FOOD ENGINEERING SERIES

Pulsed Electric Fields Technology for the Food Industry

Fundamentals and Applications

Javier Raso
Volker Heinz
Editors

PULSED ELECTRIC FIELDS TECHNOLOGY FOR THE FOOD INDUSTRY

Fundamentals and Applications

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PULSED ELECTRIC FIELDS TECHNOLOGY FOR THE FOOD INDUSTRY

Fundamentals and Applications

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Library of Congress Control Number: 2005938811

ISBN-10: 0-387-31053-3
ISBN-13: 978-0387-31053-4

Printed on acid-free paper.

Printed in the United States of America.

9 8 7 6 5 4 3 2 1

springer.com

PREFACE

In the last decade, in an attempt to improve, or replace, existing food processing methods, several novel technologies have been investigated. Some of these technologies, now known as "emerging technologies," have become, especially in highly competitive markets, a very interesting alternative for the food industry, as a means of developing new foods, or improving the safety and quality of existing ones, while reducing energy consumption.

This book presents the information accumulated on Pulsed Electric Field technology (PEF) during the last 15 years, by experienced microbiologist, biochemists, food technologists, and electrical and food engineers with the object of offering to anyone interested in this subject a comprehensive knowledge in this field.

PEF is a nonthermal food processing technology involving the application, to a food placed between two electrodes, of short duration high intensity electric fields. Such treatments cause, in cells, a phenomenon known as "electropermeabilization." Electropermeabilization is a temporary or permanent permeabilization of the cell membrane. This permeabilization has shown to have very useful effects in food technology such are the inactivation of microorganisms or the extraction of cell components.

In the food industry, processing operations seeking the stabilization of foods by microbial inactivation are of the outmost importance. Thus, the capability of PEF to inactivate microorganisms at temperatures that do not cause any deleterious effect on flavor, color or nutrient value of foods, opens very interesting possibilities.

On the other hand, in some food processes, an essential and very common pretreatment step to improve mass transfer rates is the breakdown of cell membranes by different techniques such as grinding, or thermal or enzymatic treatments. Electroporation is also an alternative to these procedures, and it is more so in those processes where the complete disintegration of cell membranes is not desired.

The contents of this book are presented in three sections.

The first section, consisting of three chapters, includes the introduction and the fundamental aspects of PEF technology. In Chapter 1, the historical evolution of PEF is reviewed and a general overview of the state of the art of this technology is given. In Chapter 2, a fundamental understanding of the electrical circuits of PEF generation systems is presented and the specifications of the major components is given. Chapter 3 revised theories proposed to explain "electroporation" in eukaryote cell membrane but it is mainly focused on recent discoveries on electroporation of cell membranes of prokaryotes.

Second section is devoted to the effects of PEF on microorganisms, enzymes, main components responsible for food quality, and extraction of intracellular components.

In Chapter 4, the effect of PEF on microorganisms is treated. The design of an adequate PEF inactivation treatment requires a prior knowledge on the influence of different factors on inactivation kinetics in order to be able to develop mathematical models that allow to calculate the effect of treatments. These are aspects covered in detail in this chapter as well as the most successful combination of PEF with other preservation technologies.

In Chapter 5, the influence of PEF treatments on some food spoilage enzymes and on different components responsible for nutritional and sensorial properties of foods are discussed.

The last chapter of this section, Chapter 6, is devoted to an interesting application of PEF such is the extraction of cellular components: Fundamentals of this procedure, as well as possible industrial applications, are described in this chapter.

In the *last section*, the possible applications of PEF in food technology, in general, are discussed and information on food processing equipment is given. Several potential applications of PEF are revised in Chapter 7 and an economical analysis is also included to help potential users of the PEF to estimate costs. Finally, in Chapter 8, the main characteristics of PEF processing equipment for the food industry are discussed to help to identify requirements for the correct design and construction of safe and reliable PEF equipment.

Thanks are given to the collaborators in this book and to all the researchers of different disciplines who have contributed in the development of this technology for the food industry. With their efforts they deserve that this book becomes the comprehensive interdisciplinary reference source for researchers and industrials interested in the application of PEF technology.

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PART I

INTRODUCTION

CHAPTER 1

PULSED ELECTRIC FIELDS PROCESSING OF FOODS: AN OVERVIEW

Gustavo V. Barbosa-Cánovas and Bilge Altunakar

1. INTRODUCTION

The ever-increasing trend toward nutritionally qualified foods has challenged food technology to produce fresh-like foods by replacing thermal treatments with alternative methods of preservation. Thermal processing is a major technology that has been commonly used in the food industry to increase shelf life and maintain food safety with low processing costs (Knorr *et al.*, 1994). To qualify as an alternative method, a new technology should have significant impact on quality while at the same time maintain the cost of technology within feasibility limits. In recent years, several technologies have been investigated that have the capability of inactivating microorganisms at lower temperatures than typically used in conventional heat treatments (Lado and Yousef, 2002). Therefore, nonthermal methods correspond to the expectations for minimally processed foods of fresh quality, which have higher nutritional value because of color and flavor retention. Among all emerging nonthermal technologies, high intensity pulsed electric fields (PEF) is one of the most appealing technologies due to its short treatment times and reduced heating effects with respect to other technologies. High intensity pulse electric fields is highly appreciated as a nonthermal food preservation technology that involves the discharge of high voltage electric short pulses through the food product.

With the use of electric fields, PEF technology enables inactivation of vegetative cells of bacteria and yeasts in various foods. As bacterial spores are resistant to pulsed electric fields, applications of this technology mainly focus on food-borne pathogens and spoilage microorganisms, especially for acidic food products. In addition to the volumetric effect of PEF technology in controlling the microbiological safety of foods in a fast and homogenous manner, successful application provides extended shelf life without the use of heat to preserve the sensory and nutritional value of foods. PEF technology has the potential to economically and efficiently improve energy usage, besides the advantage of providing microbiologically safe and minimally processed foods. Successful application of PEF technology suggests an alternative substitute for conventional thermal processing of liquid food products such as fruit juices, milk, and liquid egg (Mertens and Knorr, 1992; Bendicho *et al.*, 2002a; Hodgins *et al.*, 2002).

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This chapter gives an overview of the basics of pulsed electric field processing of foods within the food industry. Evolution of technology and certain factors involved are summarized with emphasis on a general review of PEF technology.

1.1. Historical Evolution and Chronological Developments in Pulsed Electric Field Processing

There are several ways to use an electrical source for food pasteurization, in the form of ohmic heating, microwave heating, and high intensity pulsed electric fields (HIPEF). Among these, ohmic heating is one of the earliest methods. Ohmic heating relies on the use of heat generated when an electric current passes through the food, and has already been approved for viscous and particulate products, especially for aseptic processing. Application of electric fields to preserve foods first appeared with the Electro-pure method for pasteurization of milk. In this early process, heat generated by an alternating electrical current (220–4200 V) was used as a method of thermal sterilization in which heat flowed through the milk. The electrical chamber consisted of a vertical rectangular tube with opposing walls of carbon electrodes insulated with heavy glass. In this process, 100 gallons of preheated raw milk at 52°C was heated to 70°C and held for 15 s. Successful results were obtained using the Electro-pure method in the inactivation of *Mycobacterium tuberculosis* and *Escherichia coli.* The method's efficiency in inactivating some bacterial populations resistant to other thermal pasteurization methods generated the use of electric current itself (Palaniappan and Sastry, 1990). However, due to the low capacity of the system and the lack of proper equipment for temperature adjustment and control, the technology did not draw much attention or become commercially popular at that time (Getchell, 1935).

By the 1940s, electric fields were being used in the food processing process for purposes other than inactivation of microorganisms. Flaumenbaum successfully used pulsed electric fields in a process that increases the permeability of plant tissues, facilitating subsequent extraction of cellular fluid. Today, many applications of PEF focus on increasing the efficiency of juice extraction from fruits by using PEF as pretreatment (Heinz and Knorr, 2001). The electrohydrolytic method was introduced in the 1950s to inactivate microorganisms suspended in liquid systems. The inactivation was achieved through shock waves generated by an electrical arc, which also caused formation of highly reactive free radicals from the chemical components in foods. This method enabled the use of pulsed electric discharges at different energy levels to inactivate *E. coli*, *Streptococcus feacalis*, *Bacillus subtilis*, *Streptococcus cremoris*, and *Micrococcus radiodurans* suspended in sterile distilled water (Gilliand and Speck, 1967).

A variety of pulsed electric field equipment and methods was described by Doevenspeck in 1960 (Germany), including a study addressing the nonthermal effects of pulsed electric fields on microbes. The study concentrated on the interaction of pulsed electric fields and microbial cell walls. Sale and Hamilton (1967, 1968) conducted a systematic study to assess the nonthermal bactericidal effects induced by electric fields and reported the lethal effects of homogeneous electric fields on bacteria, which included *E. coli*, *Staphylococcus aureus*, *Sarcina lutea*, *Bacillus subtilis*, *Bacillus cereus*, and *Candida utilis*. Along with this study, a series of papers were published on the use of electrical field technology, which concluded that bacterial cells exposed to direct electrical fields lose their membrane properties, leading to cellular death. The killing effect of PEF was mentioned as being independent of current density, thus the bacterial destruction was due to nonthermal effects. Sale and Hamilton are considered pioneers in the field and most of their findings have been accepted, which are still being used today to assess the critical process parameters for effective PEF inactivation. They emphasized that electric field strength, pulse duration, and morphological parameters of the target microorganisms are the main factors involved in PEF application (Sale and Hamilton, 1968).

Table 1.1. International groups working on PEF (Barbosa-Cánovas *et al.*, 1999).

Institution	Country
Catholic University of Leuven	Belgium
University of Guelph	Canada
AGIR	France
University of Bordeaux	France
University of Montpellier	France
CPC Europe	Germany
Technical University of Hamburg	Germany
Technical University of Berlin	Germany
ICE Tech	Iceland
ATO-ĐLO	The Netherlands
Unilever Research Vlaardingen	The Netherlands
University of Aberdeen	Scotland
University of Lleida	Spain
University of Zaragoza	Spain
Tetra Pak	Sweden
University of Lund	Sweden
Nestle	Switzerland
Campden and Chorleywood Food Research Assoc.	UK
Natick Laboratories	USA
National center of Food Safety and Technology	USA
Ohio State University	USA
PurePulse Technologies	USA
University of Wyoming	USA
Washington state University	USA

Once the basic principles and potential of PEF technology were understood, researchers in the area of genetic engineering and medical science began exploring the action mechanism at a cellular level. Zimmermann *et al.* (1974) introduced the dielectric rupture theory using a method developed to promote cell fusion, which would provide control over the permeability of cell membrane in localized zones. This process then referred as electrical breakdown or reversible electroporation is still being used to explain the action mechanism of electric fields on cells. In the following years, several studies followed the work of Zimmerman on the action mechanism of electrical pulses, combining the theory of membrane disruption by electric fields with the previous work on process parameters, sensitivity of microorganisms, and inactivation mechanisms (Kinosita and Tsong, 1977; Sugar and Neumann, 1984; Tsong, 1990; Ho and Mittal, 1996). By the 1980s, the sensitivity of different kinds of microorganisms to pulsed electric fields was studied by a group led by Hülsheger, in which the earliest mathematical expressions were derived to describe inactivation kinetics as a function of electric field strength and treatment time (Hülsheger and Nieman, 1980; Hülsheger *et al.*, 1981, 1983).

In the 1980s, interest in PEF technology suddenly increased among scientific communities in the commercialization of PEF applications. Different from all existing technologies, exploration of PEF technology was complicated without the collaborative work of researchers from different fields of study. Multidisciplinary research groups, including microbiologists, food scientists, and electrical engineers supported by food companies, began investigating the unknowns of pulsed electric fields, which eventually became known as an emerging innovative nonthermal technology (Table 1.1).

These investigations were followed by several patents on PEF, mainly focused on the microbial inactivation, tissue response to electric fields, enzyme inactivation, engineering aspects, inactivation kinetics, modeling, and scale-up (Table 1.2).

Table 1.2. Patents on pulsed electric field technology (Sepulveda and Barbosa-Cánovas, 2005)

Reference	Patent
Held and Chauhan (2002)	Destruction of waste-activated sludge
Morshuis *et al.* (2002)	Device and method for pumpable food products
De Jong and Van Heesch (2002)	Pulse electric field treatment system
Lelieveld and Volanschi (2001)	Method and apparatus for preserving food
Zhang and Qiu (2001)	High voltage pulse generator
Mastwijk and Bartels (2001)	Integrated modular design for treatment chamber
Bushnell (2000)	A serial electrode treatment cell for pumpable food
Mittal *et al.* (2000)	Method and apparatus for electrically treating foodstuff
Addeo (2000)	Use of PEF coupled with rotational retorting
Bushnell *et al.* (2000)	Uniform product flow in a PEF treatment chamber
Qin *et al.* (1998)	Continuous flow electrical treatment chamber
Hayden (1998)	Method of inactivation in liquids
Yin *et al.* (1997)	High voltage PEF chambers for liquid products
Qin *et al.* (1997)	Continuous flow electrical treatment chamber
Zhang *et al.* (1996)	Batch mode food treatment using PEF
Bushnell *et al.* (1996)	Process for inactivation of microorganisms with PEF
Bushnell *et al.* (1995a,b)	Prevention of electrode fouling, and electrochemical effects in PEF systems
Bushnell *et al.* (1993)	PEF systems for extension of shelf life
Bushnell *et al.* (1991)	PEF systems for extension of shelf life
Dunn *et al.* (1991)	Methods for preservation by PEF
Doevenspeck (1991)	Electric impulse method and device for treatment
Dunn *et al.* (1989)	Methods for preservation with PEF
Dunn and Pearlman (1989)	Device for shelf life extension for liquid foods
Dunn and Pearlman (1987)	Methods for shelf life extension of liquid foods

One of the first industrial applications of PEF technology was the ESTERIL process, developed by Krupp Mashinentechnik (Hamburg, Germany) in the late 1980s for electrical sterilization and pasteurization of pumpable electrically conductive media (Sitzmann, 1995). Successful results were reported from these early applications (Hamburg University in conjunction with Krupp) and thus PEF technology proved to be a promising treatment for fluid foods such as orange juice and milk. In a later study, a microbial reduction of 4D was reported for *Lactobacillus brevis* inoculated milk when treated with 20 pulses of 20 μs at 20 kV/cm, *S. cerevisiae* inoculated orange juice treated with 5 pulses of 20 μs at 4.7 kV/cm, and *E. coli* inoculated sodium alginate treated with 5 pulses of 20 μs at 14 kV/cm (Grahl *et al.*, 1992). The ELCRACK process was also developed by Krupp Mashinentechnik (Hamburg, Germany), the disruption of vegetable and animal cell membranes subjected to pulsed electric fields. The process consisted of exposing the slurry of comminuted fish or meat to high intensity electric pulses, which caused disruption of the cell membranes and subsequent release of fat from the cells during the separation step (Sitzmann, 1995). PurePulse Technologies, a subsidiary of Maxwell Laboratories, developed CoolPure® pulsed electric field processing systems in 1995 for antimicrobial treatment of liquids and pumpable foods. That same year, the U.S. Food and Drug Administration (FDA) released a "letter of no objection" for the use of pulsed electric fields, thus approving industrial application of PurePulse Technologies. In 1996, PEF treatment on liquid eggs was also approved by the FDA with certain conditions to be accepted. Regulations required active and continuous communication between the FDA and production facility during development of this novel process within specified safety margins. Recognition by regulatory agencies enabled further implementations with an increasing interest in the technology.

From 2000 to the present, great advances have been achieved in the commercialization of PEF applications. However, translating the technical parameters into affordable and effective PEF systems within legal regulations is not easy. Currently, commercial PEF systems are available that include both bench-top and industrial systems, as those provided by PurePulse Technologies, Inc. and Thomson-CSF, besides many different lab-scale and pilot-scale PEF systems. The use of solid-state high voltage pulsed power systems, developed by Diversified Inc., has provided reliability and process consistency in PEF processing when used in a laboratory scaled up to commercial food processing applications. The present challenge is to increase treatment capacity with the use of feasible high power systems, by optimizing the overall PEF system design in light of critical process parameters.

1.2. Present Status of Pulsed Electric Field Technology and Applications

The extent of improvement in a food processing company achieved by an emerging technology generally reflects the interest in that technology by the food industry. The method of high intensity pulse electric fields used to inactivate microorganisms has been under research for nearly 45 years, initiated with the first patent received by Doevenspeck in 1960. During this time span the technology has proved to be most effective in the inactivation of vegetative bacteria, yeasts, and molds, while bacterial spores are much more tolerant (Qin *et al.*, 1996). Many successful steps have been taken in the design of system components and inactivation mechanism for different species, however, there are still many points that have not been fully explained. Inactivation kinetics and the effect of PEF on spores are some of the most discussed issues in recent studies. Methods applied to thermal processing technologies by plotting logs of the numbers of survivors against log or treatment time, or number of pulses, have been used to explain inactivation kinetics neglecting the deviations from linearity for these plots (Zhang *et al.*, 1995a). The lethal effects of PEF processing on spores are still being explored by means of effective delivery of pulses into the relatively nonconductive and dehydrated cytoplasm of spores, which was proposed as the main resistance mechanism (Hamilton and Sale, 1967). Additionally, the synergistic effect of PEF technology in combination with other mild preservation methods is one of the research interests popular in recent years. The use of antimicrobials as nisin or other bacteriosins has been proposed as having lethal effects on electroporation (Kalchayanad *et al.*, 1994).

Considering the effectiveness of PEF treatment on liquid products, such as milk, fruit juices, liquid egg, and any other pumpable food products, extensive research has been done to implement the process at an industrial level. Flavor freshness, economic feasibility, improvements in functional and textural attributes and extended shelf life are some of the main points of interest besides achievement of microbiological safety of food products (Dunn, 2001). Among all liquid products, PEF technology has been most widely applied to apple juice, orange juice, milk, liquid egg, and brine solutions (Qin *et al.*, 1995).

The rapid increase in the market segment of fruit juices has led manufacturers to seek ways to overcome the thermal effects of processing while increasing the shelf life of juices (Sadler *et al.*, 1992; Hodgins *et al.*, 2002). Application of PEF is especially promising for the citrus industry, which is concerned with the spoilage microorganisms and resultant production of off-flavor compounds such as lactic acid bacteria (Hendrix and Red, 1995). PEF processing has been successful in a variety of fruit juices with low viscosity and electrical conductivity such as orange, apple, and cranberry juice. Recent studies reported more than a 3-log reduction in orange juice (Qin *et al.*, 1998) and apple juice (Evrendilek *et al.*, 2000), and a 2-log reduction in cranberry juice (Jin and Zhang, 1999). *Lactobacillus brevis* inoculated in citrus juice was studied by Elez-Martinez *et al.* (2005), achieving 5.8 log reductions when processed at 35 kV/cm for 1000 μs using bipolar pulses. A 6.2-log reduction

was achieved in grape juice at 51°C when 20 pulses of 80 kV/cm were applied with nisin addition (Wu *et al.*, 2005). Additionally, the color change in fruit juices (subject to prolonged storage) was reportedly less in juices treated by PEF, as in a recent study of PEF-treated orange juice stored at 4°C for 112 days; there was less browning than thermally pasteurized juice, which was attributed to conversion of ascorbic acid to furfural (Yeom *et al.*, 2000). Retention of carotene in orange–carrot juice and color in apple juice were also reported, respectively (Evrendilek *et al.*, 2000; Esteve *et al.*, 2001).

Studies conducted on the effects of PEF on dairy products such as skim milk, whole milk, and yogurt compromise a major section of PEF applications (Alvarez and Ji, 2003). Milk is very susceptible to both spoilage and pathogenic microorganisms requiring the application of thermal pasteurization under current regulations, which ensures safety but generally results in a cooked flavor (Wirjantoro and Lewis, 1997). Dunn and Pearlman (1987) were the first researchers to conduct a challenge test prior to shelf life study on homogenized milk inoculated with *Salmonella dublin* and *E. coli* treated with 36.7 kV/cm and 40 pulses for 25 min. PEF-treated milk was reported to contain 5-log and 3-log reductions in bacterial populations for *S. dublin* and *E. coli*, respectively, when compared to untreated milk (Dunn, 1996). Another study by Qin *et al.* (1996) approved the retention of the physical, chemical, and sensory attributes of PEF-treated milk with application of 40 kV/cm of 6 pulses after 2 weeks of refrigerated storage. A significant inactivation of *Saphylococcus aureus* inoculated skim milk treated by PEF was also reported (Evrendilek *et al.*, 2004).

In addition to inactivation of microorganisms and enzymatic treatments, the application of PEF provides highly effective pretreatment of foods and food ingredients in an ecological and gentle way (Guderjan *et al.*, 2005). The ability of pulsed electric fields to destroy cell membranes by removing the cellular turgor component of the texture and exerting an estimable influence on the viscoelastic properties of the plant tissue has been used in several applications (Lebovka *et al.*, 2003). Beginning with Flaumenbaum (1968), an electrical treatment of apple mash, known as electroplasmolysis, achieved an increase in yield of about 10–12%. Further yield enhancement was achieved with treatment on carrot juice (Knorr *et al.*, 1994), red beet juice (Bouzrara and Vorobiev, 2003), and grape and sugar beet juice (Estiaghi and Knorr, 2002). The effect of combined pressure–PEF treatment resulted in permeabilization of the cell matrices, improving mass transfer across the cell membranes (Angersbach and Knorr, 1997). As a result, the treatment enhances the solid–liquid expression of different biological tissues and increases the juice yield (Wang and Sastry, 2002; Vorobiev *et al.*, 2004). The application involves the usage of electric fields at 5–10 kV/cm for 0.1–10 μs at room temperature, which could be a good alternative against traditional methods of plant tissue treatment (Lebovka *et al.*, 2005).

PEF technology has recently been used in alternative applications including drying enhancement, enzyme activity modification, preservation of solid and semisolid food products, and waste water treatment, besides pretreatment applications for improvement of metabolite extraction. The ability of PEF to increase permeabilization means it can be successfully used to enhance mass and heat transfer to assist drying of plant tissues. Studies conducted on different plant tissues such as potato tissue (Angersbach and Knorr, 1997), coconut (Ade-Omowaye *et al.*, 2000), carrots (Rastogi *et al.*, 1999), mango (Tedjo *et al.*, 2002), and apple slices (Ade-Omowaye *et al.*, 2002) reported increased yield of water removal by 20–30% when exposed to low intensity electric fields. Pretreating red peppers at the PEF-enhanced initial drying rate significantly indicated that combining PEF and partial osmotic dehydration in solutions before air drying may offer good potential in satisfactorily enhancing mass transfer rates and preserving the color quality of red peppers (Ade-Omowaye *et al.*, 2003). As stated before, PEF technology commonly focuses on processing pumpable and homogenous liquid foods free of particles and air. In the case of solid foods the mixture is mixed with

air, contributing to the low electrical conductivity of the product, hence, not limiting the maximum applicable electric field intensity. Some studies conducted on model foods (Zhang *et al.*, 1994a), viscous foods such as yogurt and rice pudding (Ratanatriwong *et al.*, 2001), or particulate foods such as pea soup with plastic beads (Dutreux *et al.*, 2000) reported successful results for PEF applications on solid or semisolid foods.

2. FUNDAMENTAL ASPECTS OF PULSED ELECTRIC FIELD TREATMENT

Thermal processing is traditionally accomplished by subjecting the food to a temperature range of 60 to above 100°C for a few seconds to minutes, involving a large amount of energy transfer to the food (Jay, 1992). Although the target of the energy is to destroy microorganisms for preservation of food, many unwanted reactions are enhanced that lead to undesirable changes, including the loss of the nutritional and organoleptic quality of food (Alwazeer *et al.*, 2003). Alternative technologies for inactivating microorganisms without reliance on heat are not new concepts, but their development for use as food preservation treatments has received considerable attention in only recent years. Nonthermal food processing technologies, with the use of ambient or near-ambient temperatures, may provide an alternative to thermal technologies by means of improving safety while maintaining product quality and economic feasibility.

Several nonthermal processing technologies were proposed on the basis of the same basic principle of keeping food below temperatures normally used in thermal processing. This would retain the nutritional quality of food including vitamins, minerals, and essential flavors while consuming less energy than thermal processing. High hydrostatic pressure, oscillating magnetic fields, intense light pulses, irradiation, the use of chemicals and biochemicals, high intensity pulse electric fields, and the hurdle concept were all recognized as emerging nonthermal technologies in recent years (Barbosa-Cánovas *et al.*, 1999). Each of the nonthermal technologies has specific applications in terms of the types of foods that can be processed. Among these, pulsed electric fields (PEF) is one of the most promising nonthermal processing methods for inactivation of microorganisms, with the potential of being an alternative for pasteurization of liquid foods. Comparable to pasteurization, yet without the thermal component, PEF has the potential to pasteurize several foods via exposure to high voltage short pulses maintained at temperatures below 30–40°C. The basic definition of PEF technology relies on the use of high intensity pulsed electric fields (10–80 kV/cm) for cell membrane disruption where induced electric fields perforate microbial membranes by electroporation, a biotechnology process used to promote bacterial DNA interchange. Induction of membrane potentials exceeding a threshold value often result in cell damage and death (Zimmermann, 1986).

