrial scale-up PEF pilot plant facility is available at The Ohio State University in the Department of Food Science and Technology,the University of Selerno,Italy Food Engineering Department and so many universities and laboratories in Australia and presently the University of Auckland in New Zealand who is working tirelessly to improve the quality of food products within the country.Food processors are invited to take advantage of the expertise of the different countries that offers the advantage of the use of PEF,and facilities to conduct confidential product evaluations for shelf life, food safety,food quality,cost reduction and to obtain guidance on food product development,below is a diagram showing the basic attributes of the PEF technique.



**Figure .2.1**. Showing the structure of pulse electric field set up for food processing (Francessca De Vito et al 2006)



**Figure 2.2.**Generalized scheme of pulse electric field treatment (Maged E.A.Mohammed et al 2000)



**FIGURE. 2.3.** Showing a commercial scale pulse electric field system.

 **2.2. High intensity pulse electric field processing**

High intensity pulsed electric field (PEF) processing is defined by the application of pulses of high voltage usually from 20 - 80 kilovolts/centimeters to liquid foods placed between two electrodes,typically inert materials for example titanium.Pulse Electric Field treatment is carried out at room temperature or slightly above room temperature for less than few seconds usually one second,and the total energy loss due to heating of foods is reduced.For food quality properties, Pulse Electric Field technology is usually thought about as a better technique as compared to usual or traditional heat or thermal treatment of foods because it avoids or greatly reduces the detrimental changes of the sensory,chemical composition and physical properties of foods (Quass 1997)other studies some has discussed that Pulse Electric Field preserves the nutritional components of the food, other effects of Pulse Electric Field on the chemical and nutritional aspects of foods must be better understood before it is used in food processing (Qin et al 1995).

Addittionally,according to the United States Food and Drug Administration,the aspects of great importance in Pulsed Electric Field Technology design are the production of high electric field intensities,chambers design that imparts clean and even treatment to liquid foods for example beverages with an increase in minimum room temperature,electrode design which typically reduces the electrolytic effect,furthermore the large pulses are typically achieved through directly storing a large amount of energy in a bank of capacitance usually an array of capacitors in series from a Direct Current electric power supply,which is typically released in the form of high or very volt pulses (Zhang et al 1995).Studies on the required energy have shown that Pulse Electric Field has shown energy efficiency when compared to thermal pasteurization, typically when a system usually continuous is used (Qin et al 1995).

 Generally,the procedures of pulse electric field techniques involves the use of short,exponentially decaying ,bipolar or oscillatory pulses and square waves,an exponentially decay waves may be defined as a unidirectional voltage that rises rapidly to a maximum value and decaying slowly to zero,the diagram below shows the exponential decay waveform generation,generally a capacitor bank is charged by a direct current power supply connected to a charging resistor in series,the capacitor charge further flows electric current through the food in the treatment chambers when the signal is triggered

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**Figure .2.4.**Showing the high voltage pulse generator (Mohammed Farid et al 2009)



**Figure .2.5.**Showing the electrical circuit for the production of exponential decay waveforms.

**2.3.Microbial inactivation kinetics**

Different models has been encouraged to establish a more definite way of calculating the time required to effect a cell membrane breakdown as seen in relevant literature for example during the treatment of a liquid or semi liquid food stuff by pulse electric field processing the food to be treated is placed between two electrodes which could be made up of different metals depends on the initial set up of the technique forming a treatment chamber,and as the usual principle of the technique electric pulses are applied in order to effect cell membrane breakdown the total time or time required to effect the cell membrane breakdown maybe defined by mathematical expressions for example

***t* = *n* t*p***

where **t*p*** is the pulse duration and **n** equals the number of pulses which is applied to the food ,however from this simple equation we can detect that as the time increases the number of pulses also increases as well as the pulse duration,it is also worthy of note that while the number of pulses is increased,the total energy consumption also increases,therefore increasing the pulse duration the temperature of the food also increases.

 During the treatment of a food material the food which is placed between the electrodes is sure in close contact with the electrodes and definitely subject to an electric field,assuming the sample is homogenous(for example there is permittivity across the sample),

 therefore the overall electric strength of the field becomes E

**E=V/d V** becomes the voltage across the sample and **d** is the distance across the electrodes,this simple equation goes a long way to show that increasing the gap a higher voltage is required,to obtain the desired electric field,for example critical electric field corresponding to cell membrane breakdown.

 Furthermore,the unique design of the treatment chamber for example the geometry and the dimension of the electrodes determines a more or less uniform distribution of the electric field inside the food,however the more uniform is the electric field the more homogenous or even will be the treatment.

 On the other hand, (Hülsheger et al 1980) were the pioneers to propose an arithmetic model for inactivation of microorganisms with pulse electric field processing. This important model was based on the dependence of the survival ratio S (N/No or the ratio of living cell count before and after PEF treatment) on the electric field intensity E according to the following mathematical expression

 **ln(S) = -bE(E-Ec) ............     (1)**

where bE is defined as the regression coefficient, E is defined as the applied electric field, and Ec is the critical electric field obtained by the extrapolated value of E for 100% survival.This regression coefficient describes the gradient of the straight survival curves and is a micro-organism media constant.The critical electric field (Ec) has been found to be a function of the cell size (much lower for large cells) and pulse width (that is, with pulse width > 50 µs, Ec = 4.9 kV/cm; pulse width > 2 µs, Ec = 40 kV/cm). (Hülsheger et al 1981) further proposed an inactivation kinetic model that relates microbial survival fraction (S) with PEF treatment time (t) in the form of

 **lsS = -btln(t/tc) ....................     (2)**

where bt is the regression coefficient, t is the treatment time, and tc is the extrapolate value of t for 100% survival, or critical treatment time. The model can also be expressed as

 **........ (3)**

 İn equation 3 above,t is treatment time,is critical treatment time is tc,critical electric field intensity is denoted as Ec, and K is the kinetic constant.A little value for the kinetic constant [K] indicates a wide span in the inactivation rate curve and lower sensitivity to PEF,however a large value implies a steep decline or higher susceptibility to PEF. Also,Lower Ec values would indicate less resistance to the PEF treatment.