PEF technology is based on a pulsing power delivered to the product placed between a set of electrodes confining the treatment gap of the PEF chamber. The equipment consists of a high voltage pulse generator and a treatment chamber with a suitable fluid handling system and necessary monitoring and controlling devices (Fig. 1.1). Food product is placed in the treatment chamber, either in a static or continuous design, where two electrodes are connected together with a nonconductive material to avoid electrical flow from one to the other. Generated high voltage electrical pulses are applied to the electrodes, which then conduct the high intensity electrical pulse to the product placed between the two electrodes. The food product experiences a force per unit charge, the so-called electric field, which is responsible for the irreversible cell membrane breakdown in microorganisms (Zimmermann and Benz, 1980). Inactivation of microorganisms exposed to high voltage PEF is related to the electromechanical instability of the cell membrane, while the dose of the application is adjusted by means of electric field intensity, number of pulses, and treatment time.

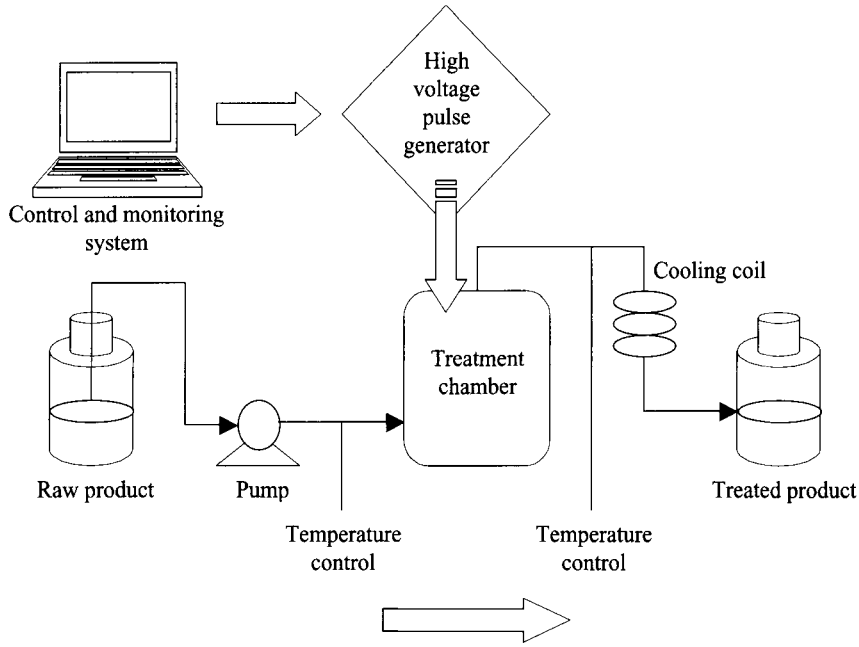


Figure 1.1. Flow chart of a PEF food processing system with basic components.

2.1. System Components

The high intensity pulsed electric field processing system is a simple electrical system consisting of a high voltage source, capacitor bank, switch, and treatment chamber. Generation of pulsed electric fields requires a fast discharge of electrical energy within a short period of time. This is accomplished by the pulse-forming network (PFN), an electrical circuit consisting of one or more power supplies with the ability to charge voltages (up to 60 kV), switches (ignitron, thyratron, tetrode, spark gap, semiconductors), capacitors (0.1–10 μF), inductors (30 μH), resistors (2Ω–10 MΩ), and treatment chambers (Gongora-Nieto *et al.*, 2002).

2.1.1. Power Supply

High voltage pulses are supplied to the system via a high voltage pulse generator at required intensity, shape, and duration. The high voltage power supply for the system can either be an ordinary source of direct current (DC) or a capacitor charging power supply with high frequency alternating current (AC) inputs that provide a command charge with higher repetitive rates than the DC power supply (Zhang *et al.*, 1996). The simplest PFN is an RC (resistance–capacitance) circuit in which a power supply charges a capacitor that can deliver its stored energy to a resistive load (treatment chamber) in a couple of microseconds, by activation of a switch (Gongora-Nieto *et al.*, 2002). Total power of the system is limited by the number of times a capacitor can be charged and discharged in a given time. The electrical resistance of the charging resistor and the number and size of the capacitors determine the power required to charge the capacitor, wherein a smaller capacitor will require less time and power to be charged than a larger one. The capacitance C_0 (F) of the energy storage capacitor is given by Eq. (1.1):

$$C_0 = \frac{\tau}{R} = \frac{\tau \sigma A}{d}, \tag{1.1}$$

where τ (s) is the pulse duration, R (Ω) is the resistance, σ (S/m) is the conductivity of the food, d (m) is the treatment gap between electrodes, and A (m^2) is the area of the electrode surface. The energy stored in a capacitor is defined by the mathematical expression:

$$Q = 0.5C_0V^2, \tag{1.2}$$

where Q is the stored energy, C_0 is the capacitance, and V is the charge voltage.

The second component of the PFN is the high voltage switching device needed to discharge the stored energy through the PFN circuit instantaneously. The switch plays an important role in the efficiency of the PEF system, and it is selected on the basis of its ability to operate at a high voltage and repetition rate. There are two main groups of switches currently available: ON switches and ON/OFF switches. ON switches provide full discharging of the capacitor but can only be turned off when discharging is completed. ON switches can handle high voltages with relatively lower cost compared to ON/OFF switches, however, the short life and low repetition rate are some disadvantages to be considered for selection. The Ignitron, Gas Spark Gap, Trigatron, and Thyratron are some of the examples from this group. ON/OFF type switches have been developed in recent years that provide control over the pulse generation process with partial or complete discharge of the capacitors. Improvements on switches, mainly on semiconductor solid-state switches, have resulted in longer life spans and better performance. The gate turn off (GTO) thyristor, the insulated gate bipolar transistor (IGBT), and the symmetrical gate commutated thyristor (SGCT) are some examples from this group (EPRI and Army, 1997; Barbosa-Cánovas *et al.*, 1999; Barsotti *et al.*, 1999; Gongora-Nieto *et al.*, 2002; Sepulveda and Barbosa-Cánovas, 2005).

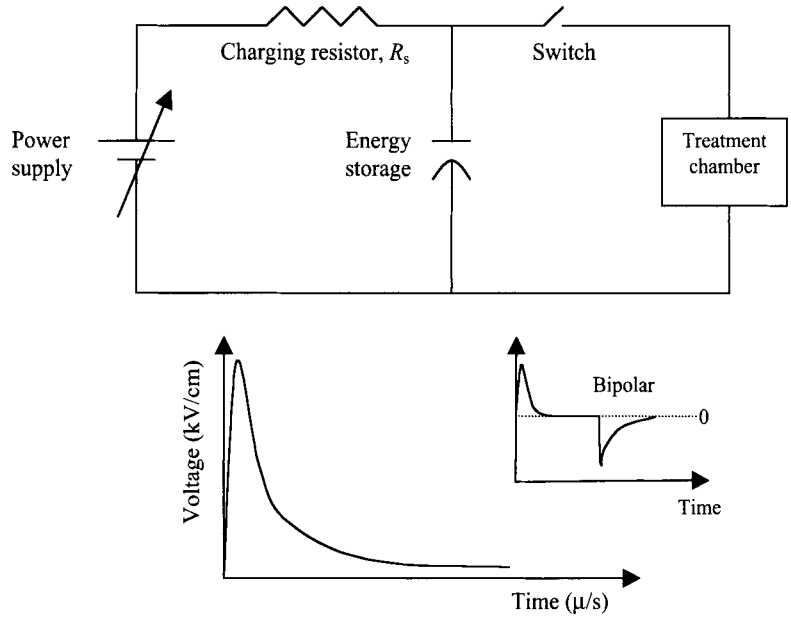
For a pulse-forming network system, the relative electrical value of each component determines the shape of the pulse. In a capacitance–resistance circuit, the pulse generated is exponentially decaying (Fig. 1.2) where the voltage across the treatment chamber as a function of time is defined as

$$V(t) = V_0e^{-t/\tau}, \tag{1.3}$$

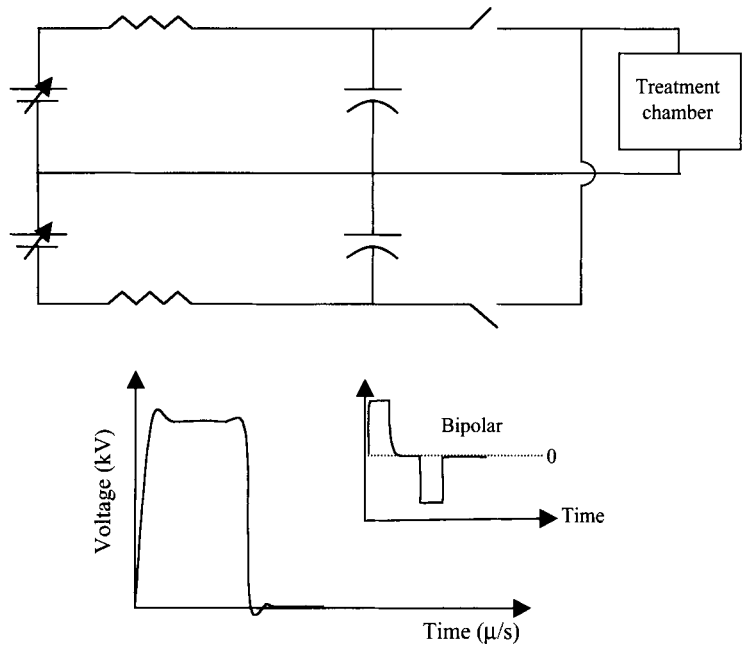
where V_0 is the voltage charged in the capacitor of the PFN, t is the pulse duration time, and $\tau = RC$ is the time constant where in an RC circuit pulse duration equals approximately five time constants (Cogdell, 1999). Considering the exponential decaying behavior of the delivered energy, τ can be adopted as the effective pulse width, calculated as the time required for the input voltage to decay to $1/e$ (37%) of its maximum value (Zhang *et al.*, 1995b; Grahl and Markl, 1996; Barsotti *et al.*, 1999; Gongora-Nieto *et al.*, 2002). More complex PFN systems can provide square pulses, bipolar pulses, and instantaneously reversal pulses, as illustrated in Fig. 1.2.

2.1.2. Treatment Chamber

One of the most important and complicated components in the processing system is the treatment chamber. PEF investigators studying inactivation and preservation effects have been highly inventive in treatment chamber design (Fig. 1.3). Several different designs have been developed through the years for this key component, wherein high voltage delivered by the power supply is applied to the product located between a pair of electrodes. The basic idea of the treatment chamber is to keep the treated product inside during pulsing, although the uniformity of the process is highly dependent on the characteristic design of the treatment chamber. When the strength of applied electric fields exceeds the electric field strength of the food product treated in the chamber, breakdown of food occurs as a spark. Known as the dielectric breakdown of food, this is one of the most important concepts to be considered in treatment chamber design. Dielectric breakdown of the food is generally characterized as causing damage on the electrode surfaces in the form of pits, a result of arching and increased



(a)



(b)

Figure 1.2. Commonly used pulse wave shapes and the generic electrical circuits: (a) Monopolar exponential decaying circuit and possible waveform; (b) Monopolar square circuit and possible waveform.

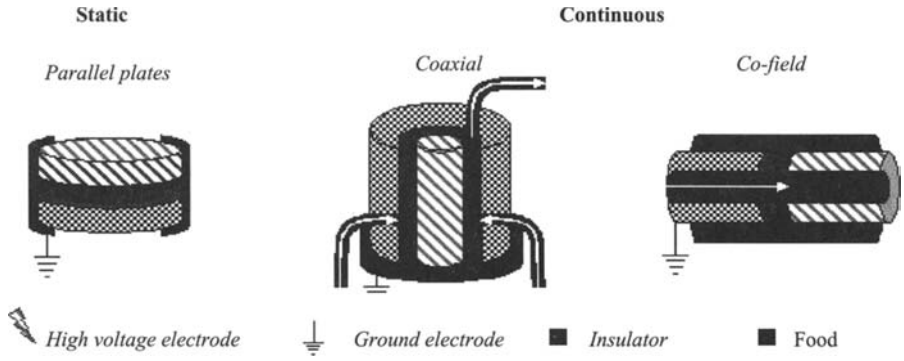


Figure 1.3. Schematic configurations of the three most used PEF treatment chambers.

pressure, leading to treatment chamber explosions and evolution of gas bubbles. Intrinsic electrical resistance, electric field homogeneity, and reduction and generation of enhanced field areas are some other important design criteria for a successful design in terms of energy consumption and low product heating (Barbosa-Canovas and Sepulveda, 2005). Treatment chambers are mainly grouped together to operate in either a batch or continuous manner; batch systems are generally found in early designs for handling of static volumes of solid or semisolid foods. Sale and Hamilton (1967), Dunn and Pearlman (1987), and Mizuno and Hori (1991) are some examples of static chamber designs, a basis for the evolution of continuous chambers that would provide advantageous pumping for efficient use in industrial applications. The concentric cylinder, concentric cone, and co-field treatment chambers are some examples of successful continuous flow chambers.

The evolution of treatment chambers began with static chambers consisting of U-shaped polyethylene spacers composed of carbon electrodes supported on brass blocks (Sale and Hamilton, 1967). The electrode area and amount of food that could be treated were regulated by using different spacers. The maximum electric field that the chamber could handle was limited to 30 kV/cm due to the electrical breakdown of air above the food. Dunn and Pearlman (1987) continued to improve designs with a chamber consisting of two stainless steel electrodes separated by a cylindrical nylon spacer. This chamber was one of the earliest designs incorporating parallel plate geometry using flat electrode surfaces separated by an insulated spacer. Liquid foods were introduced through a small aperture located in one of the electrodes, which could also be used for temperature measurement during processing. The limitations experienced with this chamber geometry were mainly due to surface tracking on the fluid, resulting in arching. Thus, conical geometry naturally evolved offering the advantage of ease in eliminating bubbles (Zhang *et al.*, 1995a). Two round-edged, disc-shaped stainless-steel electrodes were polished to mirror surfaces, and polysulfone or Plexiglas was used as insulation material. Cooling was maintained by circulating water at preselected temperatures through jackets located in the electrodes. This chamber was completely sealed and thus different from other geometries, so to prevent possible sparking or high pressure development, a pressure release device was included within the treatment chamber.

The earliest attempts at continuous treatment chamber design were by Dunn and Pearlman (1987) in their use of different geometries based on the same principle of circulating food through a closed system. The electrodes were separated from the food by ion conductive membranes made of sulfonated polystyrene, while an electrolyte was used to facilitate electrical conduction between electrodes and ion permeable membranes. New geometry designed for static operation was modified by adding baffled flow channels inside for operation as a continuous chamber (Zhang *et al.*, 1995a; Qin *et al.*, 1996). The concept of enhanced electric fields in the treatment zone was a milestone

for treatment chamber designs. The design was composed of a continuous treatment chamber with coaxial conical electrodes and introduced by Bushnell *et al.* (1993), later followed by the continuous treatment chamber with parallel electrodes coaxial cylindrical electrodes (Qin *et al.*, 1997). Among all designs to date, coaxial and co-field arrangements are currently favored over most designs. The coaxial arrangement has inner and outer cylindrical electrodes, shaped to minimize the electrical enhancements with uniform fluid flow. In a simple coaxial chamber design, the electric field intensity is not uniform and changes with change in location, as shown in the following equation (Zhang *et al.*, 1995a):

$$E = V/(r \ln(R_2/R_1), \tag{1.4}$$

where r is the radius of electric field measurements and R_2 and R_1 are the radii of the outer and inner electrodes, respectively. The advantageousness of coaxial configurations in providing a well-defined electric field distribution is based on the idea of predicting and controlling nonuniformity of electrical field distribution. Co-field designs introduced by Yin *et al.* (1997) were composed of two hollow electrodes separated by an insulator providing a tube for product flow. Based on the same principles for coaxial arrangement, co-field designs enabled handling a higher load resistance, allowing the pulser to operate at lower currents in the treatment chamber when compared with coaxial design (Dunn, 2001). Several research teams have proposed a number of different geometries for chambers, such as the glass coil static (Lubicki and Jayaram, 1997), needle-plate, and ring-cylinder continuous treatment chambers (Sato *et al.*, 2001). Limitations and the applicability of each arrangement have shaped new routes toward innovative designs.

Operation and performance of the PEF system are generally controlled by a central computer connected to the high voltage pulse generator. The computer controls the voltage and pulsing frequency in addition to operation of pumps. Data logs of temperature at different points, including flow rate, voltage, current, and power curves of applied pulses, are also recorded using appropriate probes and an oscilloscope card fed into the central computer (Barbosa-Cánovas and Sepulveda, 2005).

2.2. Effectiveness of Pulsed Electric Field Treatment

Sufficient microbial reduction by PEF has already been confirmed, however, the degree of inactivation strongly depends on several constraints. The lethality factors contributing to the effectiveness of pulsed electric field technology can be grouped as technical, biological, and media factors. Each group of determinant factors is related to type of equipment, processing parameters, target microorganism, and type and condition of media used.

2.2.1. Technological Factors

Electric field intensity has been identified as the most relevant factor affecting microbial inactivation by PEF treatment. Electric field intensity in combination with total treatment time mainly contributes to the extent of membrane disruption (Hamilton and Sale, 1967). Critical electrical field intensity must be reached for any effect to take place, and with a transmembrane potential exceeding a threshold value of around 1 V, exponential disruption is often observed resulting in cell damage and death (Hülsheger *et al.*, 1981; Zimmermann, 1986).

A good understanding of the electrical principles behind PEF technology is essential for a comprehensive analysis of the PEF system. The electrical field concept, introduced by Faraday, explains the electrical field force acting between two charges. When unit positive charge q located at a certain point within the electric field is generated in the treatment gap (E_r), it experiences force F identified by position vector r (Blatt, 1989). The electrical field per unit charge is then defined as

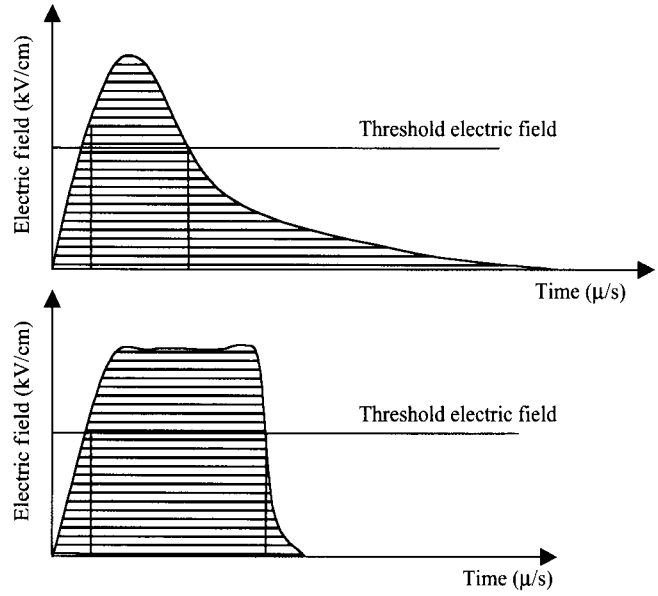


Figure 1.4. Effective energy of decaying and square pulses (Gongora-Nieto *et al.*, 2002).

shown in Eq. (1.5):

$$E_r = \frac{F_{qr}}{q}. \tag{1.5}$$

The electrical potential difference (V) between voltage across two points, separated by a non-conductive material, results in generation of an electric field between these points, with an electrical intensity (E) directly proportional to the magnitude of potential difference (V) and inversely proportional to the distance (d) between points, as given in Eq. (1.6):

$$E = \frac{V}{d}. \tag{1.6}$$

High intensity pulse electric fields can be applied in the form of exponential decay, square, wave or oscillatory—either monopolar (only positive pulses) or bipolar (alternating positive and negative pulses). Square and exponential decay pulse shapes are the two most commonly used applications due to their effectiveness to inactivate microorganisms. Square pulses are reportedly superior to exponential decay pulses since they ensure a 60% higher inactivation rate for *S. cerevisiae* and consume 15% less energy with fewer than 20 pulses applied at 12 kV/cm (Zhang *et al.*, 1994c). The superiority of square pulses over exponential decay pulses is due to the uniformity of electric field intensity during each pulse (Fig. 1.4). When maximum voltage is applied in the case of square pulses, the electric field intensity remains constant for that pulse duration. However, in the case of exponential decay, the voltage exceeds beyond an effective voltage while exponentially increasing to a peak and decreasing to zero, and therefore has no bactericidal effect, thus contributing to the heating effect and considered to be waste (Qin *et al.*, 1994; Pothakamury *et al.*, 1996).

Treatment time for a PEF application is defined as a function of pulse width and number of pulses. In RC circuits, the total pulse duration equals approximately (5τ) five time constants (Cogdell, 1999). Considering the exponential decaying behavior of the delivered energy, τ can be adopted as the effective pulse width, calculated as the time required for the input voltage to decay to $1/e$ (37%) of its maximum value (Zhang *et al.*, 1995a; Grahl and Markl, 1996; Barsotti *et al.*, 1999; Gongora-Nieto

et al., 2002). In general, PEF treatments are applied in the form of short pulses to avoid excessive heating or undesirable electrolytic reactions (Barbosa-Cánovas and Sepulveda, 2005).

2.2.2. Biological Factors

Biological factors that include the individual characteristics of target microorganisms and their physiological and growth states are determinant factors affecting PEF application. The susceptibility of a microorganism to PEF inactivation is highly related to the intrinsic parameters of the microorganism such as size, shape, species or growth state. Generally, Gram-positive vegetative cells are more resistant to PEF than Gram-negative bacteria, while yeasts show a higher sensitivity than bacteria. Induction of electric fields into cell membranes is greater when larger cells are exposed to PEF treatment (Sale and Hamilton, 1967; Hülsheger *et al.*, 1983; Zhang *et al.*, 1994b). Most of the studies on yeast activation have been conducted on *S. cerevisiae*. Among bacterial species, yeasts were reported to be more susceptible to PEF inactivation than bacterial cells, probably due to their larger size, while Gram-negative species are more resistant than Gram-positive species (Jacob *et al.*, 1981; Hülsheger *et al.*, 1983; Lubicki and Jayaram, 1997). Initially, the extensive research by Sale and Hamilton (1967) stated that neither the growth phase nor the temperature of the medium affects the inactivation mechanism. However, the higher sensitivity of microorganisms in the logarithmic phase, as compared to ones in the stationary phase, was observed in later years (Hülsheger *et al.*, 1983). *E. coli and S. cerevisiae* are two of the most studied microorganisms in the area of PEF processing. Extensive studies have been concentrated on several species including Gram-negative pathogens *Salmonella dublin*, *S. typhimurium*, and *E. coli* O157:H7; Gram-positive microorganisms *S. aureus* and *L. monocytogenes*; spore-forming pathogens *B. cereus*; and non-pathogenic flora like *P. flourescens*. In general, the effectiveness of PEF treatment is highly dependent on the individual characteristics of biological systems, which may deviate from general resistance guidelines.

In comparison with the extensive research devoted to the destruction of microorganisms by PEF, there are few reports on the inactivation of enzymes by PEF (Yeom *et al.*, 1999). Conventionally, enzymes in foods are inactivated by thermal processing (Ho *et al.*, 1997). Studies to explore enzyme inactivation by PEF first began with Gilliand and Speck (1967), however the reported studies were not consistent concerning the potential effectiveness of PEF treatment on enzymes. In recent years, Vega-Mercado *et al.* (1995) reported a 90% reduction of plasmin in addition to 60 and 80% reductions of maximum proteolytic activity of protease extracted from *Pseudomonas flourescens* dispersed in skim milk and tyriptic soy broth, respectively. In a study conducted by Ho *et al.* (1997), several enzymes dispersed in buffer solutions were assayed. For eight different enzymes, a 70–80% activity reduction was observed for lipase, glucose oxidase, and α-amylase, while the activity reduction was 30–40% for peroxidase and polyphenol oxidase and 5% for alkaline phosphatase. Lysozyme and pepsin were further reported to increase their activity after PEF treatment in this study. Giner *et al.* (2000) reported the inactivation of pectin methyl esterase (PME) from tomato. Another microbial enzyme studied (suspended in SMUF) is lipase from *P. flourescens* (Bendicho *et al.*, 2002b), for which an inactivation of 62% was reported in batch mode and 13% in the continuous flow process. Enzyme inactivation is generally explained as due to the effect of high intensity pulse electric fields (in the form of denaturation) to change the magnitude of forces acting in native structures, such as hydrophobic interactions, hydrogen bonding, ion pairing, or molecular structure. It has also been proposed that electric fields influence the conformational state of proteins, therefore contributing to enzyme denaturation (Tsong and Astunian, 1986). Compared to the number of studies reported for enzyme inactivation by PEF, little information is available on the mechanism of inactivation, which may be due to the lack of analysis of enzyme structural data (Yeom *et al.*, 1999).

Most of the research focuses on the inactivation of vegetative cells of bacteria, while only a few reports are available on the inactivation of spores describing a limited effect of PEF. *Bacillus cereus* spores were mostly resistant (approximately 1 log reduction) to a mild PEF treatment at electric field strength of 20 kV/cm and 10.4 pulses in a study conducted on apple juice (Cserhalmi *et al.*, 2002). Another study conducted by Pagan *et al.* (1998) found that *Bacillus cereus* spores were not affected with PEF treatment of 60 kV/cm for 75 pulses at room temperature. On the other hand, Marquez *et al.* (1997) reported 3.42-log and 5-log reductions of *Bacillus subtilis* and *Bacillus cereus* spores, respectively, with PEF treatment of 50 kV/cm for 50 pulses at 25°C in salt solution. Additionally, mold condispores were reported to be sensitive to PEF in fruit juices whereas *Neosartorya fischeri* ascospores were resistant to PEF treatments (Raso *et al.*, 1998). The variation in reported results could be explained as the influence of spore resistance by factors other than the process parameters, such as preparation of the spores or media factors.