 A further second model proposed by (Peleg et al1995) illustrates a sigmoid shape of the survival curves generated by the microbial inactivation with PEF. The model (equation 4) represents the percentage of surviving organisms as a function of the electric fields and number of pulses applied. This model is defined by a critical electric field intensity that corresponds to 50% survival (Ed) and a kinetic constant (Kn, a function of the number of pulses) that represents the steepness of the sigmoid curve:

 ** (4)**

Arithmetically , about a percentage of 90 would inactivation be achieved within the critical electric field plus 3 times the kinetic constant.In this model of generalization,Ed(n) and K(n) are simple arithmetic algebraic functions that not only depend on the electric field but also on the number of pulses or treatment time.Furthermore,this model may also be simplified by not considering the relationship between the electric field and the number of pulses,but also,a small value for the kinetic constant [K (n) or K] for example 1 as seen in equation 4 above,which indicates a wide span in the inactivation rate curve and lower sensitivity to pulse electric field,whereas a large value implies a steep decline or higher susceptibility to pulse electric field,however,lower Ed values would indicate less resistance to the pulse electric field treatment.

 **S=1/1+e(E- Ed)/K (5)**

 Finally,the number of pulses and pulse duration,for example the total treatment time,are significant parameters but apart or besides them are other electrical parameters such as the pulse shape and the pulse repetition rate for example number of pulses in one second(Francesca De Vito et al 2006), also,recent studies goes a long way to show the development of mathematical or arithmetic models to express the inactivation kinetics of a microbial cell membrane as a result of the use of pulse electric field technique,but however this is an area of research that needs extensive and rigorous further work. Nonetheless, this important further models have been proposed and sure in need of further evaluation.

 **CHAPTER 3**

 **IMPORTANT PARAMETERS THAT INFLUENCE MICROBIAL INACTIVATION USING PULSE ELECTRIC FIELD TECHNIQUES.**

 **3.1. Electric field intensity**

The electric field intensity is one of the main parameters which the pulse electric field technique works on,pulse electric field which depends solely on the application of electric current in order to effect microbial permittivity.Important researches has shown that when the electric field is increased the microbial inactivation also increases which is usually above the critical transmembrane potential(Qin et al 1998)

 Additionally,with compliance and consistency with the theory of electroporation,that talks about the induced potential difference across the cell membrane in proportionality to the applied electric field some mathematical models have been proposed to describe the relationship between the electric field intensity and microbial inactivation as discussed in the chapter one of this project,however according to the United States Food and Drug administration,the critical electric field Ec may be defined as the electric field intensity below which inactivation does not occur,is typically increased with the transmembrane potential of the cell.(Jeyamkondan et al 1999)concluded that the critical electric field and the transmembrane potential are larger for larger cells.it was further seen that pulse width also have influence on the critical electric field,for example,with pulse widths greater than 50 micro seconds (µs), Ec is 4.9 kilo volts per centimeter,with pulse widths less than 2 micro seconds (µs), Ec is 40 kilo volts per centimeter(Schoenbach et al 1997).

**3.2.Treatment time**

Treatment time which may be defined as the product of the number of pulses and the pulse duration ***t* = *n* t*p,*** therefore as discussed earlier an increase in any of these important variables increases the other and therefore increases the rate of microbial inactivation (Sale and Hamilton 1967). As discussed earlier in the effect of electirc field ,pulse width influences microbial reduction by affecting the critical electric field (Ec). Longer widths decreases the critical electric field (Ec), which therefore results in higher inactivation; however, an increase in the pulse duration may also result in an undesirable food temperature increase. Furthermore,Optimum processing conditions should therefore be established to obtain the highest inactivation rate with the lowest heating effect. (Hülsheger et al 1981) proposed an inactivation kinetic model that relates microbial survival fraction (S) with PEF treatment time (t). The inactivation of microorganisms increases with an increase in treatment time ( Hülsheger et al 1983),however, not all cases seems to be the same as seen in the case of *saccharomyces* *cerevisiae* the number of pulses that increases inactivation seem to reach saturation,Saccharomyces cerevisiae inactivation by PEF which reaches saturation with 10 pulses of an electric field at 25 kV/cm (Barbosa-Cánovas et al 1999).

 Finally,further research work by Barbosa-Cánovas et al.1999) reported that for an electric field strength 1.5 times higher than the critical electric field(Ec), the critical treatment time would remain constant.

**3.3.Temperature**

Previous and recent investigations has shown the impact of temperature on the activity of pulse electric field processing,generally all other traditional conventional methods uses high temperatures to inactivate food products and improve food process development,but this methods usually comes with food products which loose taste ,nutritional value and most importantly destruction of important vitamins

 On the other hand series of research has also shown that pulse electric field maybe more effective if high temperatures are added to effect more microbial inactivation although the technique is non thermal in nature,series of on going research has implicated the temperature as a very important parameter to effect microbial inactivation.

 Furthermore, (Jayram et al 1992) suggested that pulse electric field at moderate temperatures of ~ 50 and 60 degrees centigrade has been shown to exhibit a powerful effect on the inactivation of micro-organism,(Dunn and Pearlman 1987) also showed that with constant electric field strength,with inactivation increasing with temperature. Additionally,because the application of electric field intensity generally raises the temperature of the foods, proper cooling maybe needed to maintain food temperatures far below those generated by thermal pasteurization and sterilization

 . Nonetheless,further studies by (Vega-Mercado et al 1996) shows the effect of temperature was observed when Echerichia coli reduction was increased from 1 to 6.5-log reduction cycles with a change in tempreture from 32 to 55 degree centigrade. Additionally,a higher lethal effect of PEF treatment is accomplished by increasing the process temperature to 25 ° C, from 5 or 10 ° C. Which may be due to the increase in the electrical conductivity of the solution at the higher temperature (Marquez et al 1997),furthermore the authors was trying to suggest that the leakage of mobile ions in decoated spores may increase as the temperature is raised due to an increase in average kinetic energy of the ions. A higher temperature definately also increases the motion of the solvent molecules in both the surrounding cortex and the core so that the molecules could migrate from one electrode to the other.

 Finally, (Hulsheger et al 1981)showed the additional effects of high treatment temperatures which are changes in cell membrane fluidity and permeability, which definitely increases the susceptibility of the cell to mechanical disruption and Jeyamkondan et al 1999) further showed that a low transmembrane potential decreases the critical electric field (Ec) and therefore increases inactivation

**3.4.Waveshape of pulses**

**Firstly,In the pulse** Electric field technique pulses will definately be applied in the form of decaying and exponential, square-wave, oscillatory, bipolar, or instant reverse charges. Usually Oscillatory pulses are the least efficient for microbial inactivation,with square wave pulses beign the most efficient both energy and lethally than exponential decaying pulses.

 Additionally,(Ho et al 1995)further investigated that bipolar pulses are more lethal than monopolar pulses because a pulse electric field technique causes movement of charged molecules in the cell membranes of microorganisms, and reversal in the orientation or polarity of the electric field causes a corresponding change in the direction of charged molecules.

 Furthermore,(Ho and mittal 1997) further investigated the difference reported in Bacillus *spp*. Spores, and E. coli was reported by (Qin et al 1994). Also,With bipolar pulses, the alternating changes in the movement of charged molecules cause a stress in the cell membrane and enhance its electric breakdown.Additionally,Bipolar pulses also offer the advantages of minimum utilization of energy,reduced deposition of solids on the electrode surface, and decreased food electrolysis (Barbosa-Cánovas and others 1999).

Additionally ,the instant charge reversal pulse maybe described as partially positive at first and partially negative immediately thereafter. Therefore this characteristic of the waveshape maybe influenced by the electrical conductivity of the treated food. Further ,in line with this discussion,an increase in conductivity likely decreases the duration of the positive part of the pulse as well as the span of the negative part, with an overall increase in the peak/voltage ratio.

 Nonetheless,the difference between a bipolar and instant charge reverse pulse is the relaxation time between pulses, which is only present in the former. (Ho et al 1997) further researched on the inactivation effect of an instant-reversal-charge which was believed to be due to a significant alternating stress on the microbial cell membrane that causes structural fatigue.( Ho and Mittal 1997)finally reported that instant-reversal-charge may reduce the critical electric field strength required for microbial cell electroporation.Also,the effectiveness of this waveform to destroy microorganisms compared to other pulse waveforms can maybe save up to 1/5 or 1/6 of total energy and sure the equipment cost. Further research work is required to verify and determine the effect of reversal-charge electrical pulses on the so called inactivation ratio. Additionally,the inactivation of Bacillus subtilis and Bacillus cereus spores suspended in common salt solutions has been reported to be higher when further instant reverse pulses and a polarity of electric field chambers with high pulse frequencies are used.The instant reverse charge has been reported to be effective in the inactivation of 5-log cycles of Bacillus *spp* spores.(Ho and Mittal 1997) further researched and established that the survival fraction is not only a function of the temporal pulse area but that even when both bipolar also known as (alternating exponential) and exponential waves had the same area per pulse, alternating exponential waves yielded a higher inactivation ratio.

 Further studies that was conducted by (Zhang et al 1997) showed the effect of instant-charge-reversal pulses,square waves,exponentially decaying on the shelf-life of orange juice. Also,three waveshape pulses were used:

i.The square waves with peak electric field intensity of 35 kV/cm, an effective pulse width of 37.22micro seconds( µs), 60 ns pulse rise time;

ii. The exponential decaying waves with a peak electric field of 62.5 kilovolts(kv) per centimeter(cm), an effective pulse width of 0.57 microseconds and a pulse rise time of 40 ns; and

iii. The charge-reversal waves with a peak electric field of 37 kilovolts(kv) per centimeter(cm), an effective pulse width of 0.96 micro seconds, and a pulse rise of 400 ns. The square wave pulses were by far more effective, and yielding good products with prolonged shelf-lives than those products treated with exponentially decaying and reverse charge pulses.Finally,In compliance with this study, Love et al (1998) further quantitatively demonstrated the stronger inactivation effect of pulses from square wave over other wave shapes

**3.5. Other important parameters**

3.5.1.Conductivity,pH and ionic strength.

By definition electrical conductivity of a medium with SI unit (σ , Siems/m),is the ability to conduct electric current, which is an important variable in pulse electric field technique. Also,electrical conductivity is the inverse of resistivity for example in mathematical expressions it is denoted as r=1/R , where **r** is electrical conductivity and R is resistivity so it goes to show that when one variable is increasing the other is decreasing,and measured in ohm-meters (W .m).Further studies shows that foods with large electrical conductivities generate little peak of electric fields across the treatment chamber and therefore are not appropriate for PEF treatment (Barbosa-Cánovas et al 1999).Inactivation of Lactobacillus brevis with pulse electric field showed that as the resistance of the treatment chamber was reduced when the conductivity of the fluid increased (Jayaram et al 1992),which as a consequence lead to the reduction in the pulse width and a subsequent decrease in the rate of inactivation. Furthermore,because an increment in rate of conduction usually results from increase in the the strength of ions of a liquid,an increment in the strength of ions of a food results in a decrease in resistance and definitely an increment in the rate inactivation.Furthermore,an increment in the difference between the the rate of conduction of a medium and cytoplasm of a microorganism subsequently weakens the structure of the cell membrane obviously due to an increased flow of substance usually of ionic nature across the membrane of the cell.Therefore,the rate of inactivation of the microorganisms further shows an increment with a decreased conductivity even with an application of energy of equal amount or input (Jayaram et al 1992).Another study by (Dunne et al 1996) with a model system showed that resistance had no effect at all on Pulse Electric Field effectiveness on Echerichia coli and Listeria innocua.These apparent unexplainable and controversial or maybe sometimes misleading results may be due to the type of microorganisms,the technique set up or media used,and definitely point hands in the right direction of further research work.