2.2.3. Media Factors

Beginning with the detailed research of Sale and Hamilton (1967), most of the inactivation studies on the effectiveness of PEF treatment were accomplished using model systems that included distilled water, deionized water, phosphate buffer, and simulated milk ultrafiltrate (SMUF). The physical and chemical characteristics of food products are known to strongly influence the effectiveness of microbial inactivation during PEF application (Wouters *et al.*, 2001), thus the challenge experienced using real food systems was due to the important role of the media's chemical and physical characteristics. These factors most likely influence the recovery of injured microbial cells and their subsequent growth following PEF exposure, since the presence of food components, such as fats and proteins, has reportedly had a preventive effect on microorganisms against PEF treatment (Ho *et al.*, 1995; Grahl and Markl, 1996; Martin *et al.*, 1997). On the contrary, a possible repair process might be delayed by unfavorable growth conditions (Aronsson *et al.*, 2001). Similar to the intrinsic parameters of microorganisms, treated media has its own intrinsic factors such as conductivity, resistivity, dielectric properties, ionic strength, pH, and composition. Each of these parameters influences the PEF treatment either alone or in combination.

Conductivity and ionic strength are closely related and generally lowering the electrical conductivity of treatment media, reduces the temperature and applied power and therefore increases the electric field intensity and effectiveness of PEF application. This lowering of electrical conductivity should be at an optimal level to ensure establishment of the transmembrane potential (Hülsheger *et al.*, 1981; Jayaram *et al.*, 1993; Vega-Mercado *et al.*, 1996b; Sepulveda and Barbosa-Cánovas, 2005). The mobility of the ions in the solution is increased with increasing temperature, which in turn increases the conductivity (Heinz *et al.*, 2002).

Temperature is one factor proposed that has been correlated with microbial inactivation, and although PEF application is strictly a nonthermal processing technology, the synergistic effect of temperature on foods (due to changes in the properties of cell membranes) becomes greater when foods are subjected to high intensity pulse electric fields (Jayaram *et al.*, 1993). Therefore, the temperature of the medium in which cells are suspended has a significant influence in determining the membrane fluidity properties. At low temperatures, the phospholipids are closely packed into a rigid gel structure, while at high temperatures, they are less ordered and the membrane has a liquid-crystalline structure. This phase transition from gel to liquid is dependent on temperature and can affect the physical stability of the cell membrane (Stanley, 1991). The synergistic effect of mild temperatures (below 65°C) in combination with PEF provided promising results as an alternate technology to pasteurization (Jayaram *et al.*, 1992; Zhang *et al.*, 1995a; Aronsson *et al.*, 2001; Sepulveda *et al.*, 2002).

The influence of pH and water activity (a_w) on microbial growth was documented by Jay (1992). It was found that changes in acid content and a_w of a food dramatically affect the type of microorganism growing in that particular food, however, the relationship between a_w, pH, and pulsed electric fields was not fully explored. In earlier studies, pH was reported to have no effect on the inactivation of microorganisms (Sale and Hamilton, 1967; Hülsheger *et al.*, 1981). But more recently, the important effect of pH on inactivation kinetics was demonstrated (Vega-Mercado *et al.*, 1996a) with a study on *E. coli*, where inactivation was greater at pH 5.7 compared to pH 6.8. In a study by Wouters *et al.* (1999), microbial reduction was 3.4 log units greater for *L. innocua* at pH 4.0 than at pH 6.0. Another study conducted on mold ascospores in fruit juices (Raso *et al.*, 1998) demonstrated even higher inactivation rates with lower pH values. On the contrary, the resistance of *Salmonella senftenberg* was reportedly higher with PEF treatment at pH 3.8 than at pH 7.0 (Alvarez *et al.*, 2000).

2.2.4. *Inactivation Kinetics and Modeling*

The use of first-order kinetics to describe the relationship between inactivation and electric field strength, or treatment time, is common in most inactivation studies. The significant reduction in the inactivation rate at a given electric field when treatment times are long, so-called the tailing effect, has been mentioned by several researchers in recent years (Peleg, 1995; Cole, 1999). Considering the fact that not all microorganisms show identical resistance to inactivation, to the applied stress, it is not surprising to observe a spectrum of resistance. The tailing behavior under less stressful treatments was reported to be more obvious, compared to highly stressful conditions in which microorganisms die close together. The need for models describing the death kinetics with PEF treatment has been responded to by several researchers (Peleg, 1995; Sensoy *et al.*, 1997; Wouters *et al.*, 2001; Rodrigo *et al.*, 2003). Hülsheger and Nieman (1980) proposed the first model for PEF inactivation of microorganisms based on the dependence of the survival ratio $S(N/N_0)$ on the electric field intensity E, according to the following expression:

$$\ln(S) = -b_{\mathrm{E}}(E - E_{\mathrm{c}}), \tag{1.7}$$

where b_{E} is the regression coefficient, E is the applied electric field, and E_{c} is the critical electric field for the condition of 100% survival. The gradient of straight survival curves is expressed as regression coefficient and the critical electric field (E_{c}) was reported to be a function of cell size and pulse width. Another version of this model was introduced by Hülsheger *et al.* (1981) relating the microbial survival fraction (S) with PEF treatment time (t), as in the following equation:

$$\ln(S) = -b_{\mathrm{t}} \ln\left(\frac{t}{t_{\mathrm{c}}}\right), \tag{1.8}$$

where b_{t} is the regression coefficient, t is the treatment time, and t_c is the critical treatment time obtained for t when survival fraction is 100%. Rearrangement of these models enabled derivation of kinetic constants for cited microorganisms (Hülsheger *et al.*, 1983). The susceptibility of a microorganism to inactivation was correlated to kinetic constants where a small value for kinetic constant indicates a wide span in the inactivation rate curve.

Peleg (1995) proposed a second model describing the sigmoid shape of the survival curves for PEF treatment. The model basically represents the percentage of surviving organisms as a function of the electric fields and applied number of pulses. In this model, the steepness of the sigmoid shape is represented by a critical electrical field intensity corresponding to 50% survival (E_d) and a kinetic

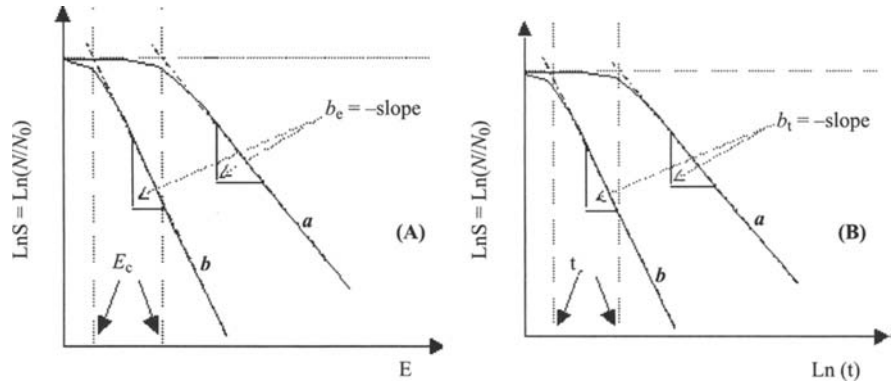


Figure 1.5. Dependence of microbial survival fraction on the (A) electric field and (B) treatment time. Curves, *a*, correspond to resistant microorganisms and curves, *b*, to sensitive microorganisms *S*, survival fraction; *N*, microbial count; *E*, electric field; *b*, kinetic constant; *t*, time. Subscripts: 0, initial; c, critical; t, time; e, electric field.

constant as a function of number of pulses (K_n), as given in the following equation:

$$S = \frac{1}{1 + e^{\frac{E - E_d}{K}}}, \tag{1.9}$$

where a small value for K indicates a wide span in the inactivation rate curve and lower sensitivity to PEF, and a small value for E_d indicates less resistance to PEF treatment (see Fig. 1.5).

3. FUTURE ASPECTS AND ECONOMIC ANALYSIS OF PEF PROCESSING

Today's consumers' expectations that food products provide convenience, variety, adequate shelf life and caloric content, reasonable cost, and environmental soundness make the modifications of existing technologies and the adoption of novel processing technologies essential. Industrial food producers in competitive markets increasingly have to consider novel processing technologies in order to increase food safety and the ability to offer better products to consumers. As stated earlier in this chapter, the potential of PEF technology to replace traditional processing methods has further inspired researchers from both academia and industry to explore the unknowns of pulsed electric fields, while achieving improvements in current techniques. Possible factors related to the effectiveness of PEF technology have already been scanned and further research is being conducted to reach a complete understanding and to minimize drawbacks. At this point, in order to prove the applicability of PEF technology for practical use, laboratory outcomes must achieve a certain level of optimization for the process to gain importance.

3.1. Combination Studies

A major trend in the application of inhibitory techniques is to employ new combinations of techniques that deliver effective preservation without the extreme use of any single technique (Leistner, 2000). Even though nonthermal inactivation is claimed in PEF processing, the inactivation effect of PEF on microbial flora and the shelf life extension of refrigerated products can be increased by applying PEF in combination with other stress factors, such as the presence of antimicrobial compounds like nisin and organic acids, increased water activity, pH, and mild heat.

Coupling of electric field treatments at moderate temperatures (50–60°C) is one of the most appealing combinations studied so far due to the synergistic effect on the inactivation of microorganisms, where at constant electric field strength, inactivation increases with rise in temperature. Temperatures used in combinations with PEF are held below those used in the pasteurization and sufficient cooling is provided to ensure proper food temperatures. Sensoy *et al.* (1997) achieved nearly a 2-log cycle increase in the inactivation of *Salmonella dublin*, by increasing the process temperature from 10 to 50°C with 100 μs PEF treatment at 25 kV/cm. Reina *et al.* (1998) reported the same results with *Listeria monocytogenes* inoculated in whole milk and treated with 600 μs PEF treatment at 30 kV/cm, where 3.5-log and 4-log reductions were achieved at 10 and 50°C. Other microorganisms such as *Salmonella enteridis*, *E. coli*, and *Lactobacillus brevis* have shown similar effects. The use of mild sublethal thermal conditions in combination with PEF has proved to enhance the effectiveness of treatment, however the mechanism behind inactivation has not been determined yet. Schwan (1957) proposed the hypothesis that reduction in the charging time of bacterial membranes due to increased electrical conductivity of the media caused by increased temperatures was a factor. Slightly higher temperatures were suggested to change the phase state of cell membranes (Jayaram *et al.*, 1992) or to weaken the cell membrane, thus favoring destabilization and cell death (Pothakamury *et al.*, 1996; Vega-Mercado *et al.*, 1996b).

In the presence antimicrobial agents affecting the microbial membrane, such as nisin or organic acids, an increased inactivation in response to PEF was observed (Pol *et al.*, 2000). The exposure of *L. innocua* to nisin after PEF treatment has an additive effect on the inactivation of microorganisms compared to PEF alone (Calderon-Miranda *et al.*, 1999; Terebiznik *et al.*, 2000). Even though the mechanism behind this synergistic effect has not been fully understood, PEF treatment was suggested to facilitate the incorporation of nisin into the cytoplasmic membrane, resulting in formation of larger pores on the microbial membrane (Pol *et al.*, 2000). As stated as a media factor before, lowered pH is one of the substantial hurdles enhancing the efficacy of other antimicrobial processes. PEF inactivation of *E. coli* O157:H7 in a 10% glycerol solution was enhanced synergistically by lowering pH from 6.4 to 3.4 using sorbic and benzoic acids (Liu *et al.*, 1997). In a study conducted by Evrendilek and Zhang (2003), lowering pH of *E. coli* O157:H7 to 3.6 before PEF treatment resulted in higher inactivation rate compared to pH of 5.2 or 7.0. Similar to the nisin effect, 5% added ethanol increased the efficiency of PEF treatment on vegetative *Bacillus subtilis* cells due to the ability of ethanol to alter fluidity of microbial membranes (Heinz and Knorr, 2000). Acidification of the medium with hydrochloric acid, on the other hand, did not show extra inactivation of raw milk's microflora when compared to PEF alone (Smith *et al.*, 2002). Considering the different effects of lowered pH on PEF, much more research is required for commercial adaptation (Yousef, 2001).

The effect of combined high pressure and PEF treatment on the inactivation of vegetative *Bacillus subtilis* cells after exposing the cells to a high pressure of 200 MPa for 10 min with increasing specific pulse energy was increased inactivation. Kinetic changes in membrane components such as phase transition of lipids or proteins suggested these factors contributed to the level of inactivation by PEF (Heinz and Knorr, 2000).

3.2. Drawbacks/Limitations

A lot of research in the field of food engineering has focused on new preservation technologies, but very few of these methods have been implemented by the food industry until now due to their limitations and drawbacks. Besides numerous possibilities and the advantages of PEF technology there are some shortcomings that future research should address. The main limitations of this technology are scaling up of the system; bubble formation leading to electrical breakdown of the treated product concerning safety issues; particulate foods; availability of commercial units; and resistance of some microbial species including bacterial spores.

The major concern for commercialization of PEF technology, considering competition in the market, is the initial investment cost. Generation of high voltage pulses with sufficient peak power is the main limitation in processing large quantities of fluid economically. Industrial PEF equipment used to be rare and expensive, having a limited capacity of around 1800 L/h (Mittal *et al.*, 2000), however with proper equipment, PEF is an energy efficient process compared to thermal pasteurization. The price of PEF equipment from bench-top to pilot plant models ranges from 40,000 to $500,000, and operating cost is estimated around $0.02/L (Gongora-Nieto *et al.*, 2002). Despite high capital investments, operation costs proved to be lower than traditional thermal processing technologies (Barbosa-Cánovas *et al.*, 1999). With recent developments, commercial-scale PEF systems processing between 1000 and 5000 L of liquid foods per hour are available, which would add only 0.03–$0.07/L to final food costs (OSU, 2005).

The application of the technology is restricted to food products with low electrical conductivity capable of withstanding high electric fields. PEF technology has demonstrated to be applicable for pumpable foods while not much information exists on solid and particulate foods. PEF-treated products are therefore limited to some extent in order to provide uniformity of electric field distribution within the treatment chamber (Qin *et al.*, 1995).

The inactivation mechanism of PEF is assumed to be due to hyperpolarization and subsequent dielectric breakdown. However, in some cases, this high intensity short time pulsing can generate electrochemical reaction products with bactericidal properties, depending on the medium composition. Therefore, the model medium for inactivation studies should be conducted in properly chosen systems to exclude indirect inactivation by electrochemical reaction products. In the case of treatment of foods, more attention is required to design treatment conditions that minimize electrochemical changes in order to avoid loss in product quality and safety (Kristien *et al.*, 2004).

As a final perspective, one of the major hurdles to commercialization is the lack of experimental standardization between PEF studies to date, which in effect disables the development of a common language for parameters and measures. Numerous critical process factors, diversity of experimental conditions, and a wide range of equipments complicate the process of comparing the effectiveness of treatments, hence limiting the conclusions drawn on the influences of different factors on microbial inactivation (Wouters *et al.*, 2001). In general, evaluation of PEF treatment delivery has relied on estimations of treatment time and the electric field experienced by a food product while confined within a treatment chamber. However, experts in this technology agree on the experimental inconsistencies due to the lack of methods that can accurately measure the treatment delivery (CFSAN-FDA, 2000). Additionally, energy density or energy per pulse is rarely reported in most of the studies except for ones mentioned qualitatively correlated with the inactivation level (Evrendilek *et al.*, 2000; Giner *et al.*, 2000; Heinz *et al.*, 2002; Zhang *et al.*, 1994c). In fact, low energy of certain pulse waveforms and overall energy consumption of the PEF treatment are very attractive to the food industry due to the possibility of lower costs compared to most other technologies (Ho *et al.*, 1997; Gongora-Nieto *et al.*, 2004).

The fundamental aspects of PEF technology, both from a historical and technological point of view, were reviewed in this chapter. Continued research on the use of pulsed electric fields for food preservation combined with development of more effective and affordable processing systems would help to overcome hurdles and make commercial processing available in the near future.

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CHAPTER 2

GENERATION AND APPLICATION OF HIGH INTENSITY PULSED ELECTRIC FIELDS

Markus J. Loeffler

1. INTRODUCTION

The generation of pulsed electric fields at high-power levels is one of the tasks of pulsed power engineers. In this chapter, we define pulsed power technologies to be those electrical devices that are used to treat organic and inorganic materials with electric fields >10 kV/cm and/or magnetic fields >10 T. As a rule, they work at power levels >0.1 GW, which are power levels that are not available from small- or medium-sized conventional energy sources (Bödecker, 1985). Typical durations of single or repetitive power pulses are in the range between nanoseconds and milliseconds. Most pulsed power devices use capacitors to store electrical energy that is delivered to their respective electric loads via a high-power switch.

The energy stored in capacitors is used to generate electric or magnetic fields. Electric fields are used to accelerate charged particles, leading to thermal, chemical, mechanical, electromagnetic wave, or breakdown effects. Electromagnetic fields transfer energy as electromagnetic waves. X-ray, microwaves, and laser beam generation are typical examples. Magnetic fields facilitate the generation of extremely high pressures ranging from 0.1 GPa to many GPa. These effects are applied to modify molecules to remodell, compress, weld, segment, fragment, or destroy materials; and to modify the surface of organic and inorganic parts and particles (Weise and Loeffler, 2001).

In general, those devices that are used to electrically pulse treat liquid foods or other aqueous substances have an electrode gap filled with a more or less conductive liquid. From an electrical point of view, these devices can be compared to water resistors driven either in the "normal operation mode" (e.g., electroporation) or in the "failure mode" (e.g., shock wave treatment). In the first case, a current is driven across the entire cross-section of the liquid, whereas in the second case an electrical breakdown occurs followed by high intensity current flowing through the small cross-section of an arc. The specific power requirements of such "resistors" together with information about the required mass flux and the dimensions of the treatment zone define the type and the specific parameters of the power supply.

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The objectives of this chapter are to provide basic information

1. about the power and energy requirements of PEF systems
2. about high-power sources
3. about low-power sources.

2. ELECTRIC LOAD REQUIREMENTS

From the point of view of an electrical engineer, organic material in water is a bad insulator surrounded by a highly permittive medium, i.e., water. The effect of stressing organic materials under water with an electric field is shown in Fig. 2.1 (Loeffler *et al.*, 2001). The rubber membrane was positioned between two electrodes under water. A voltage was applied between the electrodes. Because of the high permittivity of the water, the electric field was concentrated in the rubber membrane itself thus causing electrical breakdown at several locations on the foil. Increasing the field and the treatment time, increased the number of holes in the foil.

The electrical specifications of devices used to inactivate microorganisms by perforating their cell membranes require some fundamental information about their electric load: strength of the electrical field, specific power consumption, mass flow rates, average power consumption, voltage waveforms, and treatment durations. Because of the different treatment profiles needed for different applications, a "characteristic application" with "characteristic values" shall be defined by averaging and evaluating values and information gained from a large number of experiments published in the open literature.

2.1. Specific Power Consumption

The specific power consumption of liquids treated with electrical pulses is defined by

- their specific electrical conductivity and
- the electric field strength required to kill the cells contained in the liquid.

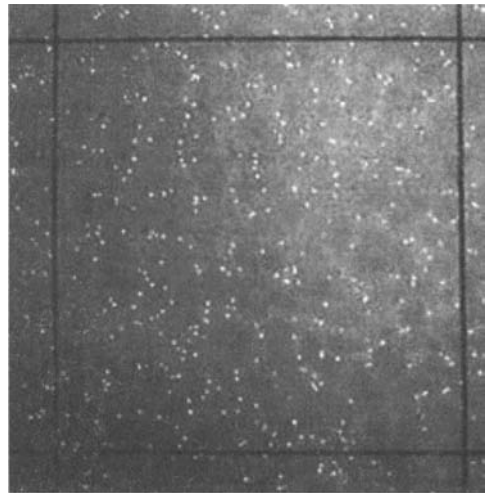


Figure 2.1. Effect of pulsed electric fields on rubber membrane under water.

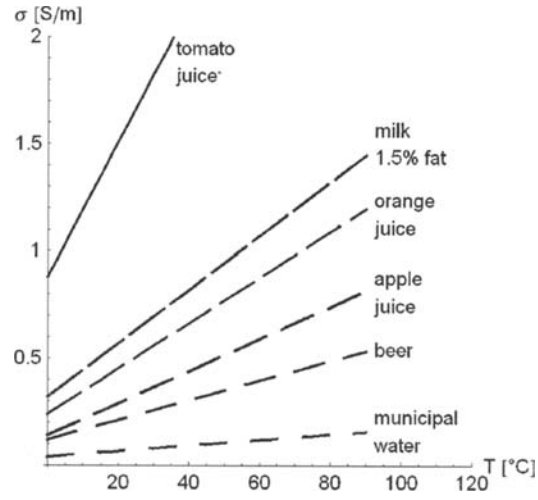


Figure 2.2. Conductivities of selected liquids.

In the following, the focus will be on "electroporation devices" rather than shock wave devices. Figure 2.2 shows the conductivities of some selected liquid foods at different temperatures (Reitler, 1990). For comparison, Fig. 2.3 shows in a logarithmic scale the range of specific electrical conductivities σ of aqueous liquids together with the conductivities of insulating, semiconducting, resistive, and conducting materials. Depending on their ingredients, aqueous liquids have conductivities comparable to semiconducting materials. They vary between 10^{-2} and 10 S/m.

To treat aqueous liquids successfully, electrical field strengths E, which is defined to be the applied voltage u divided by the distance d between the electrodes, should be in the range between 10^0 and 10^2 kV/cm (Heinz *et al.*, 2001; Gaudreau *et al.*, 2004). Figure 2.4 gives an overview of the results from several experiments in which different microorganisms in different fluids were treated (Barbosa-Cánovas *et al.*, 2000). This figure shows a reduction in viability by a factor of about $10^{3.5}$

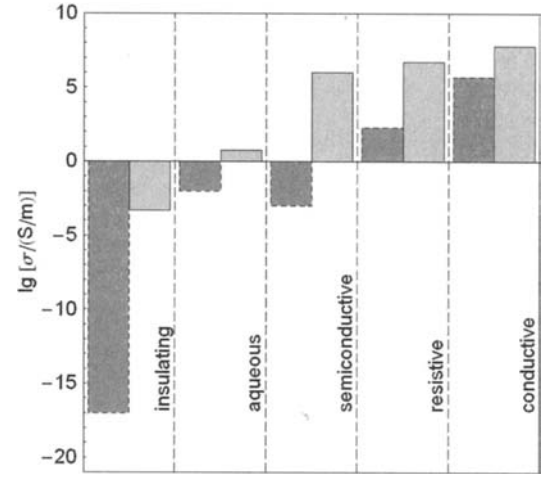


Figure 2.3. Conductivities of different materials.

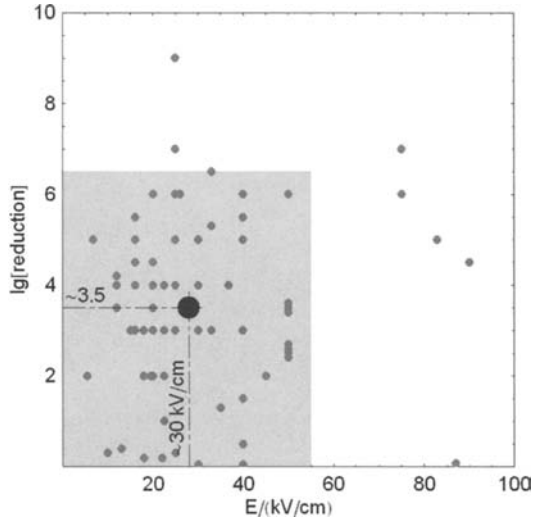


Figure 2.4. Overview of the reduction effect of different electrical field strengths on different materials.

when the electrical field strengths were about 30 kV/cm. Note that these data do not represent optimal values, because in most of these experiments exponential voltage waveforms were used (for more information about the influence of voltage waveforms on the inactivation of biological material see below).

The power densities p required to apply field strengths E across materials with conductivities σ is given by

$$p = \sigma \cdot E^2. \tag{2.1}$$

Figure 2.5 combines the values of the specific conductivities given in Fig. 2.3 with the electric field strengths given in Fig. 2.4. Applying formula (2.1) to the mean values of the conductivity and of the field strength ($\sigma_{av} \approx 0.3$ S/m, mean value taken from the logarithmic values; $E_{av} \approx 30$ kV/cm, mean value taken from the linear values) yields in an average power density of $p \approx 3$ GW/L. To treat 1 L of a typical liquid requires for short time (1–1000 µs) powers at the GW level. Large power plants deliver electrical power in the GW range. By this it is obvious that power converters are essential to convert the power available from the public network into the power required at the treatment facility. In principle, comparable considerations of "shock wave devices" yield the same results.

The generation of electrical power at such levels requires capacitors and/or coils.