 A further studies by (Vega-Mercado et al 1996) studied the effect of pH and ionic strength of the medium during Pulse Eelectric Field treatment.The ratio of inactivation increased from not present to cycles of 2.5 logarithm when the strength of ions solutions were reduced from 168 to 28mM. Also,at 55 kilovolts per centimeters and typically 8 electrical pulses,as the pH was decreased or reduced from 6.8 to 5.7, the ratio of inactivation increased from 1.45 to 2.22 logarithm cycles.Furthermore,the pulse electric field treatment and strength of the ions were responsible for electroporation and further compression of the cell membrane,but however, the pH of the medium affected the cell cytoplasm when the electroporation was complete.(Dunne et al 1996)further researched and reported that depending on the microorganism,low pH enhanced microbial inactivation.But however,there was a lack of information of what microorganisms were affected or what range of pH was used.

3.5.2 Liquid particulate foods

**İnvestigations has been carried out by different scientist and literature reviews has been non exhaustive a further work by (Dunne et al 1996) was on the** inactivation of microorganisms in particulate liquid systems.Echerichia coli,Listeria innocua,Staphyloccocus aureus,and Lactobacillus acidophilus were suspended in a 2 mili meter diameter beads model,also the effect of variables in Pulse Electric Field inactivation of microorganism was carried out.The researchers discussed and concluded that the process and technique was effective in killing microorganisms especially when embedded in forms of particulate nature.However,to be able to achieve more than a cycle of 5 logarithm reduction,high inputs of energy were indeed needed an estimation of about 70 - 100 J/ml,depending on the type of bacteria.Furthermore,with this high Pulse Electric Field intensities,the probability of dielectric breakdown sees a limitation that is waiting to be eliminated.Other reports of significance where carried out by (Qin et al 1995) that dielectric breakdown indeed occurs when air or liquid vapor is available in the liquid food because of the difference in dielectric constant between gas and liquid.Likewise,dielectric breakdown may occur at a liquid to particle interface due to differences in the constants of electric current.

 Finally,from this vast knowledge of important literature and scientific studies it is further suggested that the pulse electric field would work better with a combination of several hurdles such as lower pH,increased supply of direct currect e.g increased electric field,longer time,and even the addition of antimicrobials but this can be continuous and will however be a necessary direction for further research work.

 **CHAPTER 4**

 **MICROBIAL SUSCEPTIBILITY TO PULSE ELECTRIC FIELD TECHNIQUE**

 **4.1.The type of micro-organism**

A microorganism which is derived from the greek word “*mikros*” and “*organismós*”maybe defined as an ubiquitous and diverse group of microscopic organism,which may be either single cell or multicellular usually viewed with the aid of a microscope (Anthonnie Van Leeuwenhoek 1675)and the study of this organisms is called microbiology,they may include prokaryotes,eukaryotes,archaea e.t.c

 Today this organisms are exploited in biotechnology,food and beverage production,water treatment,genetic-engineering,but however some of this micro organisms are pathogenic,pathogenicity which maybe defined as the ability of an organism to cause a disease,in the light of all this good uses and importance they are largely responsible for major illnesses that affects human beigns as well as important farm animals that serve as food to humans,among this organisms there are gram positive(+) and gram negative(-) bacteria,with gram negative proving to be more pathogenic and resistant to antibiotics as a result of their structure to be more effective the complex lipopolysaccharide layer above their thin peptidoglycan layer protects them from usual antibiotics,dye and normal detergents.but simple drugs may be used to destroy this organisms and reduce their ability to cause a disease examples of this drugs are neomycin,ampicillin,chloramphenicol e.t.c.

 Furthermore,in relation to this project topic among bacteria, Hülsheger et al 1983 showed that those that are gram-positive are more resistant than those that are gram-negative,without much evidence but from important literature it will be believed that its as a result of their thicker peptidoglycan layer since electroporation is concerned about cell membrane permeabilization,then as a common sense it will be believed that the thicker the cell membrane or cell protection the more resistant they may be. Furthermore,(Sale and Hamilton 1967) as well as (Qin et al 1995) showed that yeasts are more sensitive to electric fields than bacteria due to their larger size,and further illustrated that at low electric fields they seem to be more resistant than gram-negative cells.Further research showed a comparison between the inactivation of 2 yeast spp. of different sizes proving that the field intensity needed to achieve the same inactivation level was inversely proportional to cell size. Those results are logical but however inconsistent with results by Hülsheger et al (1983). This goes to suggest that further research is needed in this area to explain the controversies in this area in order to better understand the effect of the type of microorganism on microbial inactivation.

**4.2.Number of micro-organisms**

under logical explanations it will be believed that the a lot of material the less it is to attain even distribution ,therefore, the concentration of microorganisms in food could have an impact on their inactivation with electrical fields.Different reports wherever given to indicate that the concentration of micro-organism could have a large result on the electrical field in all probability thanks to uneven distribution of the electrical field,Barbosa-Cánovas et al (1999) reported that inactivation of *E*. *coli* in a very model food system of simulated milk ultra filtrate (SMUF) wasn't affected once the concentration of microorganisms was varied from 103 to 108 cfu/ml once being subjected to seventy kV/cm, 16 pulses, and a pulse breadth of two micrseconds(µs). Increasing the quantity of *S*. *cerevisiae* in fruit crush resulted in slightly lower inactivation (25 kV/cm, 1 pulse, and pulse breadth of twenty five micro seconds µs).The result of microorganism concentration on inactivation could also be associated with cluster formation of yeast cells or probably hid microorganisms in low field regions.