2.2. Average Power

Another requirement of the load is its specific energy consumption, accumulated over all pulses, together with the rate of flow of the substances to be treated. In a logarithmic scale, Fig. 2.6 gives information about the specific energies applied in several experiments. The data taken from Heinz *et al.* (2002, 2003), Ulmer *et al.* (2002), Min *et al.* (2003a,b), Ngadi *et al.* (2003), Toepfl *et al.* (2004), and Gaudreau *et al.* (2005) are marked with vertical lines. Note that the data are not optimum values, because energetic optimization was not the aim of these experiments. For standardization purposes, the units of the original values, given in kJ/L, were transformed into MJ/t, assuming that

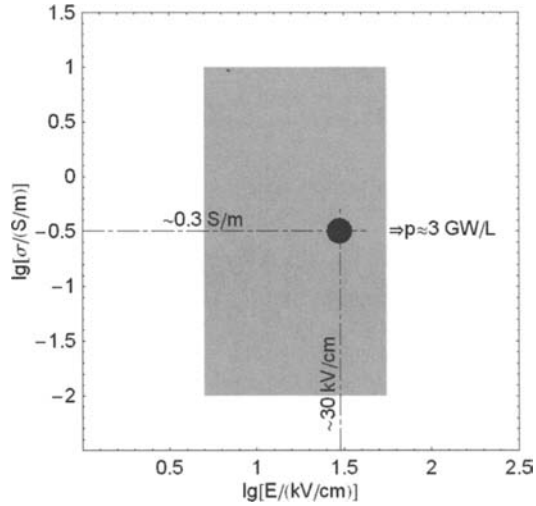


Figure 2.5. Range of specific conductivities and electric field strengths.

the density of the respective liquids was ~1 kg/L (Heinz *et al.* 2002). As an average value taken from the logarithmic scale, $w \approx 50$ MJ/t may be a typical specific energy. Common industrial flow rates for different applications are marked with horizontal lines in a logarithmic scale (Heinz, V., personal communication, 2005). As an average value taken from the logarithmic scale, $\dot{m} \approx 20$ t/h may be a typical mass flow rate. The average power $\bar{P}$ required to treat mass flows $\dot{m}$ with specific energies w can be calculated with

$$\bar{P} = \dot{m} \cdot w. \tag{2.2}$$

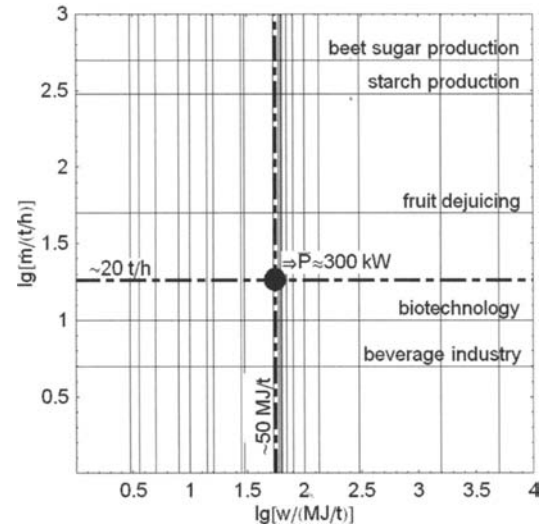


Figure 2.6. Range of specific energies and of rates of flow.

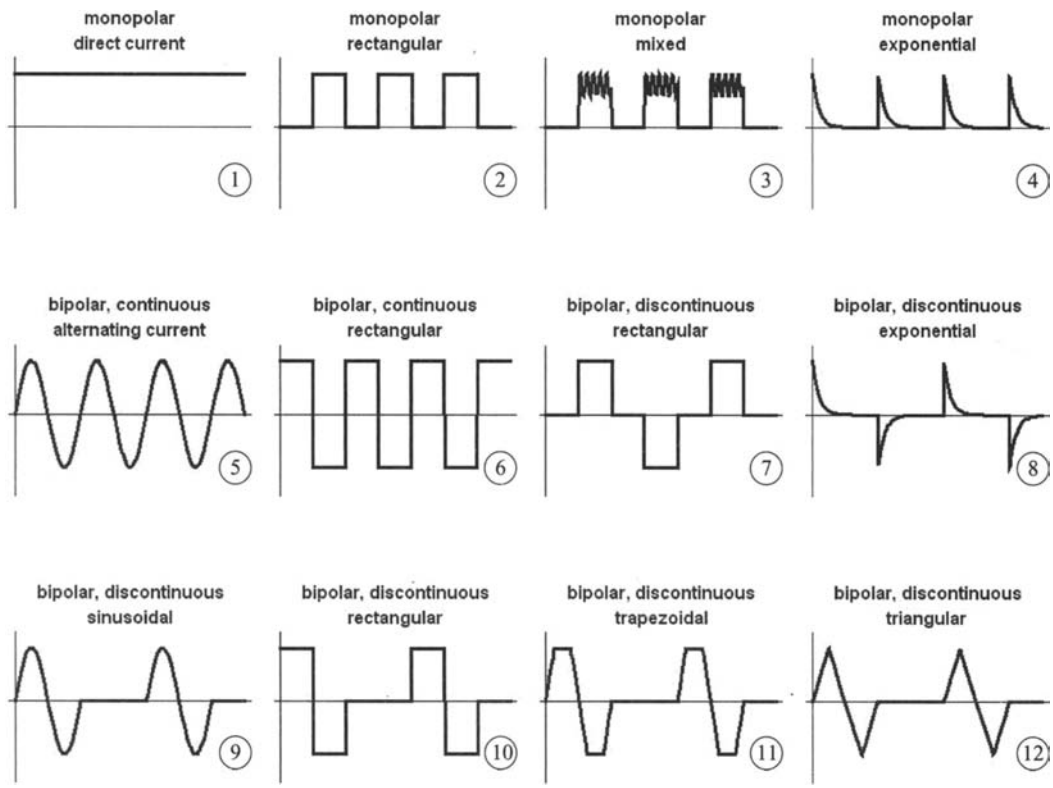


Figure 2.7. Typical voltage waveforms.

Applying Eq. (2.2) to the average values, yields a typical value of the average power, which in this case is $\bar{P} \approx 300$ kW.

2.3. Voltage Waveforms

Typical voltage waveforms used to generate high intensity pulsed fields are either monopolar or bipolar (see Fig. 2.7). Monopolar waveforms have constant, rectangular, exponential, or mixed waveshapes, whereas bipolar waveforms are sinusoidal, triangular, trapezoidal, continuous rectangular, discontinuous rectangular, or discontinuous exponential (Lazarenko *et al.*, 1977; Papchenko *et al.*, 1988a,b; McLellan *et al.*, 1991; Zhang *et al.*, 1995; Barbosa-Cánovas *et al.*, 2000; Bazhal, 2001; Kotnik *et al.*, 2001a,b; Jemai *et al.*, 2002; Kotnik *et al.*, 2003; San Martín *et al.*, 2003).

The effects of these waveforms on several substances mainly were tested in small-scale experiments. Inactivation effects were only measured if the voltage amplitudes reached a value greater than the critical values given by the specific electric field strengths specified above. For large-scale applications as defined by the typical values given above, continuously acting waveforms without pause between single monopolar or bipolar pulses will not be practicable due to the extreme power requirements. Compared to monopolar pulses, bipolar pulses have a slight effect on the permeabilization of the cell membranes at moderate voltages, whereas none (Kotnik *et al.*, 2001b) to some (Qin *et al.*,

1994) significant effects were registered relative to the number of cells killed at voltages higher than the critical voltage. However, applying bipolar pulses, like the voltage waveform in Fig. 2.6, remarkably reduces the erosion of the electrodes (aluminum, stainless steel) in the treatment chamber (Johnstone and Bodger, 1997; Kotnik *et al.*, 2001a). The voltage steepness du/dt at the beginning and at the end of rectangular pulses has no detectable influence on the efficiency of electropermeabilization, if this steepness is essentially smaller than the pulse length itself (Kotnik *et al.*, 2003). For same pulse lengths, trapezoidal, triangular, and mixed voltage waveforms yield the same effectiveness as rectangular voltage waveforms, but only at higher peak voltages (Kotnik *et al.*, 2003). Exponential voltage waveforms are not too energy efficient, since they have a long tail at electric field strengths lower than the required critical values. During this time, they only heat-up the aqueous substance without any further effect on the biological material to be inactivated (San Martín *et al.*, 2003).

From these findings it can be stated that voltage waveforms (2), (7), and (10) seem to be best suited for industrial applications. However, voltage waveforms (4) and (9) should also be considered due to their widespread usage, ease of generation, and relative low cost.

2.4. Pulse Lengths and Repetition Rates

Independent of the voltage waveforms, typical pulse lengths vary between 1 and 1000 µs at pulse rates between 1 and 300 and at pulse repetition rates between 1 and 2000 pps (Barbosa-Cánovas *et al.*, 2000; Kotnik *et al.*, 2001a; Gaudreau *et al.*, 2004).

2.5. Conclusion

To conclude, it can be stated that industrial power modulators for the treatment of liquid foods and other biological liquids or aqueous materials should have the following typical values:

- Specific electrical data for medium treatment
  - Specific conductivity of the medium
    - range: 10^{-2}–10 S/m
    - typical value: 0.3 S/m
  - Electric field strength in the medium
    - range: 1–100 kV/cm
    - typical value: 30 kV/cm
  - Power density
    - range: 2 MW/L–30 GW/L
    - typical value: 3 GW/L
  - Specific energy consumed in the medium
    - range: 0.5–5000 MJ/t
    - typical value: 50 MJ/t
- Specific data for facilities
  - Mass flow
    - range: 5–300 t/h
    - typical value: 20 t/h
  - Average power
    - range: >20 kW
    - typical value: 300 kW

- Treatment time
  - range of single pulse durations: 1–1000 μs
  - range of pulse rates: 1–3000
  - typical value of treatment time (rectangular pulses): 10 μs
- Pulse repetition rate
  - range: 1–2000 pps
  - typical value: 1 pps (remark: this value yields for laboratory devices; for industrial applications higher repetition rates in the order of ~100 pps are required).

For each specific application, these values have to be confirmed by experiments.

3. PULSED POWER SYSTEMS

Figure 2.8 shows the general block diagram of a pulsed power system that generates pulsed electric fields. The resistive electrical load—the PEF treatment chamber—is powered by a high-power/high-voltage source. The high-power source itself is powered by a low-power source connected to a 50 Hz (60 Hz) public or local network.

The PEF treatment chamber consists of one or more electrode gap(s) filled with the substance to be treated. The electrodes must be shaped to ensure a more or less homogeneous electrical field. Contamination of the substance to be treated by the electrode material should be as small as possible.

The high-power source has to deliver the voltage required by the load at the right amplitude (1–100 kV), in the right form and at the right time (μs–ms). The main parts of the high-power source are one or more capacitors, as primary energy storage, on-switches and/or off-switches, and inductors as secondary energy stores.

The low-power source converts the AC voltage of the network to DC voltage or DC current. The DC current charges the capacitor(s) of the high-power source in the right amount of time to the voltage required to drive the high-power source.

The public or local network has to deliver the peak power requested from the low-power source.

The following chapter sections will describe the following in more detail:

- PEF treatment chambers
- high-power sources
- low-power sources.

Although not all the technical possibilities relative to the construction of electric pulsers can be covered in this overview, the reader will be acquainted with their technical possibilities and limitations.

One of the critical components of pulsed power generators is the switch. Therefore, we will begin with a discussion of switches.

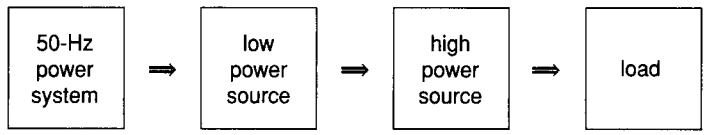


Figure 2.8. Basic set-up of pulsed power systems for the generation of pulsed electrical fields.

3.1. General Remarks About Switches in Pulsed Power Generators

As a rule in working with pulsed power devices, the load has to be electrically separated from the high-power source while it is being charged by the low-power source. After completion, the low-power source should be separated from the high-power source by switches and/or by properly chosen ohmic resistances. In general, this task can be solved by using commercially existing components.

If, under large-scale industrial conditions, the load consumes energy at power levels in the multi-MW to GW range, the final switch between the high-power source and the load has to transfer this energy at this power level. If, as an example, the load consumes energy at a peak power of 1 GW and at a voltage of 100 kV, the on/off-switch has to handle before/after switching a voltage of 100 kV and after/before switching a current of 10 kA. This task does not change, if, instead of a single switch, multiple switches are used in parallel and/or in series. This step has to be done, if switches for such voltages and currents are not available. In addition to the other parameters that need to be taken into consideration (di/dt, cost integral, recovery time, etc.), the replacement of a single switch by multiple switches can lead to other demanding tasks like the synchronization of the switches.

3.2. PEF Treatment Chambers

From the electrical point of view, the PEF treatment chamber represents the electrical load consisting of two or more electrodes filled with the liquid substance to be treated. The chamber has to be constructed in such a way that the electrical field acting on the liquid is more or less homogeneous across the entire active region. This goal can be reached in principle with planar, coaxial, and axial electrode geometries. Figure 2.9 shows sketches of the cross-sections and of the top views of these basic geometries.

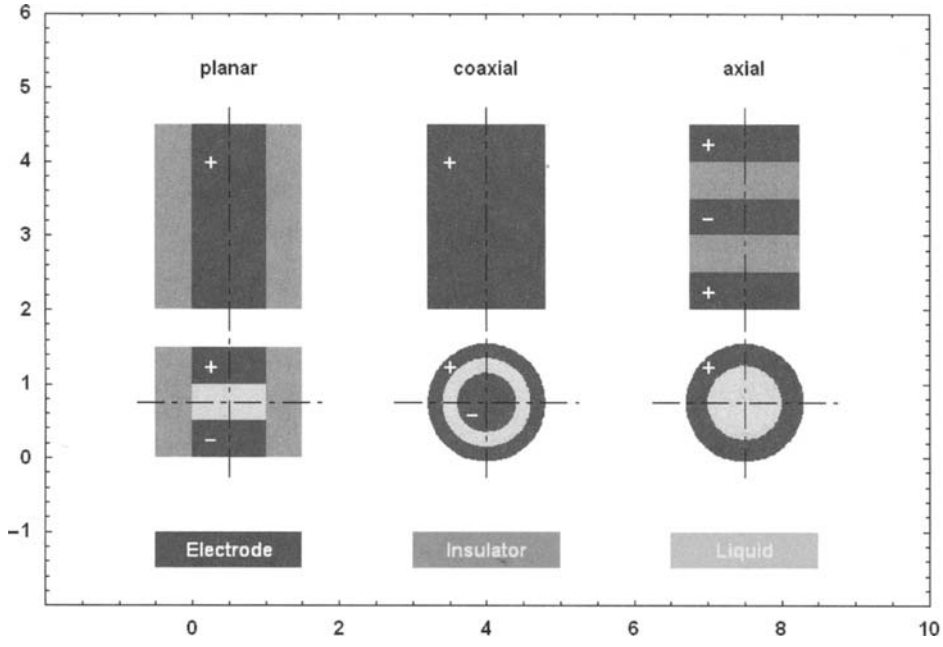


Figure 2.9. Typical electrode geometries.

Planar electrode configurations consist of two parallel electrodes fixed by insulators. The insulators and the electrodes form a channel for the streaming liquid. Coaxial electrode configurations consist of two coaxial electrodes. The liquid streams between these electrodes that are fixed by insulators not shown in the figure. Axial electrode configurations consist of several electrode rings on alternating potentials separated by insulating rings.

Planar electrode configurations ensure homogeneous fields provided their separation distance is not significantly larger than their width. In the case of coaxial electrodes, the relation between the outer radius and the inner radius of the active zone should be smaller than 1.23 to ensure that the electric field does not decay by more than 10%. To ensure homogeneous fields in the main treatment zone of the axial electrode configurations, the direct proportionality of the electrode distance to the diameter of the channel should be maintained (Gaudreau *et al.*, 2004).

In addition to geometrical effects, the homogeneity of the electrical field also strongly depends on the permittivity and on the conductivity of the liquid. The separation distance of the electrodes is restricted by the voltage available from the high-power supply. In commercial applications, voltages on the order of 100 kV are difficult to handle and, in many cases, offer a hard restriction. For exponentially damped voltages, field requirements of ~30 kV/cm lead to electrode gap separations of ~3 cm. Another restriction is the amount of power available from the high-power source. Assuming a maximum power of $\hat{P} \approx 1$ GW $\approx$ (e.g., this would relate to currents of ~10 kA at voltages of ~100 kV) and assuming a typical power density of $p \approx 3$ GW/L consumed by the liquid, the volume of the active treatment zone would be restricted to

$$V = \frac{\hat{P}}{p} \approx 300 \text{ mL}.$$

For electrode separation distances of ~3 cm, the active area of the electrodes would be restricted to ~100 cm^2.

An overview of some PEF treatment chambers investigated so far is given in (U.S. Food and Drug Administration, 2000). Actually, their volume is between 0.1 and 200 mL with electrode gaps between 0.1 and 1 cm.

Electrode materials also play an essential role. If monopolar voltage waveforms are applied, electrode corrosion can become critical and the substance to be treated can be contaminated. In commercially available electroporation devices with small probes, aluminum, stainless steel, carbon, gold-plated electrodes, and even silver electrodes are used (Barbosa-Cánovas *et al.*, 2000; Puc *et al.*, 2004). Stainless steel electrodes suffer with problems such as electrolysis, formation of deposits, electrode corrosion, and transfer of particles into the treated media (Barbosa-Cánovas *et al.*, 2000). Carbon electrodes are better for the inactivation of cells, since they have seemingly lower erosion rates, than stainless steel electrodes (Barbosa-Cánovas *et al.*, 2000). Erosion can be minimized by applying bipolar voltage waveforms (Johnstone and Bodger, 1997; Kotnik *et al.*, 2001a).

3.3. High-Power Sources

Several methods exist for generating pulsed electric fields. Depending on the specified type of voltage wave form, as well as on the available elements (capacitors, inductors, transformers, switches), the possibilities vary between simple circuits and very sophisticated networks. In the following, several important possible pulsed power circuits will be discussed. The explanations are restricted to the essentials. Practical devices, based on the circuits presented herein, are more complex due to technical safety and failure handling necessities. There are also other possible schemes that are driven by the physical and technical needs of the components, as well as that of the continuously changing ohmic properties of the load.

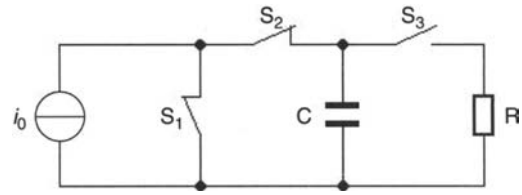


Figure 2.10. Capacitive circuit.

Basically, these circuits can be distinguished as being

- basic pulsed power circuits
- circuits with voltage multipliers
- pulse forming circuits or
- networks with pulse forming switches.

3.3.1. Basic Pulsed Power Circuits

3.3.1.1. Capacitive circuits

Capacitive circuits are the most simple and popular way to generate high-power pulses. Figure 2.10 shows the basic scheme of a capacitive circuit.

When switch S_1 opens, capacitor C is charged by the low-power current source i_0 (e.g., a pulse width modulator, see below; charging with constant voltage via a series resistor would reduce the energy efficiency to 50%). During time t, the capacitor voltage u_C increases according to $u_C = i_0 \cdot t/C$. The power demand also increases in accordance with the formula $P_C = i_0^2 \cdot t/C$. The average power can be calculated by using $\bar{P} = i_0 \cdot u_0/2$, whereas the peak power of the current source is two times the average power. If voltage u_0 equals the initial voltage $u_{R,0}$ at the load, the capacitor stores energy according to this relation $W_C = C \cdot u_0^2/2$. Switch S_2 is opened and switch S_1 is closed. Switch S_3 is activated. The capacitor exponentially discharges via the load R. The path of the standardized

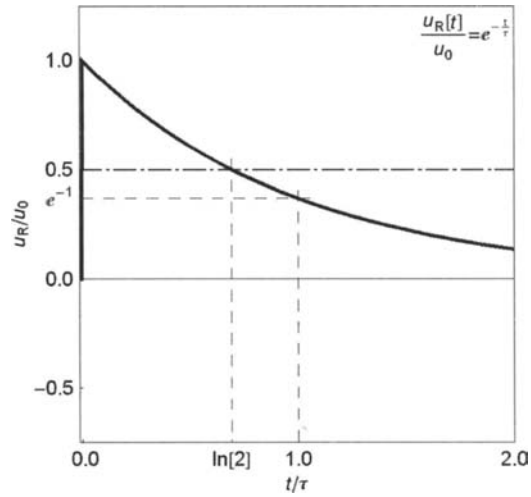


Figure 2.11. Standardized load voltage in a capacitive and inductive circuits.

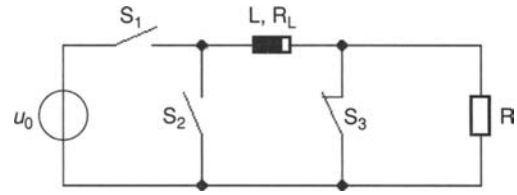


Figure 2.12. Inductive circuit.

voltage u_R/u_0 across the load is shown in Fig. 2.11, where it plotted as a function of the normalized time t/τ. The time constant τ of the discharge circuit is defined by $\tau = R \cdot C$. The voltage decays to 50% of its initial value at $t/\tau = \ln(2) \approx 0.693$. At this time, the capacitor has delivered 75% of its energy to the load. The capacitive power and energy at this moment have decreased to 25% of its initial value.

3.3.1.2. Inductive circuits

Figure 2.12 shows the basic scheme of an inductive circuit. After closing switch S_1, the coil represented by its inductance L and resistance R_L is charged by the low-power voltage source u_0 (e.g., bridge converter; charging with a constant current source would reduce the energy efficiency to 50%) via switch S_3. The coil current i_L increases according to the formula $i_L = u_0 \cdot (1 - e^{-\delta \cdot t})/R_L$, where the coil's self-damping constant is $\delta = R_L/L$. When the working current $i_0 = u_{R,0}/R$, with initial voltage $u_{R,0}$ at the load, is reached, the inductance stores an energy $W_L = L \cdot i_0^2/2$. Switch S_1 is opened and switch S_2 is closed (the inductance is "crowbared"). Switch S_3 is opened, commutating the current into the load. The inductance discharges exponentially via the load R. The path of the voltage across the load is the same as that shown in Fig. 2.11. Here the time constant τ of the circuit is defined by $\tau = L/(R + R_L)$. Again the voltage reaches 50% of its initial value at $t/\tau = \ln(2) \approx 0.693$. As in the case of capacitive circuits, at this time the inductance has delivered 75% of its energy to the load and its own resistance and the power stored in the inductor has decreased to 25% of its initial value.

The inductor can also be charged by a charged capacitor instead of by the low-voltage source u_0. In general, fast repetitive pulsed inductive circuits are not as effective as capacitive circuits. The reason is that nonsuperconducting coils of reasonable shape have a power consuming resistance, that decreases the overall energetic efficiency of the process.

3.3.1.3. Ringing circuits

In addition to capacitive circuits, series and parallel ringing circuits are another simple and popular way to generate high-power pulses. Figure 2.13 shows the basic diagram of a series circuit.

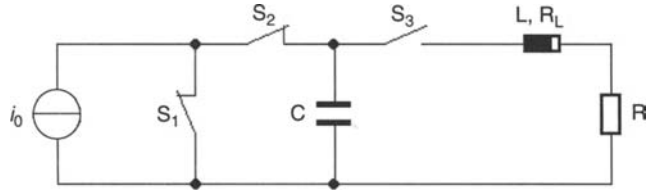


Figure 2.13. Serial ringing circuit.

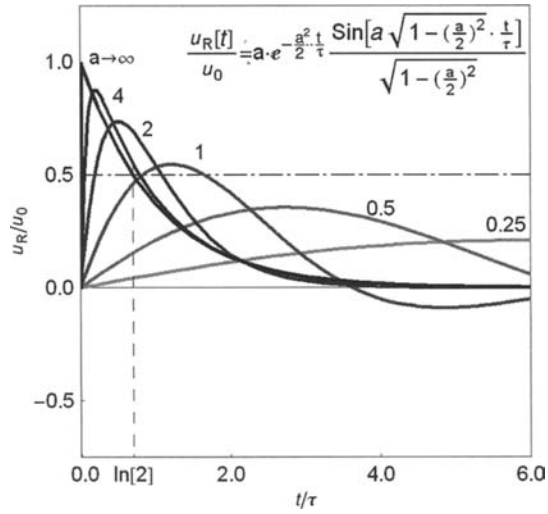


Figure 2.14. Standardized load voltage in a serial ringing circuit.

A capacitor C is charged via a low-power current source i_0 and the switches S_1 and S_2 (compare to the capacitive circuit).

If the capacitor reaches the voltage u_0, switch S_2 opens, switch S_1 closes, and switch S_3 is activated. The capacitor discharges via the coil (L, R_L) and the load R. The history of the normalized load voltage is shown in Fig. 2.14 as function of the normalized time t/τ, where $\tau = (R + R_L) \cdot C$ and $a = (R + R_L)/L$. Note that the formula given in the figure also holds for negative arguments under the root sign. The highest voltage can be achieved for $a \to \infty$ when the inductance L is small. In this case, the ringing circuit behaves like a capacitive circuit. The load peak voltage decreases significantly when $a < 2$.