**4.3. Growth phase of micro organisms**

In real sense,scientific research shows that the logarithmic phase cells are more sensitive to fret than stationary and log phase of cells.Growth of microorganisms in logarithmic phase is outlined by a high rate of cells undergoing growth and division,during which the semipermeable membrane is more liable to the applied electric field. (Hülsheger et al 1983) concluded that cells from log growth phase are more sensitive to pulse electric field than from the stationary growth part. Likewise, *E*. *coli* cells within the logarithmic part were more sensitive to pulse electric field treatment when put next to cells within the stationary and lag phases (Pothakamury et al 1996). Additional studies with *S*. *cerevisiae* have shown that the susceptibleness of actively growing cells to pulse electric field additionally happens with yeast cells (Jacob et al 1981; Gaskova et al 1996). as an example, Gaskova et al (1996) reported that the killing result of pulse electric field within the logarithmic phase is half-hour bigger than of those in stationary part of growth.Finally this vital reports by relevant analysis groups has established to an inexpensive doubt that microorganism cells are more inclined within the log part to pulse electric field process as a results of high rate growth and cellular division essentially as a logical rationalization,but however the most reason for susceptibleness might not have been totally explained and nonetheless not properly understood,this will at least point towards the direction of additional analysis and work.

 **Microbial inactivation data analysis**

Different scientific models are investigated over the years to grasp and point hands towards the correct part of analysis and more work,during the analysis of various necessary datas whereas some point in one direction others might contradict already existing models making it terribly difficult and therefore the want for more work becomes terribly imperative,furthermore, varied inactivation levels of *S*. *cerevisiae* are achieved in food models and foods employing a kind of pulse field chambers and experimental conditions (Mizuno and Hori 1991) as well as (Zhang et al 1994) plus (Qin et al). Also,other yeasts of importance in food spoilage have conjointly been reduced,suggesting PEF's potential to stop or delay yeast-related food spoilage.Additionally, (Fernandez-Molina et al 1999) reported two.6- and 2.7-log reductions for various microorganisms like *L*. *innoccua* and *pseudomonas* *fluorescens* with two μs one hundred pulses at fifty kV/cm at close temperature.The influence of the food composition was shown by (Calderon-Miranda 1998) studies when *L*. *innoccua* was reduced by 2.4 and 3.4-log cycle reductions in raw milk and liquid whole egg, severally, below constant experimental conditions.Further investigations by (Fernandez-Molina et al 1999) reported a pair of 6 and 2.7-log reductions for various microorganisms like *L*. *innoccua* and *Pseudomonas* *fluorescens* with a pair of μs one hundred pulses at fifty kV/cm at close temperature. The influence of the food composition was shown by (Calderon-Miranda et al 1998) studies where *L*. *innoccua* was reduced by a pair of 4 and 3.4-log cycle reductions in raw skimmed milk and liquid whole egg, severally, beneath a similar experimental conditions.

In addition,(Grahl et al 1992) reported the influence of pulse variety in microorganism inactivation of *E*. *coli*.Furthermore, they were able to scale back populations of *E*. *coli* in UHT milk by one, two and three log cycles once, five, ten, and fifteen pulses (22 kV/cm) where applied. (Qin et al 1998) achieved over a six log cycle reduction in E. coli suspended in milk ultra filtrate in simulated form (SMUF) exploiting field intensities of thirty six kV/cm with a 5-step (50 pulses) pulse field treatment. The temperature within the chamber was maintained below forty degree centigrade throughout the pulse electric field treatment, that is below the temperature of commercial pasteurization seventy to ninety degree centigrade for milk. (Hülsheger et al 1983) reported a four log reduction of *E*. *coli* in an electrical field strength of forty kV/cm accompanied with an extended treatment time of 1080 μs. A pulse field methodology appropriate to inactivate up to 7-log cycles of *E*. *coli* with fewer pulses (20 versus 70) in a stepwise recirculation whereby the merchandise is processed many consecutive times (Barbosa-Cánovas et al 1999). (Liu et al 1997) reported that PEF and organic acids (benzoic and sorbic) achieved a pair of cycle reductions in 5.6 and 4.2 logarithm, compared to a one-log cycle reduction once PEF was used alone, suggesting increased effects with the mixture of PEF and organic acids.Further research showed the higher efficiency of bipolar pulses versus monopolar pulses was suggested by (Qin et al 1994).Cells of B. subtilis were reduced to 3- and <2-log cycles when bipolar and monopolar pulses were respectively applied.

 Some further work shows the inactivation rate and the effects of pulse electric field on bacterial spores are scarce with conclusions and results that varies.The pioneer studies by (Sale and Hamilton 1967) reported the resistance of *Bacillus* *spp*.spores to Pulse Electric Field of exponential waves with 30KV/cm of electric field strength and only after germination they were found to be sensitive to Pulse Electric Field,(Simpson et al 1995) confirmed the high resistance of Bacillus subtilis spores to Pulse Electric Field,and subsequently studied a hurdle approach with some heat shock,the addition of lysozyme,EDTA,and pH,but however,only a combination of 80 degrees centigrade heat shock,addition of lysozyme,followed by Pulse Electric Field at 60 degree centigrade was able to achieve a 2-4 logarithm cycle reduction of spores.Also,the resistance of spores to pulse electric field was shown by (Pothakamury et al 1995),that reported only 3-4 logarithm reduction cycles for Bacillus subtilis spores that was subjected to about 60 electrical pulses of about 16 kV/cm electric field intensity and 200 to 300micro seconds (μs) and pulse widths.Furthermore,(Pagán et al 1998) found that spores of Bacillus subtilis were not affected or destroyed when Pulse Electric Field of (60 kV/cm and 75 pulses) was used in combination with high hydrostatic pressure at 1500 atm for 30 min and 40 ° C,on the other hand these treatments however induced the germination of the spores of Bacillus subtilis by more than 5-logarithm cycles,making them more sensitive to further pasteurization as a result of heat treatment.Thus,the combinations of Hydrostatic Pressure,Pulse Electric Field,and heat treatments brings about an alternative to the final stability of food products by heat to inactivate spores,further research by (Marquez et al 1997) successfully inactivated 3.4 and also 5 logarithm cycles of Bacillus subtilis and Bacillus cereus spores at room or ambient temperature,an electric field intensity of about 50 kV/cm with instant and reversed electrical impulses at 30 and 50 respectively.