Figure 2.15 shows the basic diagram of a parallel circuit. After opening switch S_1, capacitor C is charged by the low-power current source i_0. After reaching voltage u_0, switch S_2 opens, switch S_1 closes, and switch S_3 closes. The capacitor discharges via the inductance L, its resistivity R_L, and the load R. The history of the normalized load voltage for $R_L \ll R$ is shown in Fig. 2.16, as a function of the normalized time $\tau = R \cdot C$ and as function of $a = 4 \cdot R^2 \cdot C/L$. The highest voltage can be achieved for $a \to 0$ when the inductance L is very large. In this case, the ringing circuit behaves like a capacitive circuit. In practice, this behavior is maintained up to values $a < 0.1$.

Ringing circuits are less effective than capacitive circuits, either due to voltage reduction in the inductor (series) or due to the reduction in time the voltage is maintained beyond the $u_0/2$ level (parallel).

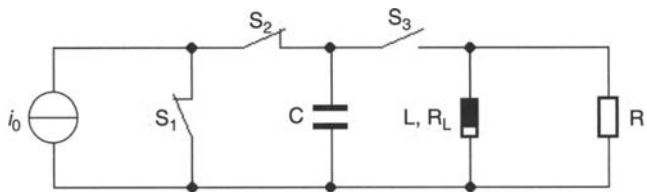


Figure 2.15. Parallel ringing circuit.

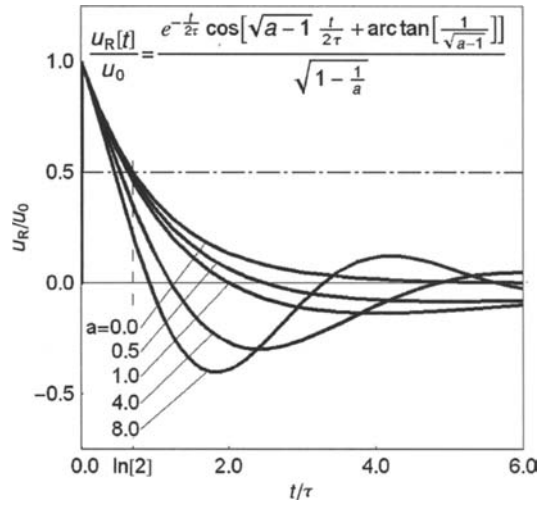


Figure 2.16. Standardized load voltage in a parallel ringing circuit.

3.3.2. Circuits with Transformers or Other Voltage Multipliers

In the circuits discussed above, the on- and off-switches have to maintain a full load voltage (capacitive and inductive circuits); that is, the voltage across the capacitors (capacitive and ringing circuits). At voltages above 100 kV and for repetition rates of ~1 Hz or more, the availability of high-lifetime switches, as well as operation of the facilities become more and more critical. In this case, transformers or voltage multiplying circuits should be taken into consideration. In the following, coils will only be represented by their inductances, assuming that their ohmic power losses are neglectable compared to the energy input into the PEF load.

3.3.2.1. Circuits with pulse transformers

One way to multiply voltages is to use transformers that are switched between the high-power source and the load. As an example, Fig. 2.17 shows the situation for capacitive circuits.

Capacitance C can be charged and discharged, as in capacitive circuits without transformer. If the windings of the transformer, with inductances L_1 and L_2, are coupled perfectly (where the mutual inductance between the windings is $M \approx \sqrt{L_1 \cdot L_2}$), the transformer amplifies the voltage by a factor of $m \approx \sqrt{L_2/L_1}$. Because the discharge frequencies usually exceed 1 kHz, such transformers must be either air core transformers or ferrite transformers. Looking backward from the load to the capacitor, the circuit behaves like a parallel ringing circuit (Fig. 2.15), where inductance L

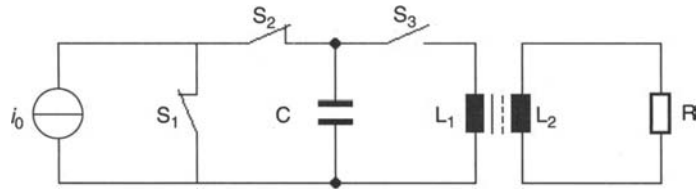


Figure 2.17. Capacitive circuit with pulse transformer.

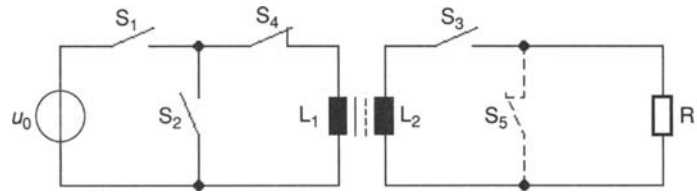


Figure 2.18. Circuit with storage transformer.

is replaced with $m^2 \cdot L_1$ and the capacitance C is replaced with C/m^2 at the same voltage level as the load. This circuit shows a nearly capacitive behavior, if $a = 4 \cdot R^2 \cdot C/(m^4 \cdot L_1) < 0.1$ and if $L_2 = m^2 \cdot L_1 > 40 \cdot R^2 \cdot C/m^4$. Compared to the capacitive circuit, the capacitance C and the on-switch S_3 only have to achieve a voltage u_2/m.

However, in real circuits with pulse transformers, the power consumed in the on-switch is slightly higher than in the capacitive circuits due to the unavoidable stray inductances and ohmic resistances in the pulse transformer. Furthermore, the additional pulse transformer increases the cost. Capacitive circuits with pulse transformers are only cost-effective, if the on-switches with the required hold-off voltages are not available or, due to other technical problems, cannot be connected in series. They also are financially practical if the current source is not able to deliver the charging power at the required voltage level.

3.3.2.2. Circuits with storage transformers

Figure 2.18 shows a circuit with a storage transformer and no capacitor. After closing switch S_1, the low-power voltage source u_0 drives a current i_1 across the inductance L_1 of the transformer. Switch S_3 is open, so that no current is induced in the secondary coil L_2. The transformer behaves like an inductor L_1, so that the circuit behaves like an inductive circuit. After charging L_1 to the desired current level, S_1 is re-opened and switches S_2 and S_3 are closed. Assuming perfect magnetic coupling between the transformer windings ($M = \sqrt{L_1 \cdot L_2}$) and after the powerless opening of switch S_4, the magnetic flux and the energy of primary inductance L_1 transfers to the secondary inductance L_2. The current across L_2 can be calculated by using $i_2 = i_1 \cdot \sqrt{L_1/L_2}$. Opening switch S_5 commutates i_2 into the load, resulting in an initial load voltage $u_R = R \cdot i_2$. The circuit again behaves like an inductive circuit with the time constant $\tau = L_2/R$ and with an exponentially damped current i_2 (Figs. 2.11 and 2.12). When switch S_5 is introduced into the circuit, the relationship between the primary and the secondary inductance should be chosen so that $L_2 \ll L_1$ which means that the switches on the primary side of the transformer can act at lower voltages, lower currents, and at lower power during the charging of L_1 and during switching at the end of the charge cycle.

In this scheme, the circuit has no advantage over inductive circuits, because switch S_5 has to overtake the full voltage at the load. In principle, the circuit also works without this switch. Without switch S_5, switch S_4 would have to overtake the voltage $u_{S4} = \sqrt{L_1/L_2} \cdot u_2$. In this case, the primary inductance, in order to act as a voltage multiplier, has to be essentially smaller than the secondary inductance: $L_1 \ll L_2$. Although this relieves the high voltage requirement for switch S_4, it has to be considered that the overall switching power of this switch, at least, keeps the same value as that of the switching power of switch S_5 in the preceding situation.

As in the case of capacitive circuits with pulse transformers, the transformer has stray inductances and ohmic resistance. Both increase the power requirements of the switches and of the voltage source on the primary side of the transformer. With respect to high-voltage applications, PEF circuits with storage transformers are less effective than inductive circuits.

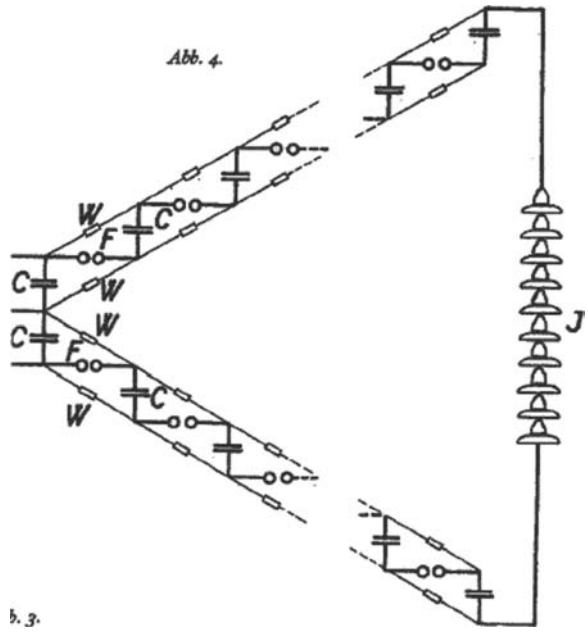


Figure 2.19. MARX-generator (from Marx, 1923).

3.3.2.3. Voltage multiplier I (MARX-generator)

The basic operating principles of the MARX-generator were first published by E. Marx in 1923 (Marx, 1923). The original application of this voltage multiplying circuit was to test isolators and other electrical high-voltage devices. Figure 2.19 shows the original scheme. In two branches n capacitors C are connected in parallel via resistors W. Switches F connect the high potential and the low potential terminals of successive capacitors (in this case spark gap switches). The ends of the capacitor branches connect to an isolator J (respectively, the load). The capacitors are charged by a current source via the resistors to the same voltage. Igniting switches F connects the capacitors in series. By doing this, voltage multiplication occurs at the load. The resistors W discouple the capacitors if their resistance is essentially higher than the (transient) resistance of the load. The overall capacitance discharging via the load is given by C/n. The resistors can also be replaced with opening switches if necessary. In this case, these switches have to open shortly before the switches F are ignited. After switching, the discharge behavior is equivalent to the discharge behavior of the capacitive circuit.

In MARX-generators, the on-switches have to maintain only $1/n$ of the voltage at the load. They have to conduct the load current. The low current source charging the capacitors only has to deliver $1/n$ of the voltage than that required for the basic capacitive circuit at n times higher charging current. During charging, the overall capacitance of the capacitors switched in parallel has to be n^2 times higher than in the capacitive circuit.

As opposed to capacitive circuits, the capacitance and the main on-switch are divided into several smaller switches and larger capacitances connected in series when system discharges. The overall volume, as well as the overall mass, of the capacitors will be slightly larger than that for basic capacitive circuits when the same energy is to be stored in the capacitors due to added buswork. The overall switching power remains the same. As in the case with pulse transformers, MARX-generators should only be chosen, if, with regard to the voltage requirements at the load, no adequate switches and/or capacitors and/or current sources are available.

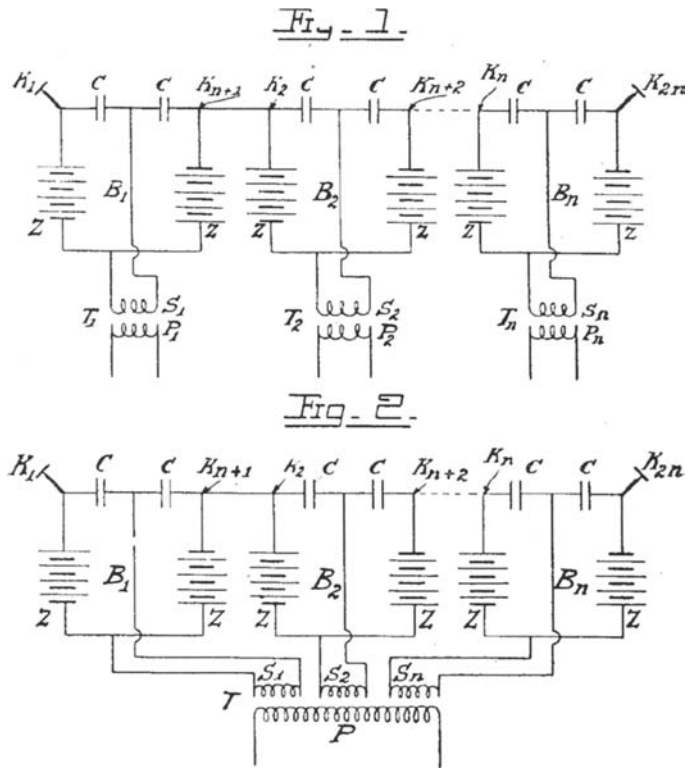


Figure 2.20. Original GREINACHER cascade (from Greinacher, 1919).

3.3.2.4. Voltage multiplier II (GREINACHER cascade)

The GREINACHER cascade was invented by H. Greinacher in 1917 (Greinacher, 1919). Figure 2.20 shows a picture from the original source, whereas Fig. 2.21 shows its circuit diagram as it is used today. An AC-voltage source with a voltage amplitude $\hat{u}_{\sim}$ drives, via a charging resistor R_{ch}, a column consisting of capacitors C and diodes D. After charging the capacitors via the diodes D, the $n = 3$ capacitors C on the right side are each charged to $<2 \cdot \hat{u}_{\sim}$ and are discharged in series via a switch S to the load R. The resulting initial voltage on the load is $<6 \cdot \hat{u}_{\sim}$, with a resulting capacitance of $C/3$. n modules, consisting of n capacitors and n diodes, provide an initial voltage $\hat{u}_{\mathrm{load}} < 2 \cdot n \cdot \hat{u}_{\sim}$ across the load at an overall capacitance C/n. The diodes in the circuit are driven at low power, because they only have to overtake the charging current and double the voltage amplitude at the voltage source. The main switch S has to handle the entire energy transfer at load powers. The efficiency of Greinacher cascades decreases with increasing number of stages due to the unavoidable ohmic losses in the diodes that hinders charging each capacitor to $2 \cdot \hat{u}_{\sim}$. From this point of view, MARX-generators provide higher efficiencies.

3.3.2.5. Current multiplier (XRAM-generator)

Published by E. Marx and W. Koch in 1966 (Marx and Koch, 1970), Figure 2.22 shows the original scheme, n coils with inductance L_1 are connected in series via opening switches (2.2). At the ends of each inductor, closing switches (3) (in this case spark gaps) are connected. The inductors are charged in series by the voltage source (2.1) to a current $i_{1,0}$. After reaching the desired current

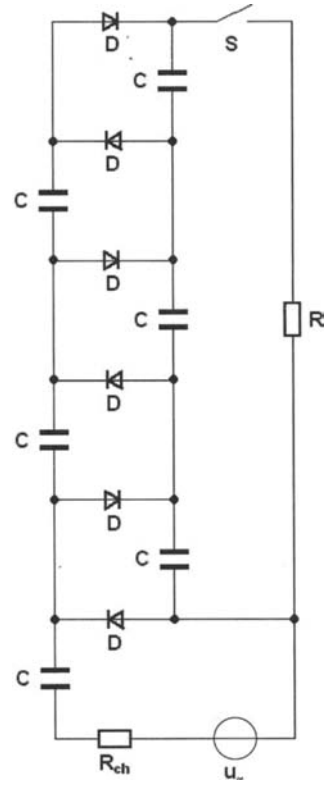


Figure 2.21. GREINACHER cascade.

level, switches (2a) and (2.2) open and switches (3) close. By doing this, the inductors are switched in parallel, generating an initial current $i_{2,0} = n \cdot i_{1,0}$ across the load (4). The current generates an initial load voltage $u_{R,0} = n \cdot R \cdot i_{1,0}$. After switching, the discharge behavior is equivalent to that of the basic inductive circuit.

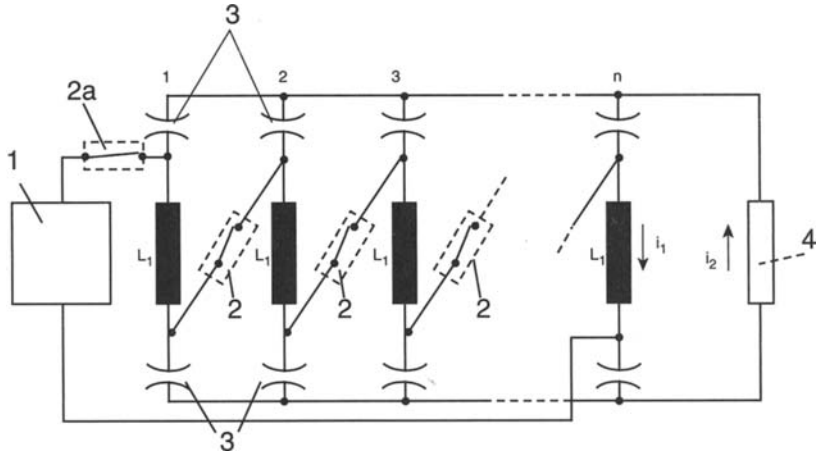


Figure 2.22. XRAM-generator (from Marx and Koch, 1970).

In XRAM-generators, the opening switches have to maintain only $1/n$ of the current across the load. However, they have to withstand the load voltage. The closing switches have to withstand $\leq$50% of the charge voltage at $1/n$ of the current level at the load. The low-power voltage source, charging the inductances, has to deliver $1/n$ of the current required in the basic inductive circuit, but at n times higher charging voltage. During charging, the overall inductance of the single coils switched in series has to be n^2 times greater than that of the inductive circuit. The overall volume, as well as the overall mass, of the coils will comparable to the basic inductive circuits due to the same amount of energy being stored in the coils for the same charging times. The necessity of repetitive simultaneous switching of the off-switches causes problems that make the application of this type of generator very difficult. Thus, XRAM-generators are not applicable to PEF applications.

3.3.3. Pulse Forming Networks

Pulse forming networks allow the generation of monopolar or bipolar rectangular voltage waveforms preferred for PEF treatment applications.

The properties of pulse forming networks (PFNs) are based on the fundamental principles of transmission lines. So, prior to an explanation of the electrical behavior of PFNs, the principal electrical behavior of transmission lines will be briefly explained.

3.3.3.1. Pulse forming lines

An ideal lossless transmission line (or pulse forming line) may be represented by a coaxial cable of length l, inductance $L = L' \cdot l$, and capacitance $C = C' \cdot l$. Charging the capacitance of this transmission line to a voltage u_0, or, respectively, to an energy $W_C = C \cdot u_0^2/2$, and discharging the line via a switch S into a load with resistance $R = \sqrt{L/C} = \sqrt{L'/C'}$ generates a single rectangular voltage pulse with amplitude $u_0/2$ and a duration of $\tau = 2 \cdot \sqrt{L \cdot C} = 2 \cdot l \cdot \sqrt{\varepsilon_r}/c$. ε_r is the relative permittivity of the line's insulator and c is the velocity of light. Circuit diagrams of this circuit are presented in the two pictures at the top of Fig. 2.23.

When the switch is triggered and if the line to the load is short-circuited at its other end, a bipolar rectangular voltage waveform with amplitude $\pm u_0/2$ is generated, where each pulse has a pulse length of $\tau/2$ (see Fig. 2.23, pictures in the center).

As a third possibility, the transmission line is split in two parts and a resistance $2 \cdot R$ reconnects the lines as seen in the bottom diagrams in Fig. 2.23. Short-circuiting the left end of the line generates a rectangular voltage waveform of duration $\tau/2$ at a voltage amplitude u_0.

These examples show the flexibility of transmission lines with respect to the generation of rectangular voltage waveforms. In all cases, the energy originally stored in the line's capacitance is completely transferred to the load. However, the following example will show the disadvantage of this solution.

With the following reasonable values

- load voltage $u_{R,0} = 100$ kV
- load resistance $R = 10\Omega$
- pulse duration $\tau \approx 15$ µs
- pulse energy $W = 5$ kJ

the specification of the pulse forming line would be as described below.

For a relative permittivity of $\varepsilon_r \approx 3$, the length of the line has to be $l \approx 1.2$ km. To store 5 kJ at a voltage of 200 kV (only 50% of this voltage acts on the load), C' is calculated to be $C' = 250$ µF/km.

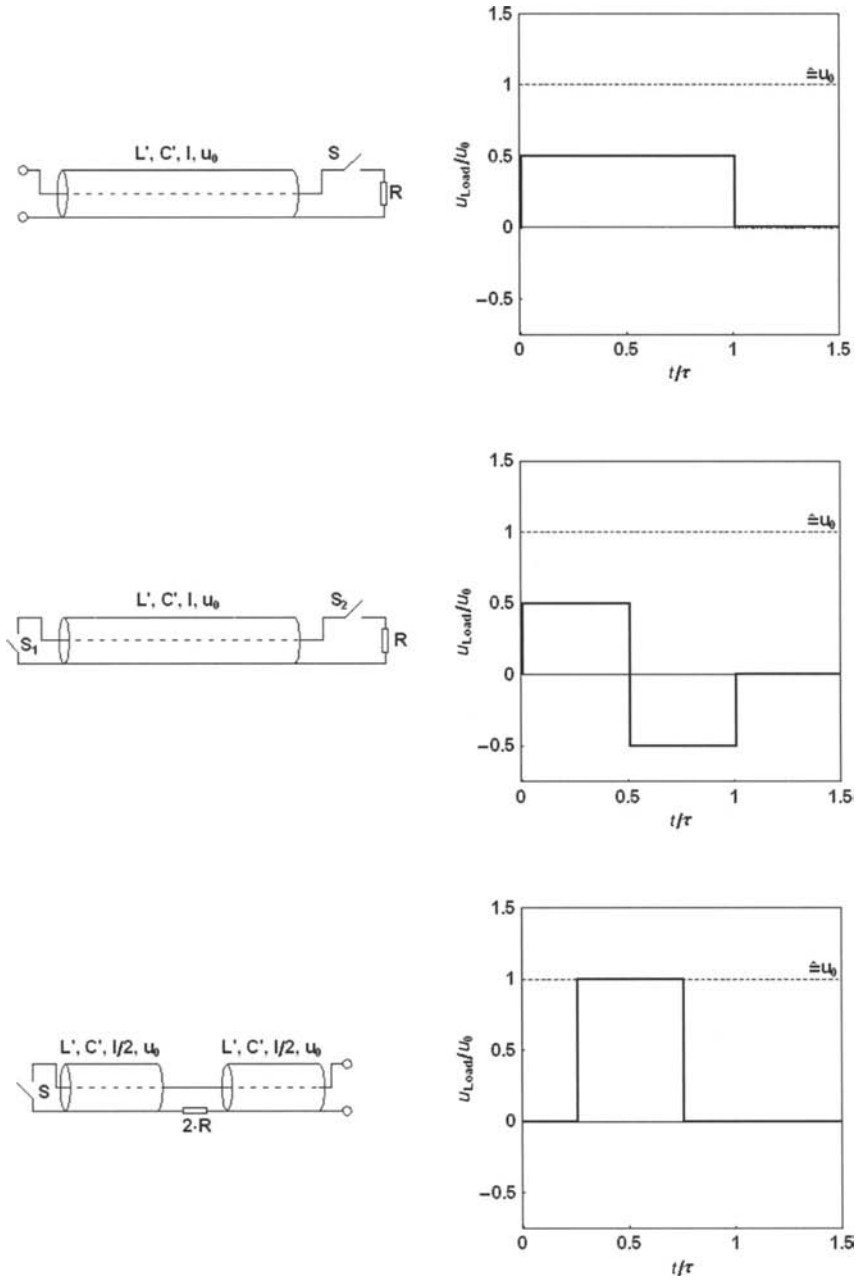


Figure 2.23. Pulse forming lines and voltage waveforms.

Conventional 20-kV cables, like N(A)HKBA cables, have capacitances up to about 2 μF/km (source: Felten & Guilleaume Energietechnik AG, Taschenbuch, 1995). The cables can withstand a peak voltage of about 30 kV. So the ends of seven cables have to be switched in series, decreasing C' to about 0.3 μF/km. To reach 250 μF/km, about 80 cable groups of seven would have to be switched in parallel. Overall, such a device would result in the handling of 560 cables with a length of 1.2 km

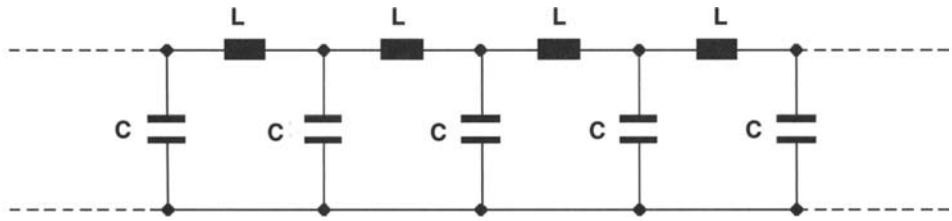


Figure 2.24. Pulse forming network.

each. At a mass of about 4 t/km this results in an overall mass of about 2700 t. The disadvantage of this solution is obvious. Furthermore, the ohmic resistance of the cables could become intolerably high for the high frequency applications.

Because of this disadvantage of a cable solution, other solutions have to be considered without losing the advantage of rectangular pulse shape generation.

3.3.3.2. Pulse forming networks

Pulse forming networks allow one to simulate the behavior of transmission lines by using capacitors and inductors with free choice of their values. Using cables, the relation between C and L is fixed due to their fixed geometry. Pulse forming networks can be derived from transmission lines as follows.

The transmission line can be split into several smaller pieces connected in a chain. Each of these pieces has its own capacitance and inductance (as well as conductor conductivity, which usually can be neglected). In principle, the diagram of a transmission line based on this design now looks like that shown in Fig. 2.24. This diagram shows a chain of several identical four-terminal networks consisting of the same L-C combinations. This chain is called a pulse forming network with a finite number of L-C combinations. A transmission line, in principle, would consist of an infinite number of these combinations with infinitesimal small inductances and capacitances.

Appendix A gives in a brief an introduction to the differential equation system used to calculate the electrical behavior of pulse forming networks, without the restriction of identical inductances and capacitances. Furthermore, conductivities parallel to the capacitances, resistances in series with the inductivities, and mutual inductances between the inductances, as well as resistances in the capacitor lines, are all considered.

Appendix B gives a simplified version of the differential equation system, if, as shown in Fig. 2.24, only identical inductances and capacitances without any ohmic losses are considered. The capacitances are charged to the same initial voltage u_0. Further considerations will be restricted to this type of a pulse forming network.

To adapt a pulse forming network to a given electric load R, the formulas defining the behavior of a pulse forming line can be overtaken, due to the similarity of both types of pulse forming devices. Assuming that there are n capacitors and inductors that $C_{\mathrm{PFN}} = n \cdot C$ is the overall capacitance, and $L_{\mathrm{PFN}} = n \cdot L$ is the overall inductance of the network, the working equations are:

$$R = \sqrt{L_{\mathrm{PFN}}/C_{\mathrm{PFN}}} = \sqrt{L/C}$$

$$\tau = 2 \cdot \sqrt{L_{\mathrm{PFN}} \cdot C_{\mathrm{PFN}}} = 2 \cdot n \cdot \sqrt{L \cdot C}$$

$$W_{\mathrm{C}} = C_{\mathrm{PFN}} \cdot u_0^2/2.$$

From these equations, L, C, and τ can be calculated as

$$C = 2\frac{W_C}{n \cdot u_0^2}$$

$$L = 2 \cdot R^2 \cdot \frac{W_C}{n \cdot u_0^2}$$

$$\tau = 4 \cdot \frac{R \cdot W_C}{u_0^2}.$$

The pulse duration τ is fixed by the values of R, u_0, and W_C.

Figure 2.25 shows some basic pulse forming networks, together with their voltage waveforms for different numbers, n, of network elements. The networks differ slightly with respect to the switches and to the transition from the network to the load. The networks in subfigures (a) and (b) simulate the behavior of the pulse forming line shown in Fig. 2.23, top. Networks in subfigures (c) and (d) simulate the pulse forming line shown in Fig. 2.22, center. And the network in subfigure (e) relates to the pulse forming line in Fig. 2.23, bottom. The calculation of the load voltage was performed by varying the number network elements between 1 and 10 (a–d), respectively, between 2 and 14 (e).

The load voltage waveforms shown in Fig. 2.25 are normalized with the initial capacitance voltage u_0. The time is normalized with the time constant τ. The dashed lines represent the voltage behavior of an ideal pulse forming line; that is, the behavior of a pulse forming network (PFN) with $n \to \infty$. The voltages in subfigure (a) are calculated by using the formulas given in Appendix B, whereas the voltages in subfigures (b)–(d), with to some minor modifications, are calculated by using the formulas given in Appendix A. In all cases, the load resistance was chosen to be $R = \sqrt{L/C}$.

In detail the figures show:

Figure 2.25a: Initially, switch S_2 is opened and the current source i_0 charges the n capacitors C via the inductors L. After charging is completed, switch S_1 is opened and switch S_2 is closed. Closing switch S_3 initiates discharge via the load R.

For $n = 1$, the network represents a well-damped ringing circuit with the respective load voltage (light-gray curve). Increasing n leads more and more to an approximation of the voltage waveform of a pulse forming line (dashed curve; compare to Fig. 2.23, top). The voltage keeps at about 50% of the initial voltage of the capacitors.

Figure 2.25b: The PFN differs from the PFN in Fig. 2.25a by the lack of an inductance between the last capacitor and the load. Charging and initialization of the discharge is the same as with the previous network.

For $n = 1$, the network represents a capacitive circuit with a respective load voltage (light-gray curve). The fact that the voltage does not yield the capacitance's initial voltage is due to a small inductance between the capacitance and the load, which is not shown in the diagram, but is included the calculation. Increasing n leads more and more to an approximation of the voltage waveform of a pulse forming line (dashed curve). Beside the unavoidable voltage peak at the beginning, the voltage remains at about 50% of the initial voltage of the capacitors nearly throughout the entire pulse duration.

Figure 2.25c: Initially, S_1 is open and the current source i_0 charges the n capacitors C via the inductors L. After charging is completed, switches S_1 and S_2 are closed simultaneously to initiate discharge via the load R.

For $n = 1$, the load voltage is ringing (light-gray curve). Increasing n leads more and more to an approximation of the bipolar rectangular voltage waveform of a pulse forming line short-circuited at one end (dashed curve; compare to Fig. 2.23, center). The voltage amplitude keeps at about 50% of the initial voltage of the capacitances. The ringing period is τ.

Figure 2.25d: The PFN differs from the PFN in Fig. 2.25c due to the lack of an inductance between the last capacitance and the load. Charging and initialization of the discharge is the same as that with the previous network.

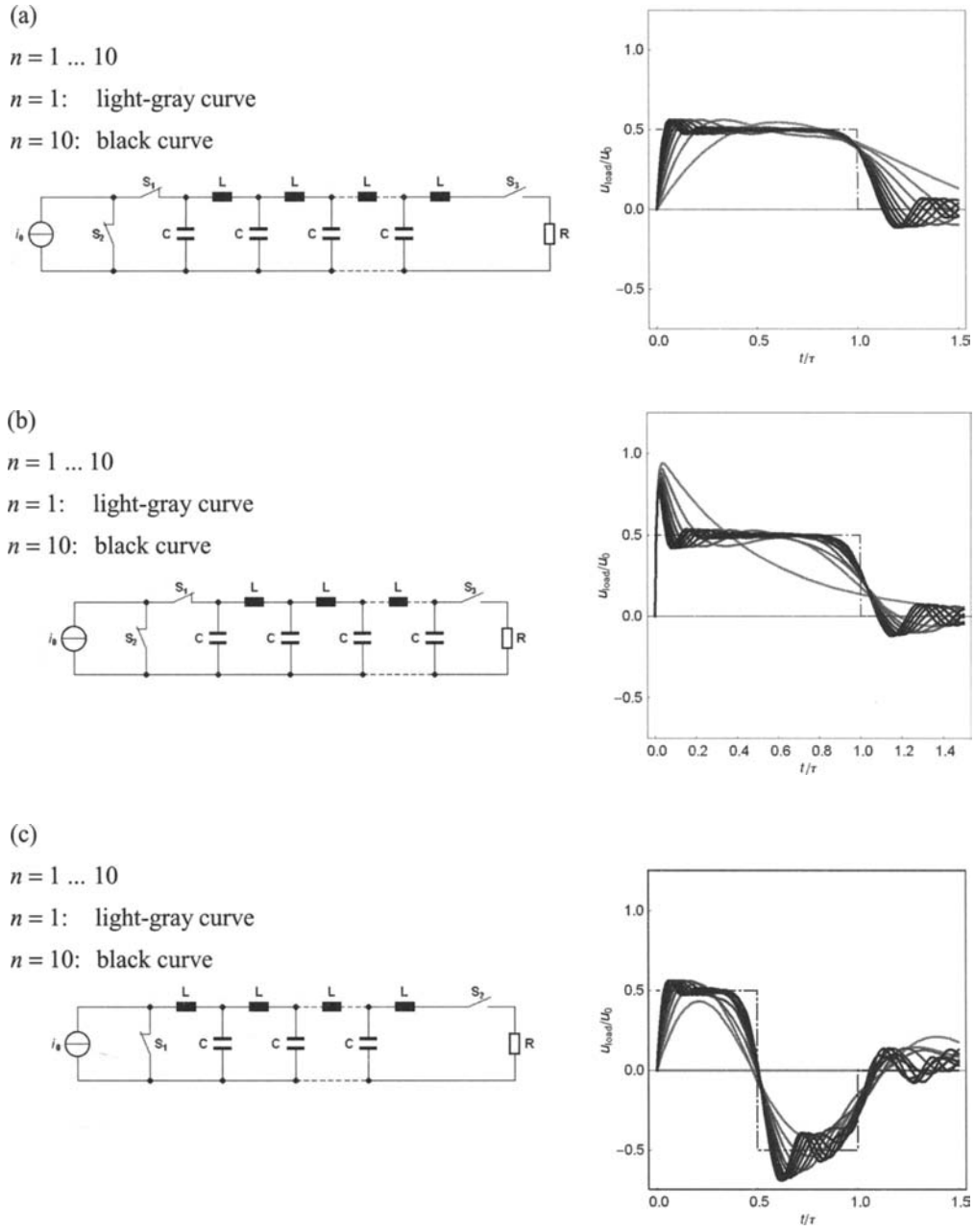


Figure 2.25. Basic pulse forming networks and related load voltage waveforms.

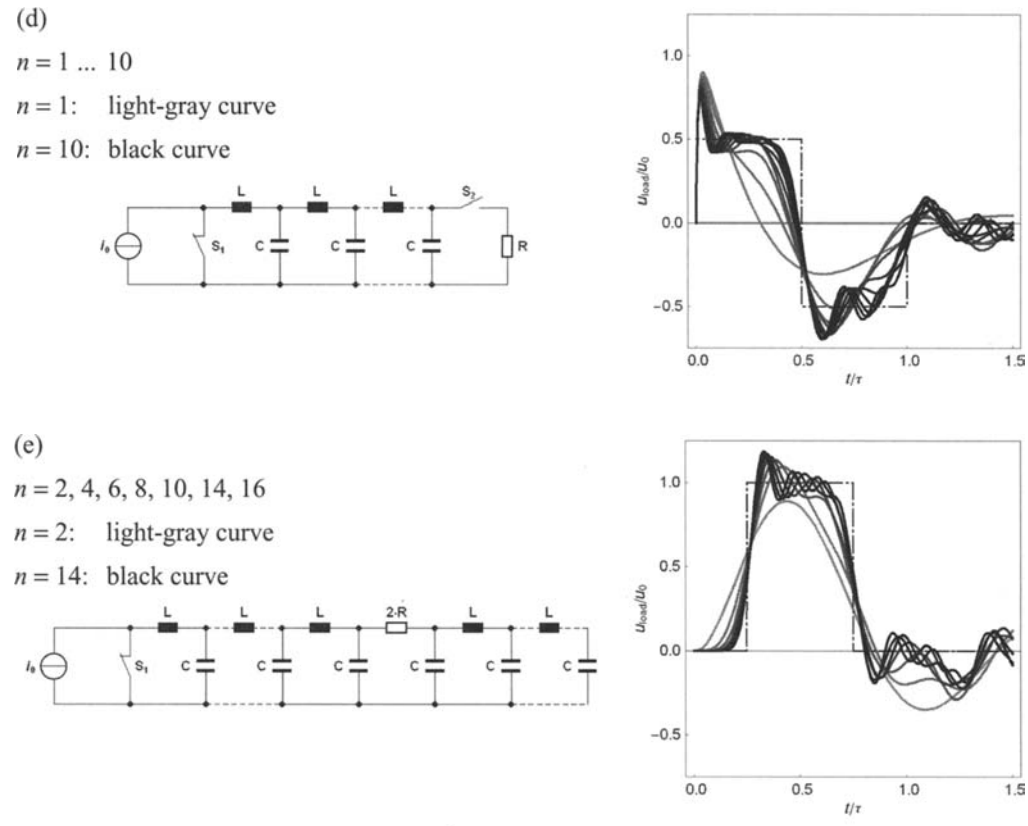


Figure 2.25. (*continued*)

For $n = 1$, the load voltage rings (light-gray curve). Increasing n leads more and more to an approximation of the bipolar rectangular voltage waveform of a pulse forming line short-circuited at one end (dashed curve). The voltage amplitude remains at about 50% of the initial voltage of the capacitances, despite some initial discharge. The ringing period is τ.

Figure 2.25e: This PFN differs from the previous PFNs in that the resistance is doubled $2 \cdot R$ in the center of the PFN. Charging and initialization of the discharge is the same as with the previous networks.

For $n = 2$, the load voltage rings slightly (light-gray curve). Increasing n leads more and more to an approximation of the monopolar rectangular voltage waveform of a split pulse forming line short-circuited at one end (dashed curve; compare to Fig. 2.23, bottom). The voltage amplitude remains at about 100% of the initial voltage of the capacitances. The ringing period is $\tau/2$. For increasing n, the start time of the load voltage is approximately $\tau/4$.

To increase the voltage steepness at the beginning and at the end, smaller capacitances are used at the ends of the PFNs.

3.3.4. Networks with Pulse Forming Switches

Another possible method for generating more or less rectangular voltage waveforms is to use capacitive or inductive circuits.

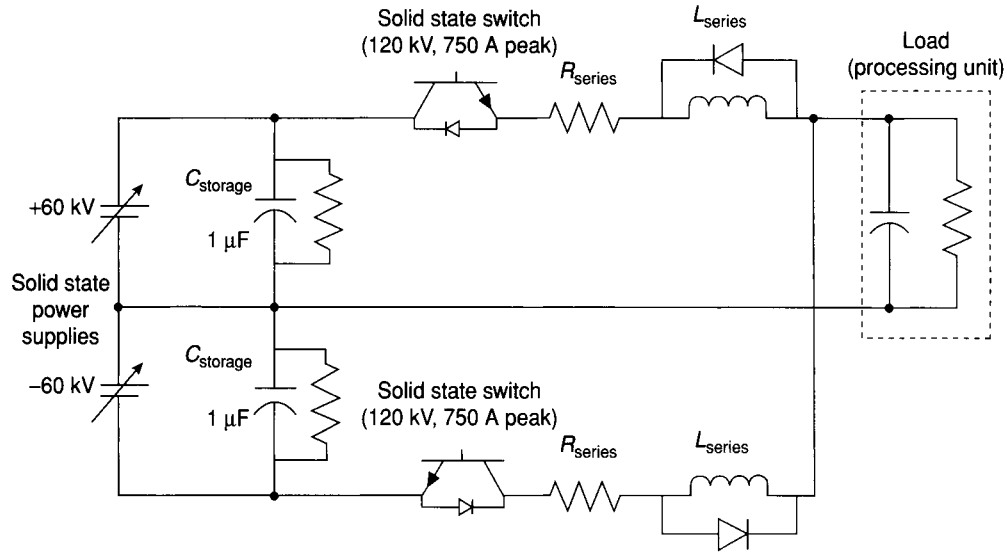


Figure 2.26. Diagram of a capacitive double-circuit capable of generating the bipolar rectangular voltage waveforms.

In capacitive circuits (Fig. 2.10), the capacitance has to be chosen in such a way that the time constant of the circuit is essentially larger than the desired pulse duration. After initializing discharge of the capacitor by closing switch S_3, this switch can in principle, be re-opened again at a specified time to interrupt the discharge of the capacitors. After this interrupt, the capacitor can be recharged to its initial voltage level and the process repeated.

As an advanced example, Fig. 2.26 shows the diagram of a capacitive double-circuit capable of generating the bipolar rectangular voltage waveforms as shown in Fig. 2.7 (Gaudreau *et al.*, 2005). The load is connected to two independent capacitive circuits. In the upper half of the circuit, a capacitor $C_{storage}$ is connected via a solid stage switch, a serial resistance R_{series} and a serial inductance L_{series} to the load, represented by its resistance and its capacitance. After charging $C_{storage}$ with a solid-state power supply (~constant current source), the switch is activated and the capacitor discharges via R_{series}, L_{series}, and the load. After opening the switch at the specified time, the current from the capacitor is interrupted. Until this time, the load voltage is kept nearly constant. To release the switch, during opening, from dumping the energy stored in L_{series} the diode in parallel to the inductance overtakes the inductor's current. To generate a bipolar voltage waveform, the lower half of the network can be activated in the same manner after switching off the upper half.

Also, inductive circuits (Fig. 2.12) can allow one to generate rectangular voltage waveforms. The inductance has to be chosen so that the time constant of the circuit is essentially larger than the desired pulse duration. After initializing the discharge of the inductance by opening switch S_3 (see Fig. 2.12), the switch can be re-closed, again at a specified time, interrupting the discharge of the inductor. After interruption, the inductance can be recharged to its initial current level and the process can be repeated.

3.3.5. Concluding Remarks

PEF treatment is possible with capacitive and inductive circuits, as well as with pulse forming networks. Voltage multiplying networks like MARX-generators or circuits with pulse transformers have to be used if no switches are available to provide the required voltage amplitudes. The exponentially damped voltage characteristic of capacitive or inductive circuits is not changed by these measures in principle. The energy efficiency is slightly lower. Ringing circuits have no advantage if the highest possible voltages are required.

Rectangular voltage waveforms can be generated by either applying pulse forming networks or capacitive or inductive circuits with "oversized" time constants and with switches that are able to interrupt the current across the load at a specified time.

In all networks or circuits, the overall power demand of the switches remains independent of the number and the arrangement of the switches as well as the type of circuit. The same is true for the energy content of the capacitors or inductors.

3.4. Components of High Power Sources

The main components of high-power sources are storage capacitors and on- and off-switches. Because of their relatively high ohmic power consumption, inductors in comparison to capacitors play a minor role.

Depending on the application, different capacitor types with different specific prices are on the market. One of the main requirements of capacitors is a long lifetime at the specified voltage and energy content. Based on a common design, the construction of different types of capacitors depends on the discharge current.

Many variants of fast switches are available: spark gaps, electron tubes (thyratrons), and semiconducting switches (diodes, thyristors). Depending on the technical specifications, these switches have specific merits and limitations. Mechanical switches are too slow for high repetition rate PEF generation.

3.4.1. High-Power Capacitors

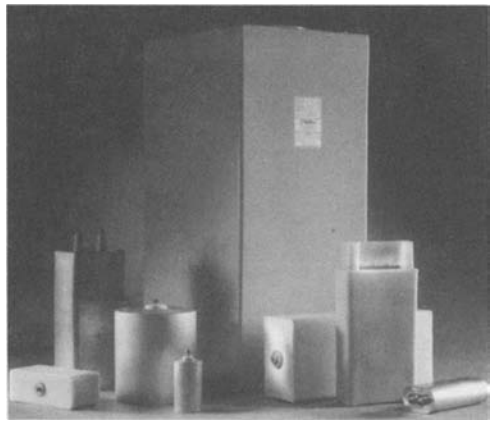
In principle high-power capacitors consist of a couple of thin sandwich-like metallic-dielectric–metallic-dielectric strips of ~100 μm thickness. The dielectrics are made, for example, from Kraft paper, polypropylene, mixtures of Kraft paper with polypropylene, or PVDF. The electrodes are made from aluminum, either as a foil or sprayed onto the dielectric. Each sandwich strip is rolled up. The resulting windings are flattened. Each flat winding represents an elementary capacitor. They can be switched in series or in parallel to construct the final capacitor. Further information about the construction of capacitors is given in MacDougall (1996).

Together with some basic data about commercially available capacitors, Fig. 2.27-1 lists some different types of capacitors of different sizes in metallic or in plastic casings.

Figure 2.27-2, left, top, gives an overview of the range of voltage and capacitance of capacitors provided by a large capacitor supplier (Ennis *et al.*, 2003). At voltages of about 1 kV, the highest capacitances are nearly 100 mF, whereas at voltages in the MV range the capacitances have values in the 100-pF range. The figure shows that, in general, the realized capacitance of single capacitors has smaller values at higher voltages.

Figure 2.27-2, right, top, gives an overview of the range of energy densities and design life of capacitors (Ennis *et al.*, 2003). For a design life of about 1000 charge/discharge cycles, energy densities up to about 2 kJ/L were achieved. To achieve very high lifetimes, that is, on the order of 10^{13} charge/discharge cycles, the energy density has to be reduced to about 0.1 kJ/L. Increasing the lifetime of capacitors is possible by decreasing the energy stored per unit volume. The lifetime of capacitors also depends on other factors like voltage, dielectric stress, voltage reversal, stressed area of the dielectric, and ringing frequency (Smith *et al.*, 2004): "A traditional power scaling guideline has been used by capacitor manufacturers to predict actual component lifetimes based on known sensitivities to these important parameters. One of its most general forms is given by

$$L = L_0 \cdot \left(\frac{V}{V_0}\right)^{-4} \cdot \left(\frac{E[V]}{E_0}\right)^{-3.5} \cdot \left(\frac{Q}{Q_0}\right)^{-1.6} \cdot \left(\frac{A}{A_0}\right)^{-1/\beta} \cdot \left(\frac{f}{f_0}\right)^{-0.5}.$$



- capacitance: up to ~0.1 F
- voltage: up to ~MV
- energy: <2.5 kJ/L
- lifetime: up to ~10^{14} c/d cycles
- specific cost: >0.05 US$/J

Figure 2.27-1. Capacitors. Left: different types of capacitors (source: Maxwell Laboratories Inc.), right: general data (taken from Fig. 2.26-2).

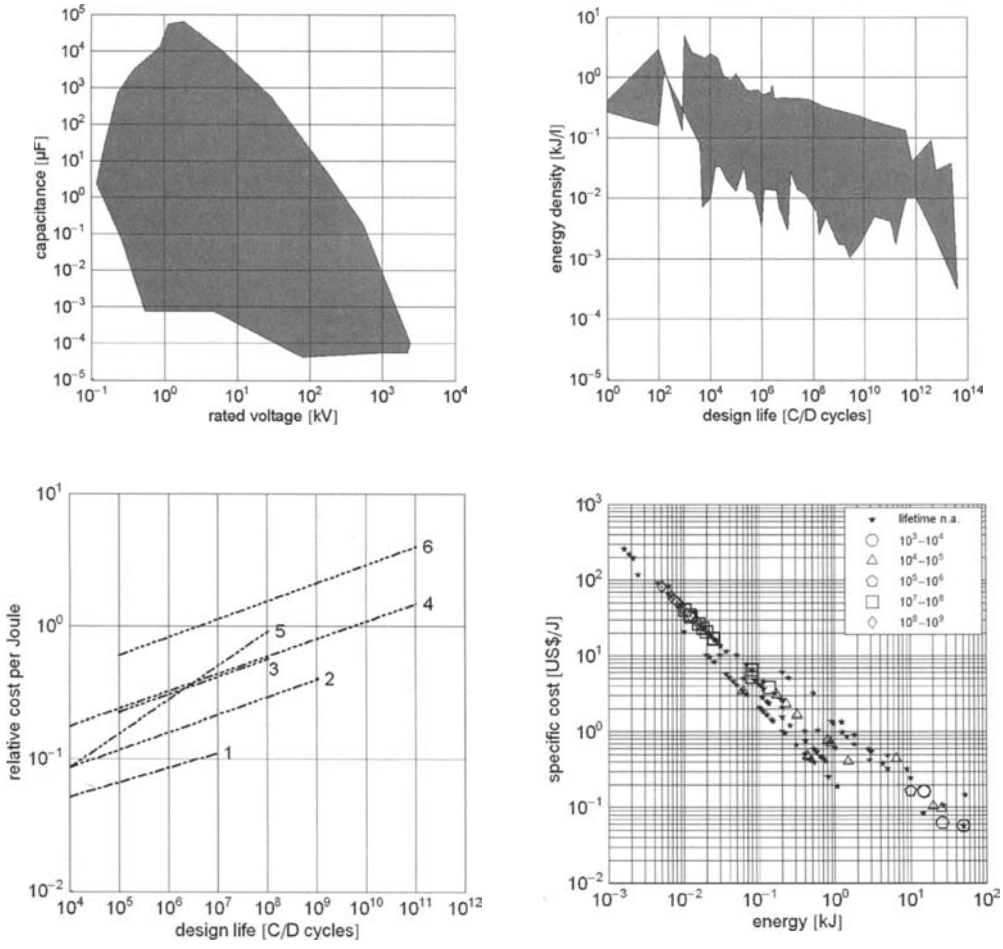


Figure 2.27-2. Typical data of capacitors; Left, top: voltage and capacitance (MacDougall, 1996); right, top: range of energy density and rated voltage (MacDougall, 1996); left, bottom: relative cost per Joule versus life of different high energy capacitor technologies; right, bottom: specific cost versus energy (Maxwell Laboratories Inc./AMS Electronic GmbH, 1997).

In this expression, L represents the expected short life after scaling; V is the capacitor operating terminal voltage; E is the dielectric electric field stress; A is the stressed dielectric area of the operating unit; β is the Weibull slope or shape factor; f is the ringing frequency of the operating unit; $Q = (\pi/2)/\ln(1/R)$ is the operating circuit quality factor; and R is the actual operating reversal (%). The symbols L_0, V_0, E_0, A_0, f_0, R_0, and Q_0 are the baseline rated design parameters." The exponents in the scaling law vary depending on the materials used. Increasing/decreasing each of these parameters compared to the baseline-rated parameters decreases/increases the expected lifetime of capacitors remarkably.