 Finally some of the positive results shows that PEF is an effective method of sterilizing food products but others might contradict and definately be misleading,pulse electric field technique which is based majorly on electroporation of a cell membrane might definitely not be active in bacillus spores and may sometimes induce the germination of its endospore especially when low pulses are applied, for example as reported by (Pagán et al 1998),although after germination it might be very sensitive which actually put them at the log phase of growth which relevant reports by (Hülsheger et al 1983) concluded in a research,even in light of all this discussion it is however believed that germination of a bacteria endospore which was reported to have effect at 60KV/cm and 75 pulses in addition to high hydrostatic pressure of 1500atm for 30 minutes and 40 degree centigrade was only meant to induce the germination of the endospores because as known and reported by relevant research studies the germination and growth of *bacillus* *spp* spores takes place between 10 and 50 degree centigrade,therefore by using temperatures of 40 degree centigrade as reported by( Pagán et al 1998)was only to aid the germination of the endospores and not necessarily to kill it,which does not stand in the purpose of the pulse electric field technique,normal cooking temperature below 100 degree centigrade leaves most *bacillus* *spp* alive only temperatures above 100 degrees and time above 30 minutes can effectively destroy the spores of the micro-organism,and therefore a combination of a series of hurdle technology might be the only reasonable approach to the effective removal of the micro-organism from food products,but however this is based on logic and more research needs to be carried out in this direction in order to provide conclusive data on the microbial pathogen elimination technique and under what conditions.

 **CHAPTER** **5**

 **EXPERIMENTAL STUDY**

 **5.1.Experimental Processing of apple juice**

As discussed earlier the nature of the food might also have a way to influence the effectiveness of pulse electric field technique,in this chapter,different types of liquid foods will be investigated to better understand the technique in one food as opposed to other food materials,while relevant research which was proved by (Simpson et al 1995) reported that apple juice from concentrate treated at fifty kilovolts per centimeter with Pulse Electric Field, ten pulses,pulse width of two micro seconds and maximum processing temperature of fourty five degree centigrade had a shelf-life of 28 days compared to a shelf-life of 21 days of fresh-squeezed apple juice and also no physical or chemical changes in ascorbic acid or sugars in the PEF-treated apple juice, a sensory panel found no significant differences between untreated and electric field treated juices.Further studies by (Vega Mercado et al 1997) reported that pulse electric field extended the shelf-life at 22 - 25 ° C of fresh apple juice and apple juice from concentrate to more than 56 days or 32 days, respectively,with results showing that there was no apparent change in its physicochemical and sensory properties.

 This goes a long way to show that Pulse electric field works better and increases the shelf life of apple juice only when the temperature is reduced,contrary to other reports that suggests that pulse electric field will be more effective with higher temperatures,as well as other hurdles,which might at the end deplete other labile vitamins and minerals that are susceptible to heat and certain enzymes.

 Further more ,other inconclusive datas has shown that *bacillus* *spp,* endospores are only destroyed at higher temperatures which goes a long way to show that the type of species of mico-organism must be investigated in order to know the type of pulse electric field treatment to apply,however this are inclusive informations and will need further evaluations and research.

**5.2.Experimental processing of orange juice**

Further research by Sitzmann ( 1995) reported on the effectiveness of the ELSTERIL(process for the electric sterilization of pumpable conductive foods) the University of Hamburg,in Hamburg Germany on the other hand are leading contributors to this technique and sure has also reported continuous process developed by their Food Engineers the reduction of the native microbial flora of freshly squeezed orange juice by 3-log cycles with an applied electric field of 15 kV/cm without significantly affecting its quality.

Furthermore,research by **(** Zhang et al 1997),studies the shelf-life of reconstituted orange juice treated with a group of PEF pilot plant system.The PEF system consisted of a chain of chambers,then temperatures were maintained close to room temperatures with cooling devices between chambers,and three pulses of waveshape were used to compare the system effectiveness,with results confirming that the square wave was the best based on effectiveness.Additionally,the authors reported that total aerobic counts were reduced by a pair of three to four logarithm cycles at four degree centigrade and 32KV/cm,both heat- and PEF-treated juices had a five months increased shelf life.Also,losses of vitamin C were lower and color was generally better preserved in PEF-treated juices compared to the heat-treated ones up to ninety days (storage temperature of 4 °C or 22 °C) or fifteen days(storage temperature of 37 °C) after processing,which suggests that PEF treated foods are better stored in lower temperatures.

**5.3.Experimental processing of milk**

Further studies by Dunn and Pearlman (1987) conducted a challenge test and shelf-life study with homogenized milk inoculated with Salmonella Dublin and treated with 36.7 kV/cm and 40 electrical pulses over a 25-minutes time period. Furthermore,Salmonella Dublin was not detected after PEF treatment or after storage at a temperature of 7 - 9 ° C for 8 days,the naturally occurring milk bacterial population increased to the value of 107 cfu/ml in the untreated milk, as opposed to the treated milk which showed about 4x102 cfu/ml.Furthermore,studies by Dunn (1996)evaluated and indicated less flavor degradation and no chemical or physical changes in milk quality attributes as seen in cheesemaking,when E. coli was used as the challenge bacteria,immediately after the treatment a 3-log reduction was achieved.

 Additionally ,Fernandez-Molina et al(1999) studied the shelf-life of raw skim milk (0.2% milk fat), treated with Pulse Electric Field at 40 kV/cm, 30 electrical pulses, and treatment time(s) of 2micro seconds( µs) using exponential decaying pulses,the shelf-life of the milk was 2 weeks stored at 4 ° C; however, treatment of raw skim milk with 80 ° C for 6 s followed by PEF treatment at 30 kV/cm, 30 electrical pulses, and pulse width of 2 µs increased the shelf-life up to 22 days,with a total aerobic plate count of 3.6-log cfu/ml and no coliform,however,the processing temperature did not exceed 28 ° C during PEF treatment of the raw skim milk.