Figure 2.27-2, left, bottom, shows the relation between the relative cost per Joule and the design life. The curves relate to different types of materials and technologies:

1. self-healing metallized electrodes, Kraft paper with film
2. self-healing metallized electrodes, metallized film dielectrics, high-resistance electrode
3. combined metallized electrodes with foil electrodes
4. self-healing metallized electrodes, metallized film dielectrics, segmented electrode
5. extended foil electrodes/tabbed foil; "single-shot," up to 100 kV, up to 1 MA, more than 20% voltage reversal; paper dielectric/mixed dielectric/all-film combined metallized electrodes with foil electrodes.

"Hybrid electrode," types 3 and 6, are especially useful in long-life industrial applications, such as water and food sterilization. In the case of type 5, a cost increase by a factor of ~6 can be expected if the design life increases by a factor of $\sim 10^6$.

Figure 2.27-2, right, bottom, shows the specific cost of capacitors relative to the stored energy for different capacitors in general and for different lifetimes (price base: year 1997). As a general rule, the specific cost decreases with increasing energy. At energies of ~100 kJ/capacitor, the decrease in the cost reaches its minimum at ~0.05 US$/J.

3.4.2. Switches

The following specific high-power switches will now be taken into consideration below:

1. Trigatrons (three-electrode spark gaps)
2. Ignitrons
3. Thyratrons and pseudospark switches
4. Thyristors and IGCTs.

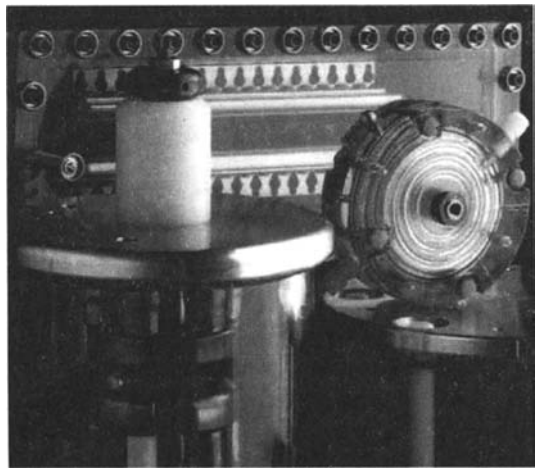
In the following, some typical electrical values of the respective switches will be given.

3.4.2.1. Trigatrons

Trigatrons or spark gaps are the workhorse in laboratories. In principle, they consist of two electrodes with an additional trigger electrode between the main electrodes or implemented into one of the electrodes. The gap between the electrodes is filled with air, synthetic air, sulfur hexafluoride, SF_6–Argon, or oxygen–argon at different pressures ranging down to vacuum pressures. Because of their simple construction, trigatrons are cheap if no demands are imposed on their lifetimes.

Figure 2.28-1 shows a selection of trigatrons of different size for different purposes together with some fundamental data.

Figure 2.28-2, left, top, shows the typical operating voltage and peak current ranges and, on the right, top, peak powers and energy transfer rates that can be handled by trigatrons in single-shot



- peak current: up to ~MA
- voltage: up to ~500 kV
- energy: <3 MJ/pulse
- peak power: <0.4 TVA
- lifetime: up to ~10^9 discharges
- specific cost: >400 US$/GVA

Figure 2.28-1. Trigatrons. Left: different types of trigatrons (source: Maxwell Laboratories Inc.); right: general data (taken from Fig. 2.28-2).

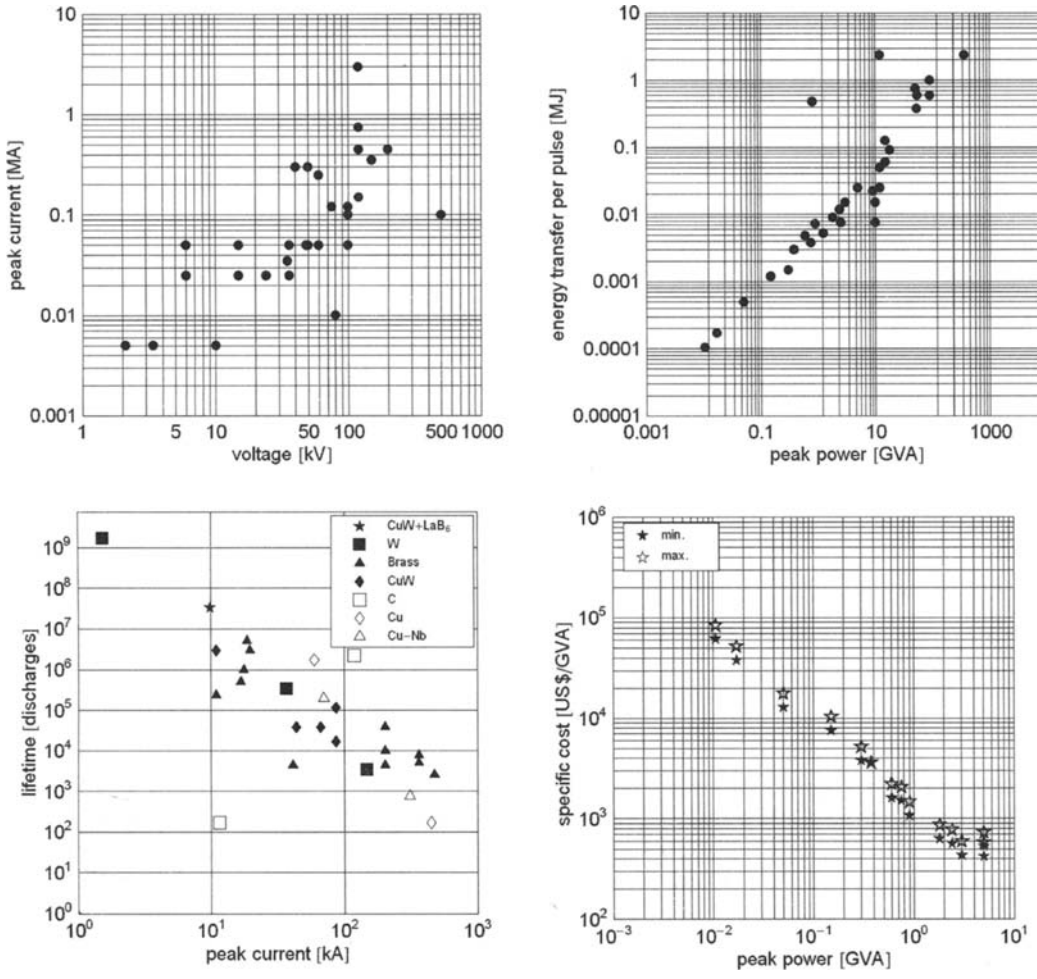


Figure 2.28-2. Typical data of trigatrons. Left, top: range of trigatron voltage and peak current; right, top: range of trigatron energy transfer and peak power; left, bottom: peak current and lifetime expectancies (from (Donaldson, 1990), modified); right, bottom: peak power and specific cost (source: Richardson Electrics Ltd., 2005).

operation. Trigatrons can work at voltages up to ~500 kV, at peak currents up to ~3 MA, at peak powers up to ~300 GVA, and at energy transfer rates up to ~2 MJ/discharge. Here, as in the following sections, the peak power is defined to be the product of the hold-off voltage and the peak current. The peak currents decrease to 10–30% as the repetition rate increases (up to 100 Hz). Nonetheless, these values are impressive and imply that trigatrons can be used for nearly any on-switch tasks in pulsed power technology. The drawback is the relatively short trigatron lifetime of $\sim 10^6$ discharge cycles at currents of 10 kA or more (Fig. 2.28-2, left, bottom). At repetition rates of ~10 Hz, the lifetime is on the order of only ~30 h, thus hindering the industrial applications for trigatrons. The prices of trigatrons are shown in Fig. 2.28-2, right, bottom. In addition, the price of trigger generators has to be taken into account with prices of about 3–4 thousand US$ per single switch (source: Richardson Electronics Inc., 2005).

3.4.2.2. Ignitrons

Ignitrons consist of an anode (usually graphite) and a mercury pool cathode. A semiconducting trigger electrode which dips into the mercury pool cathode and initiates the discharge. An electron emitting source is formed at the point where the trigger contacts the pool. This initiates an arcing between the anode and the cathode. As long as there is a significant current flow, the ignitron will remain conducting. Continuously operating ignitrons must be cooled to a well-defined temperatures. Ignitrons must be placed in a vertical position so that the mercury pool is in the right position in the tube.

Figure 2.29-1 shows an ignitron with a surrounding cooling coil together with some basic data.

Figure 2.29-2, left, top, shows typical trigger voltage and peak current data for a ignitron. Ignitrons can withstand voltages up to 50 kV and peak currents up to 700 kA. They can switch peak powers up to ~10 GVA (Fig. 2.29-2, right, top). The average power, shown in Fig. 2.29-2, left, bottom, is on the order of 0.1% of the peak power. The specific power cost of ignitrons decreases with increasing switching power. They vary between 400 and 2000 US$/GVA peak power.

Although, in general, ignitrons do not cause trouble, they have an increasing negative image due to the mercury inside the ignitrons. For the most applications, they can be replaced by other switch devices, so their use should be avoided when possible.

3.4.2.3. Thyratrons and pseudospark switches

Thyratrons are switches that are filled with hydrogen, deuterium, mercury vapor, xenon, or neon at typical pressures of 1.5–3 kPa. They consist of a heated cathode, a cold anode, and one or more gridlike gate electrodes between the cathode and anode. In many designs (hydrogen thyratrons are a common exception), the gate electrode must be biased highly negative in the off-state and then biased positive to achieve switching. Once turned on, the thyratron will remain conducting as long as there is a significant current flowing through it. When the anode voltage or current falls to zero, the device switches off. Triode, Tetrode, and Pentode variations of the thyratron have been manufactured in the past, though most are of the triode design.

Pseudospark switches are gas-discharge devices with a hollow cathode and, in some types, also a hollow anode. In the cathode cavity, a trigger unit is used to initiate breakdown in the main gap. The characteristics of the pseudospark switches are similar to those of classical pulse hydrogen thyratrons.

Figure 2.30-1 shows a view of a thyratron together with its cross-sectional view and some basic data.

- peak current: ≤700 kA
- voltage: ≤50 kV
- average power: <1 MVA
- peak power: <10 GVA
- specific cost: >400 US$/GVA

Figure 2.29-1. Ignitrons. Left: ignitron; right: general data (taken from Fig. 2.29-2).

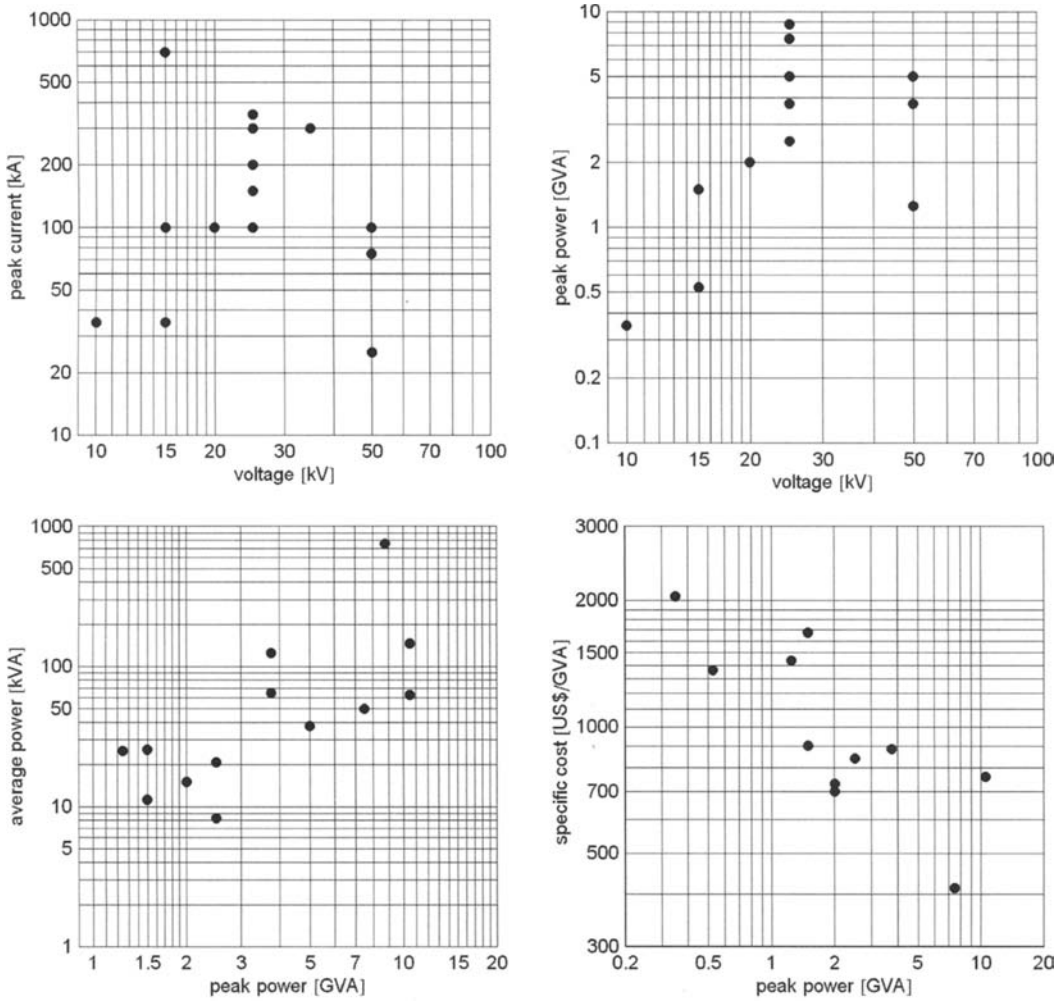
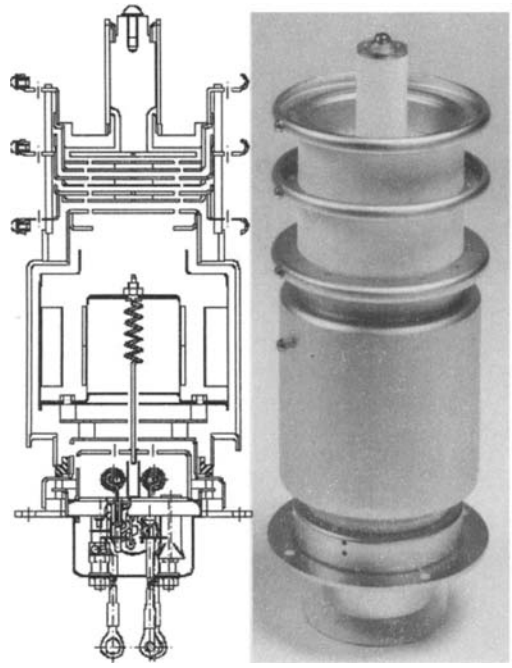


Figure 2.29-2. Typical data of ignitrons. Left, top: Voltage and peak current; right, top: voltage and peak power; left, bottom: peak power and average power; right, bottom: peak power and specific cost (source: Richardson Electronis Ltd., 2005)

Figure 2.30-2, left, shows typical ranges of thyratron voltage and peak current data. Thyratrons and pseudospark switches can handle voltages up to ~200 kV and peak currents up to ~200 kA. These switches allow energy transfers with peak powers up to ~2 GVA and average powers up to ~1 MVA (Fig. 2.30-2, right).

The current increase rate di/dt is on the order of ~100 kA/μs (Richardson *et al.*, 2004).

In comparison to the mounting position of other switches like trigatrons ignitrons or thyristors, thyratrons usually have to be mounted as shown in Fig. 2.30-3. Without an additional switch, the current used to charge a capacitor must also flow through the load. Pseudospark switches can be mounted like the other types of switches.



- peak current: ≤200 kA
- voltage: ≤250 kV
- average power: <2 MVA
- peak power: <3 GVA

Figure 2.30-1. Thyratrons. Left: thyratron; right: general data (taken from Fig. 2.30-2).

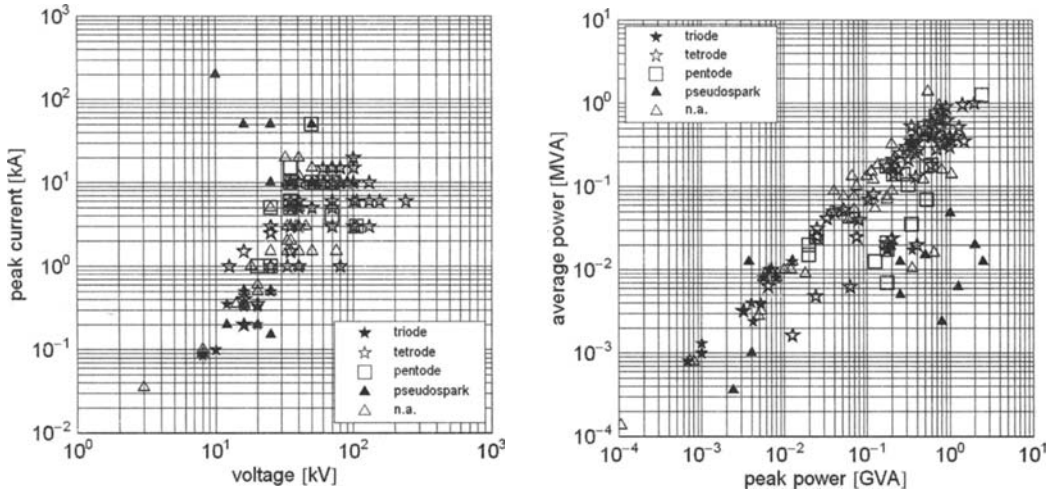


Figure 2.30-2. Typical data of thyratrons. Left: range of thyratron voltage and peak current; right: range of thyratron peak power and average power.

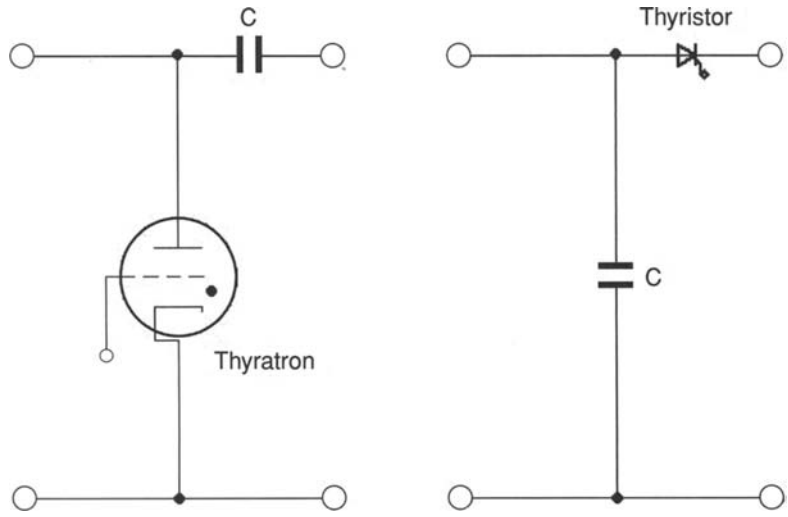


Figure 2.30-3. Mounting position of thyratrons compared to thyristors or other switches.

The average lifetime of thyratrons is on the order of $1\frac{1}{2}$ for 3 years of continuous operation (Richardson *et al.*, 2004; Welleman *et al.*, 2004). A thyratron system that operates at a voltage of 9 kV, a pulse current of 2.5 kA, a pulse length of 10 μs, a d*i*/d*t* of 5 kA/μs, and a pulse repetition rate of 400 Hz usually costs 4000 US$, whereas the tube itself costs 2300 US$ (Welleman *et al.*, 2004).

3.4.2.4. Thyristors, diodes, and IGCT switches

In principle, thyristors (=thyratron + transistor) are four-layer semiconducting devices, with each layer consisting of an alternately N- or P-endowed material, for example, N-P–N-P. The outer layers represent the cathode (N) and the anode (P). The control terminal, or "gate," is attached to one of the middle layers. After ignition, thyristors remain conducting as long as the current does not reverse. Some thyristors are optically triggered.

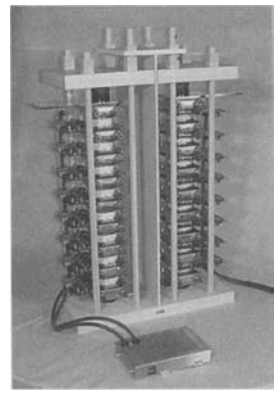
Figure 2.31-1 shows some types of thyristors with either a single semiconducting wafer in a ceramic housing, multiple wafers in a ceramic housing, or combined in a stack. Their key parameters are also given.

Figure 2.31-2 shows some typical characteristics of single thyristor disks, multi-layer thyristors, and stacked thyristors. Single thyristor disks can be stressed by voltages of 4.5 kV at peak currents up to more than 100 kA (Fig. 2.31-2, left, top). Multi-layer thyristors can be stressed by voltages of ~15 kV at peak currents up to 50 kA, whereas thyristor stacks are available for voltages up to 70 kV and for peak currents up to more than 100 kA. Note that for continuous operation, the currents and the power are a factor of 10–20 less than the peak currents and the peak powers, respectively.

The action integral

$$A = \int_0^\infty i^2 \mathrm{d}t,$$

which is proportional to the energy deposit in the thyristor and the load during one discharge at peak current, can reach values up to ~1 MJ/Ω at power levels up to 5 GVA (Fig. 2.31-2, right, top) for a single-shot. Current increase rates d*i*/d*t* are limited to ~20 kA/μs. Repetition rates can



- peak current: ~100 kA
- voltage:
  - single: 4.5 kV
  - multi: ≤13.5 kV
  - stack: ≤70 kV
- rated power: <2 MVA (single)
- peak power: <5 GVA (stack)
- specific cost: ≥0.7 €/kVA

Figure 2.31-1. Thyristors. Left: thyristor (single, multi, stack; source: ABB Semiconductors); right: general data (taken from Fig. 2.31-2).

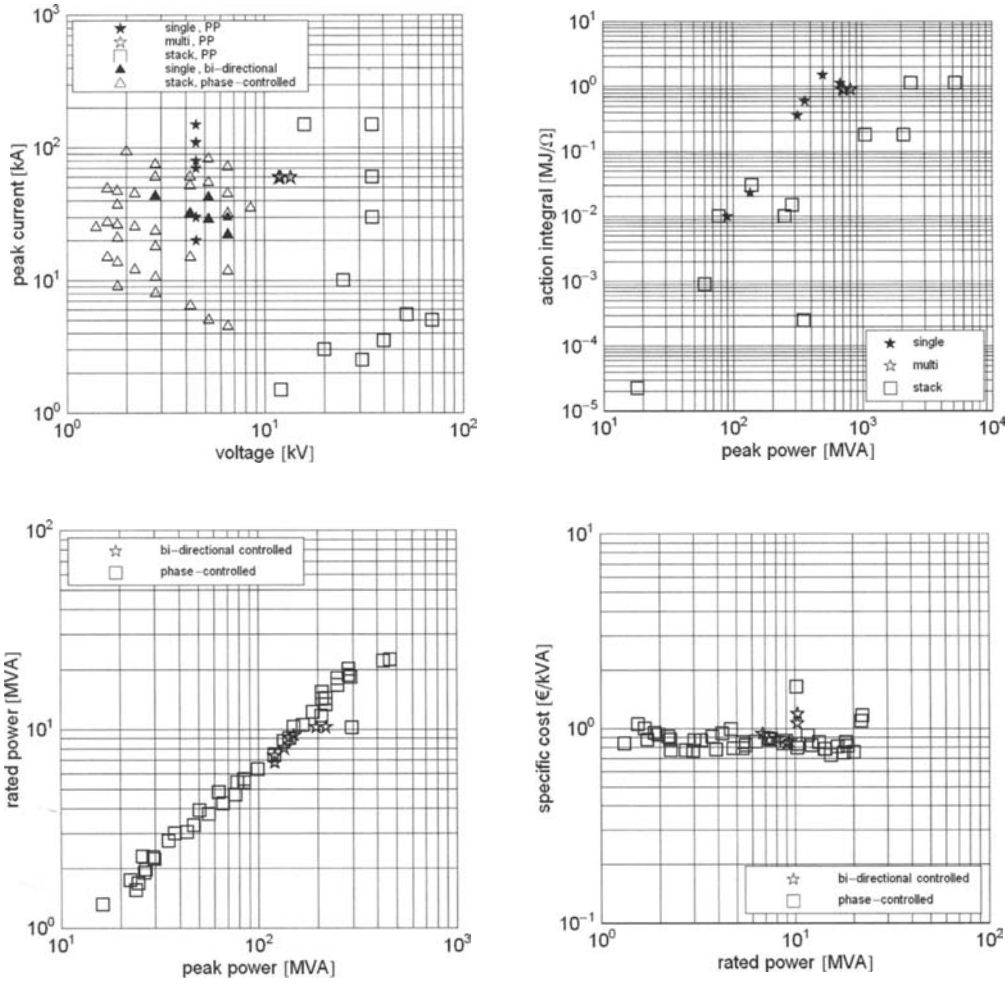


Figure 2.31-2. Typical thyristor data (source: ABB Semiconductors). Left, top: voltage and peak current; right, top: power and action integral; left, bottom: peak power and rated power; right, bottom: specific cost and rated power (rough estimate).

be up to 100 Hz at rated power. Typical values of the rated power (or average power) normalized by the peak power are shown in Fig. 2.31-2, left, bottom. As shown in Fig. 2.31-2, right, bottom, the specific cost of thyristors (single) are in the range of 0.7–1.5 €/kVA rated power. For continuous operation, the lifetime of thyristors is on the order of 10 years and more (Welleman *et al.*, 2004).