 Further research by Qin et al (1995) reported that milk (2% milk fat) subjected to 2 steps of 7 pulses each and 1 step of 6 pulses with an electric field of 40 kV/cm achieved a shelf-life of 2 weeks at refrigeration temperature,however, there was no apparent change in its physical and chemical properties and no significant differences in sensory attributes between heat pasteurized and PEF treated milk

 Other research work by,Calderon-Miranda (1998) studied the Pulse Electric Field inactivation of Listeria innocua suspended in skim milk and its subsequent and further immunological testing or to nisin. The microbial population of Listeria innocua was reduced by 2.5-log after Pulse Electric Field treatments at 30, 40 or 50 kV/cm, the same Pulse Electric Field intensities and subsequent exposure to 10 IU nisin/ml achieved 2-, 2.7- or 3.4-log reduction cycles of L. İnnocua,this appears that there may be an additional inactivation effect as a result of exposure to nisin after Pulse Electric Field.Further work by Reina et al (1998) studied the inactivation of Listeria monocytogenes Scott A milk, in pasteurized whole2%,, and skim milk with Pulse Electric Field,Listeria monocytogenes was reduced 1- to 3-log cycles at 25 ° C and 4-log cycles at 50 degree centigrade, with no significant differences being found among the 3 milks.The final effectiveness of Pulse Electric Field was a function of the field intensity and treatment time.

**5.4.Experimental processing of eggs**

Some of the pioneer studies in egg products were conducted by Dunn and Pearlman (1987) in a static parallel electrode treatment chamber with 2-cm gap using 25 exponentially decaying pulses with peak voltages of about 36 kilo Volts. Different tests were carried out on liquid eggs, and on heat-pasteurized liquid egg products, and also on egg products with potassium sorbate and citric acid added as preservatives.Furthermore,comparisons were made with regular heat-pasteurized egg products with and without the addition of food preservatives when the eggs were stored at low (4 ° C) and high (10 ° C) refrigeration temperature ranges. Furthermore,the study proved the importance of the hurdle approach in shelf-life extension,additionally,its effectiveness was even more evident during storage at low temperatures,when egg products with a final count to a value of 2.7 log cfu/ml stored at 10 degree centigrade and 4 degree centigrade maintained a low count for 4 and 10 days, respectively, versus a few hours for the heat pasteurized samples.

 Further studies on liquid whole eggs (LWE) treated with PEF conducted by Qin et al (1995) and Ma et al (1997) showed that PEF treatment decreased the viscosity but increased the color (in terms of b -carotene concentration) of liquid whole eggs when in comparism to new eggs. After panel evaluation with a triangle test, Qin et al (1995) found no differences between scrambled eggs prepared from fresh eggs and electric field-treated eggs; the latter were preferred over a commercial brand.

 Nonetheless, In addition to color analysis of eggs products, Ma et al (1997) evaluated the density of fresh and PEF-treated LWE (indicator of forming egg protein ability). Furthermore,the stepwise process used by Ma et al (1997) did not cause any difference in density or whiteness between the Pulse Electric Field-treated and fresh liquid whole eggs,the strength of the sponge cakes prepared with PEF-treated eggs was however greater than the cake made with un-processed eggs,the difference in strength was attributed to the lower volume obtained after baking with Pulse Electric Field-treated eggs, the statistical analysis of the sensory evaluation revealed no differences between cakes prepared from Pulse Electric Field processed and fresh LWE.

**5.5.Experimental processing of green pea soup**

**Studies by**Vega-Mercado et al (1996) exposed pea soup to 2 steps of 16 pulses at 35 kV/cm to prevent an increase in temperature beyond 55 ° C during treatment,the shelf-life of the PEF-treated pea soup stored at refrigeration temperature exceeded 4 weeks, while 22 or 32 ° C were found inappropriate to store the product, there were no apparent changes in the physical and chemical properties or sensory attributes of the pea soup directly after PEF processing or during the 4 weeks of storage at refrigeration temperatures.

 **CHAPTER 6**

 **RESULTS AND DISCUSSION**

Although, relevant literature was not conclusive about the relevant datas but at least from this point of view we can say the results so far is continous and will likely point towards the right part of further work,most of the data obtained from several scientist whose work was to explore the type of micro-organism which might be more susceptible to pulse electric field was not conclusive,although some basic questions was answered properly but not all was completely understood this in real sense will at least facilitate further work in that area,other important fields that need proper research is the treatment of waste water,although that is not the basis of this research project but work also needs to be done to better understand the main uses of the PEF technique,similary,number of micro-organism was not answered properly,even though logical answers might tell us that the concentration of the micro-organisms has a huge effect on the distribution of electric field intensity,but from this point of view it should be more experimental than logical,other important fields of needed research as deemed important in this project is the need to understand the overall safety of this technique as completely opposite to other techniques like pasteurization and the further research work to obtain guidance in the use of important hurdle steps to overcome every type of micro-organism and in every stage of development.

 Furthermore the use of pulse electric field often comes with its own limitations in this chapter the limitations of pulse electric field technique will be discussed in more details.

Some of the most important limitations in pulse electric field technique are

**6.1.Availability of commercial units**

The availability of commercial units, which is limited in the USA to one by PurePulse Technologies, Inc., and one by Thomson-CSF,others are diversified technologies inc,Bedford MA etc. Many pulse-power suppliers are capable of designing and constructing reliable pulsers, but except for these 3 mentioned, the complete PEF systems must be assembled independently. The systems (including treatment chambers and power supply equipments) need to be scaled up to commercial systems,however this problem has now been reduced by recent research in New Zealand and different companies has now been involved in the production of commercial units,an example is the innovative IGBT technology associated with the University of Auckland food science department with main focus on pulse electric field equipment with chambers that can operate at high electric field intensities with limited increase in liquid temperature and limited fouling of electrodes(Farid et al 2006)

**6.2.Bubbles**

The presence of bubbles which may lead to non-uniform treatment as well as operational and problems of safety,which when the applied electric field overweighs the dielectric strength of the gas bubbles,some partial discharges take place inside the bubbles that can volatize the liquid and therefore increase the volume of the bubbles. However,the bubbles may become big enough to bridge the gap between the 2 electrodes and may bring about a spark. Therefore,to be efficient air bubbles in the food must be removed, reliably with batch systems.Also,vacuum degasification or pressurizing the treatment media during processing,maybe using positive backward pressure,can minimize the presence of gas in general, however, the pulse electric field method is not suitable for most of the solid food products containing air bubbles when placed in the treatment chamber.However,reseach and subsequent treatment and innovation using PEF by the University of Auckland has shown no sparks,electrical breakdown or electrode fouling based on the ideal nature of design of the PEF technique(Farid et al 2006)

**6.3.Limitation of application**

Limited application,which is restricted to food products that can withstand high intensity of electric field,it has been proposed that the physical structure and chemical composition of a food is directly proportional to the dielectric property of the food.Furthermore,pulse electric field method has shown that homogenous liquids with lower electrical conductivity provide ideal conditions for continuous treatment.Food products of which salt has not been added usually have conductivity to a likely range of 0.1 to 0.5 S/m.u.Additionally, food products with very high electrical conductivity brings about a reduction to the resistance of the chamber and consequently require more energy to achieve a specific electrical field as discussed earlier in this project.Therefore,when food product with high salt content are undergoing processing, the salt maybe should be added after processing.

**6.4.Particle size**

 The particle size of the liquid food in both treatment and static treatment modes.The further maximum particle size in the liquid must be smaller than the gap of the treatment region in the chamber in order to maintain a proper processing operation.

**6.5.Treatment delivery measurements**

 The lack of methods to accurately measure delivery of treatment. The diversity and number in equipment, limiting the validity of conclusions that can be drawn about the effectiveness of particular process conditions.However, a method to measure treatment delivery would prevent inconsistent results due to variations in pulse electric field systems. Such a method is not however available yet.

Finally,despite the dedication by different scientist around the world since the 1980s and 1990s,further questions needs to be answered and some important research needs also will surely be clarified in order for this technique to be used commercially and under what conditions some of the important research needs are, treatment chamber design uniformity and processing capacity,confirming the mechanisms of microbial breakdown and enzyme inactivation.Identifying the pathogens of concern most resistant to pulse electric field and design of process system, evaluation, and further cost reduction

 **CHAPTER 7**

 **CONCLUSION**

The starting point of the work done in this Msc research project was a big question of how wide we could use pulse electric field technique in food processing with particular emphasis on food products and also to develop mathematical models that may demonstrate the time needed to effect cell membrane permeabilization,literature reviews was not exhaustive and the atitude of potential users of the technique was at least doubtful,but at least as a consequence while some of the basic questions where answered some where not answered properly and therefore point hands in the direction of further work,while some relevant literature contradict each other,others where also misleading and as a consequence no proper conclusion where made in respect to so many questions and as such has left potential researchers and users even more confused

 Relevant literature has indicated the use of hurdle technology as part of the technique in order to gain effectiveness of the process,but some others reported that lower temperatures are the best to effect lethal action on microbial pathogens and therefore full effectiveness of the technique.

 Some other reports also suggested that gram positive are more resistant to pulse electric field technique as opposed to gram negative ,but no further evidence was provided to show the true effectiveness of the process,but based on logic as discussed in the earlier pages of this project,from this point of view we can say that it is because of the thickness of their cell peptidoglycan layer as opposed to gram negative bacteria which has a thinner peptidoglycan layer,since pulse electric field works on a phenomenon called electroporation,which involves the widening of the cell membrane of a micro-organism,or creating new ones leading to the death of a cell after the leakage of the cytoplasmic content.

 Also from this point of view some basic questions still need further research and this will at least facilitate the performance of further work in the area of pulse electric field, some of the inconclusive questions are suggested to answer some basic questions of the type of micro-organism of concern mostly resistant and susceptible to PEF,other questions are that of resistance and conductivity of the food involved,and most importantly the use of mathematical models to determine the time and effectiveness of the technique.

 Additionally,from this point of view even though not all the questions where answered some where answered properly and from that we can give the following final statements

-That pulse electric field can be considered as an effective non thermal permeabilization technique in food processing

-The degree of cell membrane permeabilization maybe based on the direct measurement of the conductivity of the food product that is meant to be treated.

-Electrical field intensity ,time,temperature and number and type of pulses are the main process parameter for the effectiveness of the technique,others of less importance as demonstrated by relevant literature are type of micro-organism,growth rate of the micro-organism and concentration of the micro-organism,pH,ionic strength and conductivity.

-The mathematical models used to analyse the rate and time needed to effect permeabilization is also one area that needs more research as a result of inconclusive datas.

Furthermore,apart from the external electrical and different vital variables,cell cytoplasmic membrane permeabilization is influenced by the initial physical phenomenon or conductivity of the food product to be treated,a high physical phenomenon or conductivity offers a lower resistance and so a lower field strength to supply effective permeabilization as an example r=1/R,although completely different from what was reported by relevant literature that considers physical phenomenon or conductivity as a minor issue to result permeabilization of a semipermeable membrane,this so counsel that physical phenomenon or conductivity may be a major issue and therefore must be taken into consideration within the style or design of a pulse field equipment,but additional analysis must be taken so as to higher perceive the importance of physical phenomenon or conductivity within the set up of Pulse Electric Field equipment.

 Finally, we can mention that the promising results obtained so far to design a PEF technique apparatus operating can be continuous, and indeed this apparatus will facilitate the performance of further and additional analysis oriented and needed for food product development,which indeed is necessary to answer the basic questions of how useful and important will be pulse electric field techniques in the food industry and under what conditions.

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