At a voltage of 9 kV, a pulse current of 2.5 kA, a pulse length of 10 µs, a di/dt of 5 kA/µs, and a pulse repetition rate of 400 Hz, the typical cost of a thyristor stack is 8700 US$ (Welleman *et al.*, 2004).

Thyristors switch-off if the current reverses, but are not able to switch-off the current before zeroing. Integrated Gate Controlled Thyristors (IGCT) have the capability to switch-off currents actively. As the IGCT is the improved version of GTOs (Gate Turn-Off Thyristor), the GTO technology is not used anymore for pulsed applications (Welleman *et al.*, 2004). Although their turn-on behavior and di/dt is reduced compared to the discharge switches mentioned earlier, they offer the possibility to drive circuits with pulse forming switches. Single GTO switches are designed to switch-on and to switch-off currents up to 4 kA. Blocking voltage per device is 4.5 kV or 6 kV. They are usually used in medium- and high-power drivers and frequency converters. The switches can be stacked so that, with a reverse blocking IGCT device consisting of seven 4.5-kV IGCTs, forward/reverse blocking voltages of 31 kV at nominal 1600-µs pulse currents of 2.5 kA can be switched at repetition rates of 15 Hz (Welleman *et al.*, 2004). Frequencies up to 400 Hz are possible.

As a perspective optically triggered Emitter Turn-Off Thyristors (ETO) can operate at currents up to 4 kA and at voltages of 6 kV at frequencies of more than 1 kHz with less power consumption. The maximum current increase rate di/dt is 1 kA/µs.

Diodes are passive switches, that switch on automatically at negative voltages. Figure 2.32, left, shows the peak current and voltage of several types of diodes with a single wafer. Peak currents up to 90 kA can be switched for blocking voltages up to 6 kV. The rated power over the peak power is shown in Fig. 2.32, right. The rated power (average power) has values up to 25 MVA at peak power levels up to 400 MVA. As a rule of thumb, the specific power cost of diodes relative to their average power can be estimated as 3/4 of the cost of comparable thyristors.

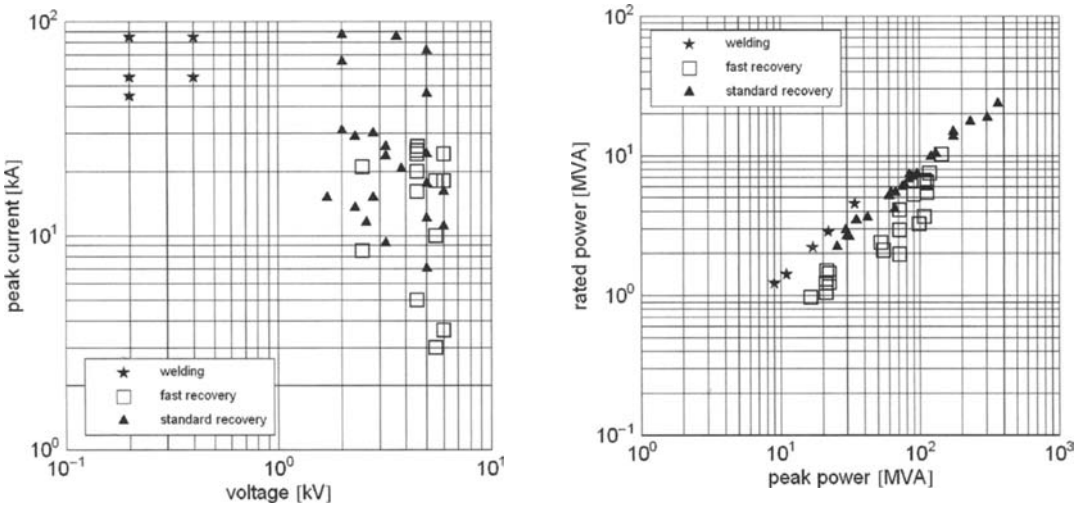


Figure 2.32. Typical data of diodes (source: ABB Semiconductors). Left: voltage and peak current; right: peak power and operational power.

3.4.2.5. Concluding remarks

For PEF applications, thyratrons and semiconducting switches offer the most promising possibilities. Trigatrons suffer from relatively short lifetimes, whereas ignitrons are more and more out of favor due to the mercury inside the tube. The choice between thyristors and thyratrons depends on the specific requirements of the load. At voltages of ~100 kV, there are clear advantages for using thyratrons, especially with respect to the investment cost. At voltages lower than 30 kV, thyristor switches may yield lower operation cost than thyristors due to their comparably higher lifetime.

3.5. Low-Power Source

3.5.1. Basic Considerations

The low-power source (AC/DC converters) converts an alternating voltage into a constant current, constant voltage, or constant power to charge the capacitors or inductors of the high-power source. The term "low power" means low power compared to the power of the high-power sources. Several types of such converters exist. Their technical details are discussed, for example, in [48]. Figure 2.33 shows typical values of the power and output frequencies of commercially available converters (Brosch *et al.*, 2000). Power levels up to ~100 MVA have been realized in large facilities.

In high repetition rate circuits, like PEF circuits, the converter is the most expansive part. The cost depends on the maximum current and the maximum voltage, which defines the rated power of the device, that the converter can handle. For cost-effectiveness, the power utilization factor should be as high as possible to achieve high energetic efficiency.

It is well known that the charging of capacitors with constant voltage via a resistor yields an energetic efficiency of not more than 50% and a power utilization not more than 25%. On the other hand, charging with constant currents yields energetic efficiencies of more than 90% at comparably high-power utilization factors. Charging with constant power is in between

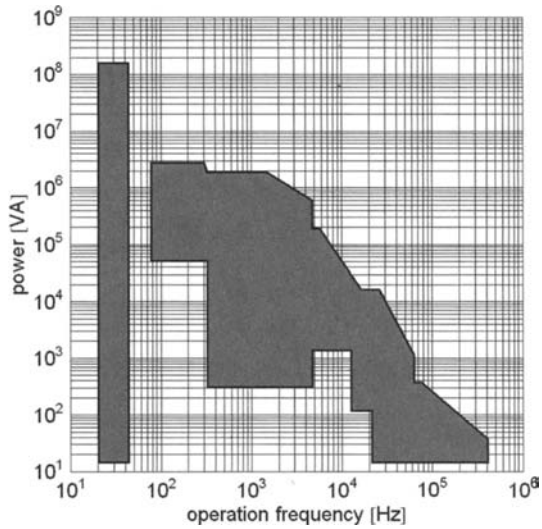


Figure 2.33. Converters. range of frequency and power (from (Brosch *et al.*, 2000, Mitsubishi), modified in the low frequency region with values from ABB Semiconductors).

these two types. From this, it clearly turns out that capacitors should be charged by a constant current source.

For constant charging current i_0, the power in the capacitor is

$$P_C = u_C \cdot i_0.$$

The capacitor voltage increases according to the following formula

$$u_C = \frac{i_0}{C} \cdot t,$$

so that the power can be calculated with

$$P_C = \frac{i_0^2}{C} \cdot t.$$

The power increases linearly with time. If the charging voltage u_0 is attained, the peak power at the capacitor is

$$\hat{P}_C = u_0 \cdot i_0.$$

This power, plus some additional power to account for ohmic losses, has to be installed in the constant current power supply. The minimum cost of this device can be calculated by applying the rule of thumb that define the specific cost to be 0.5 €/W.

The average power is half the peak power. So, as an example, a device with an average power of 300 kW (compare Fig. 2.6) would need an installed power of 600 kW. The cost would be in minimum 300 k€.

3.5.2. Typical Devices

To close this chapter, some typical systems that have already been constructed and that are in use will be briefly described.

Figure 2.34 shows a power supply for PEF applications that allows one to charge capacitors up to 18 kV at an average power of 35 kJ/s and at repetition rates up to 100 Hz. On the left side of the figure, the power supply is shown as a customer usually sees it. The cost of a device is about

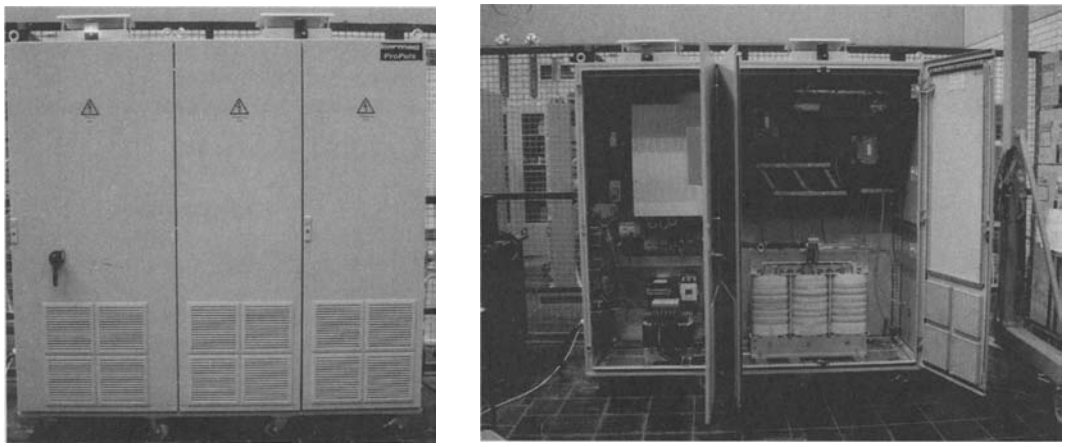


Figure 2.34. 35 kJ/s/18 kV power supply (source: BARMAG, Propuls).

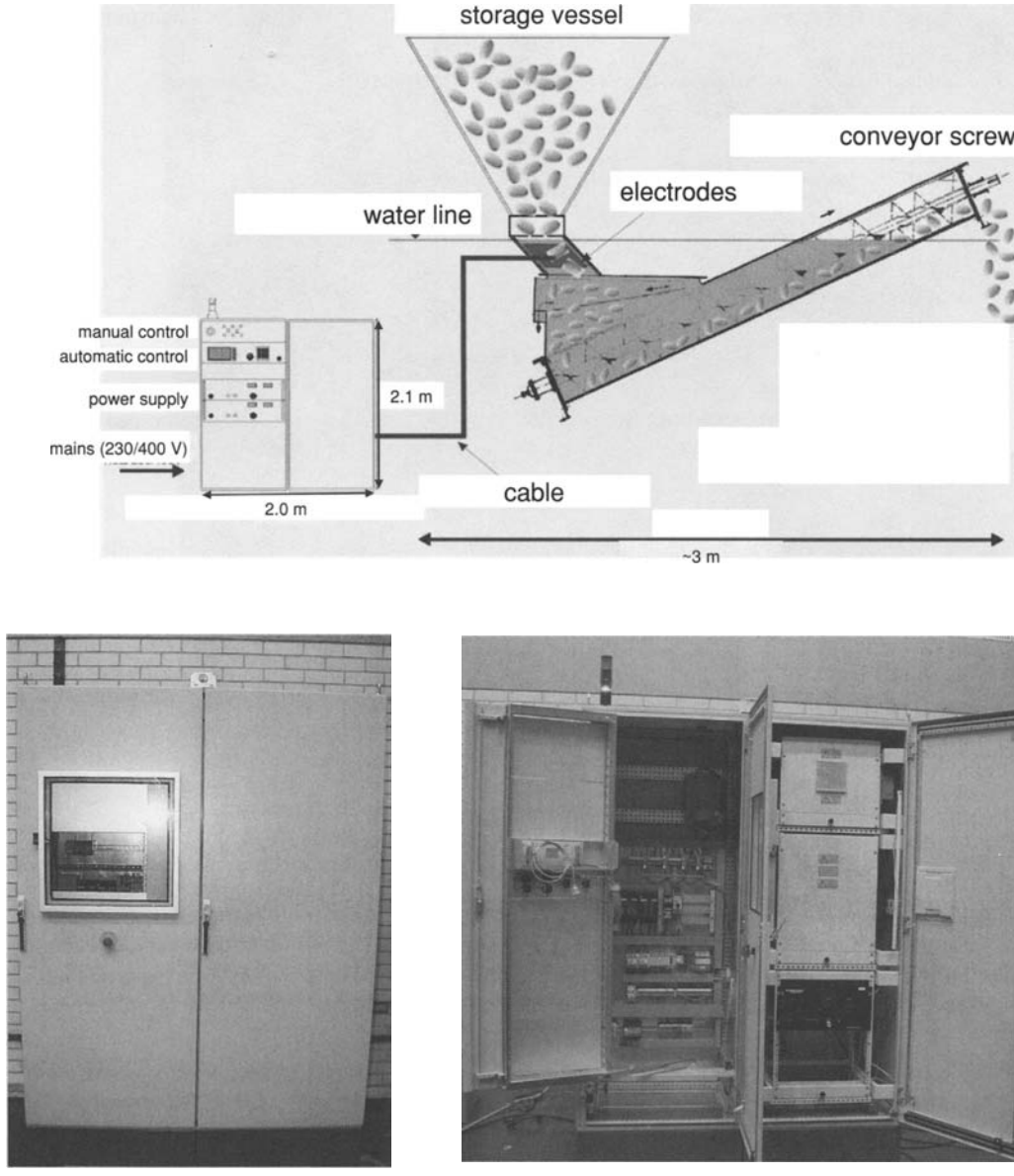


Figure 2.35. 20 kJ/s/20 kV monopolar solid-state PEF pulser (source: AMS Electronics/Propuls).

30 k€ according to the specific cost per unit power estimate given above. Doubling the power would increase the cost by ~50% (Neumann, J. personal communication, 2005).

Figure 2.35 shows a solid-state pulser delivering monopolar exponentially damped pulse voltages of 20 kV. The 50-Hz pulser has an average power demand of 20 kW. Its purpose is the treatment of potatoes with PEF for the starch industry (see Fig. 2.35, top). The potatoes are positioned by a steady flow of water between two electrodes. After the PEF treatment, a conveyor screw transports the potatoes to the next stage where the softened potatoes are treated mechanically. A low-voltage power and the electronic controls are located on the left side of the pulser (see Fig. 2.35 right, bottom).

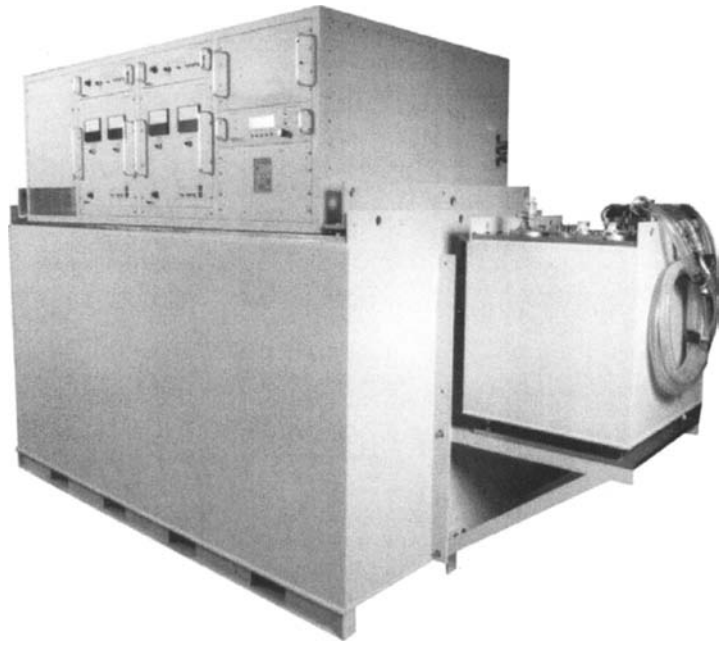


Figure 2.36. 75 kJ/s/60 kV bipolar solid-state PEF pulser (Gaudreau *et al.*, 2004, 2005) (source: Diversified Technologies).

A 20-kJ/s average power supply is positioned below a capacitor and a solid-state switch part on the right side. The cost of the complete system was about 150 k€.

Figure 2.36 shows a solid-state PEF pulser with an average power of 75 kJ/s (Jin *et al.*, 2002; Gaudreau *et al.*, 2004, 2005). Its high-power circuit is shown in Fig. 2.26. The pulser generates bipolar or monopolar square waveform voltages with 60 kV amplitude at currents up to 750 A. The pulse repetition rate is between 500 and 2000 pps. The pulse width varies between 2 and 8 μs. Each pulse delivers 10–100 J of energy. The system is nominally sized to process orange juice at a rate of 2000 L/h. For highly resistive foods, like apple juice, the unit can process up to 5000 L/h. The total cost of the system, including pulse generator, aseptic drink processing unit, PEF treatment chamber unit, and aseptic packaging machine, was about 700 k€.

APPENDIX A: DIFFERENTIAL EQUATION SYSTEM OF INHOMOGENEOUS PULSE FORMING NETWORKS

Figure A-1 shows the most generalized form of a pulse forming network replacing pulse forming lines. Different admittances $C_k||G_k$ connected in parallel are connected with different resistances W_k and with switches S in serial. Different impedances L_k–R_k are connected in series. The capacitances C_k are charged to different charging voltages $u_{k,0}$. Switches S simultaneously switch on and initiate a current via the load R. The $n^2 - n$ mutual inductances $M_{k,l}$ between the single inductances are represented by the collective letter M.

To define the linear differential equation system of the circuit the following matrices and vectors are introduced:

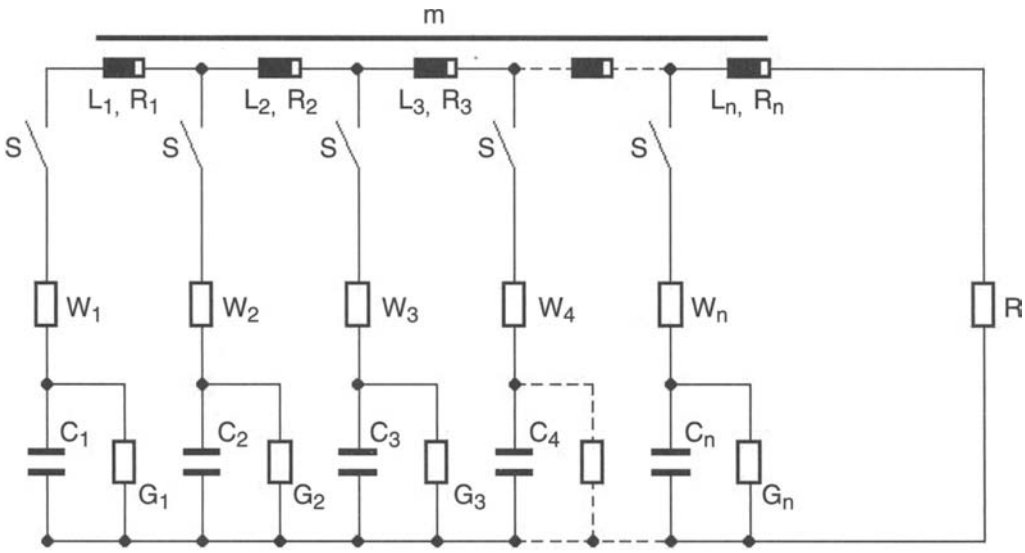


Figure A-1. Inhomogeneous pulse forming network

Matrix of capacitances:

$$C = \begin{pmatrix} C_1 & 0 & 0 & \dots & 0 & 0 \\ 0 & C_2 & 0 & \dots & 0 & 0 \\ 0 & 0 & C_3 & \dots & 0 & 0 \\ \vdots & \vdots & \vdots & \ddots & \vdots & \vdots \\ 0 & 0 & 0 & \dots & C_{n-1} & 0 \\ 0 & 0 & 0 & \dots & 0 & C_n \end{pmatrix}$$

1-Matrix:

$$E = \begin{pmatrix} 1 & 0 & 0 & \dots & 0 & 0 & 0 \\ -1 & 1 & 0 & \dots & 0 & 0 & 0 \\ 0 & -1 & 1 & \dots & 0 & 0 & 0 \\ \vdots & \vdots & \vdots & \ddots & \vdots & \vdots & \vdots \\ 0 & 0 & 0 & \dots & -1 & 1 & 0 \\ 0 & 0 & 0 & \dots & 0 & -1 & 1 \end{pmatrix}$$

Matrix of conductivities:

$$G = \begin{pmatrix} G_1 & 0 & 0 & \dots & 0 & 0 \\ 0 & G_2 & 0 & \dots & 0 & 0 \\ 0 & 0 & G_3 & \dots & 0 & 0 \\ \vdots & \vdots & \vdots & \ddots & \vdots & \vdots \\ 0 & 0 & 0 & \dots & G_{n-1} & 0 \\ 0 & 0 & 0 & \dots & 0 & G_n \end{pmatrix}$$

Matrix of inductances and mutual inductances:

$$L = \begin{pmatrix} L_1 & M_{1,2} & M_{1,3} & \dots & M_{1,n-1} & M_{1,n} \\ M_{1,2} & L_2 & M_{2,3} & \dots & M_{2,n-1} & M_{2,n} \\ M_{1,3} & M_{2,3} & L_3 & \dots & M_{3,n-1} & M_{3,n} \\ \vdots & \vdots & \vdots & \ddots & \vdots & \vdots \\ M_{1,n-1} & M_{2,n-1} & M_{2,n-1} & \dots & M_{n-1,n-1} & M_{n-1,n} \\ M_{1,n} & M_{2,n} & M_{3,n} & \dots & M_{n,n-1} & L_n \end{pmatrix}$$

Matrix of Zeroes (n × n-matrix):

$$O = \begin{pmatrix} 0 & 0 & 0 & \dots & 0 & 0 \\ 0 & 0 & 0 & \dots & 0 & 0 \\ 0 & 0 & 0 & \dots & 0 & 0 \\ \vdots & \vdots & \vdots & \ddots & \vdots & \vdots \\ 0 & 0 & 0 & \dots & 0 & 0 \\ 0 & 0 & 0 & \dots & 0 & 0 \end{pmatrix}$$

Matrix of resistances:

$$W = \begin{pmatrix} -(W_1 + R_1 + W_2) & W_2 & 0 & \dots & 0 & 0 \\ W_2 & -(W_2 + R_2 + W_3) & W_3 & \dots & 0 & 0 \\ 0 & W_3 & -(W_3 + R_3 + W_4) & \dots & 0 & 0 \\ \vdots & \vdots & \vdots & \ddots & \vdots & \vdots \\ 0 & 0 & 0 & \dots & -(W_{n-1} + R_{n-1} + W_n) & W_n \\ 0 & 0 & 0 & \dots & W_n & -(R + R_n) \end{pmatrix}$$

Vector of capacitance voltages and initial capacitance voltages:

$$U\,[t] = \begin{pmatrix} u_1\,[t] \\ u_2\,[t] \\ u_3\,[t] \\ \vdots \\ u_{n-1}\,[t] \\ u_n\,[t] \end{pmatrix}, \quad u_0 = \begin{pmatrix} u_{1,0} \\ u_{2,0} \\ u_{3,0} \\ \vdots \\ u_{n-1,0} \\ u_{n,0} \end{pmatrix}$$

Vector of inductance currents and initial inductance currents:

$$I\,[t] = \begin{pmatrix} i_1\,[t] \\ i_2\,[t] \\ i_3\,[t] \\ \vdots \\ i_{n-1}\,[t] \\ i_n\,[t] \end{pmatrix}, \quad i_0 = \begin{pmatrix} 0 \\ 0 \\ 0 \\ \vdots \\ 0 \\ 0 \end{pmatrix}$$

Applying these matrices and vectors the differential equation system is defined with:

$$\frac{d}{dt}\mathbf{v}[t] = \mathcal{A}^{-1} \cdot \mathcal{B} \cdot \mathbf{v}[t], \mathbf{v}[0] = \mathbf{v}_0$$

with the block matrices

$$\mathcal{A} = \begin{pmatrix} \mathcal{C} & 0 \\ 0 & \mathcal{L} \end{pmatrix}$$

and

$$\mathcal{B} = \begin{pmatrix} -\mathcal{G} & -\mathcal{E} \\ \mathcal{E}^T & \mathcal{W} \end{pmatrix}$$

and with the block vectors

$$\mathbf{v}[t] = \begin{pmatrix} u[t] \\ i[t] \end{pmatrix}$$

and

$$\mathbf{v}_0 = \begin{pmatrix} u_0 \\ i_0 \end{pmatrix}$$

APPENDIX B: DIFFERENTIAL EQUATION SYSTEM DESCRIBING HOMOGENEOUS PULSE FORMING NETWORKS

Figure B-1 shows the most popular form of a pulse forming network. Here all capacitances and inductances are identical: $C_k = C$, $L_k = L$. Resistances R_k and W_k, conductivities G_k and mutual inductances $M_{k,l}$ are neglected. The initial capacity voltages are now identical: $u_{k,0} = u_0$. Hence the switches S now can be combined in a single switch at the load R.

To define the linear differential equation system of the circuit the matrices and vectors of the inhomogeneous pulse forming network can be simplified as follows:

Matrix of capacitances:

$$C = \begin{pmatrix} C_1 & 0 & 0 & \dots & 0 & 0 \\ 0 & C_2 & 0 & \dots & 0 & 0 \\ 0 & 0 & C_3 & \dots & 0 & 0 \\ \vdots & \vdots & \vdots & \ddots & \vdots & \vdots \\ 0 & 0 & 0 & \dots & C_{n-1} & 0 \\ 0 & 0 & 0 & \dots & 0 & C_n \end{pmatrix}$$

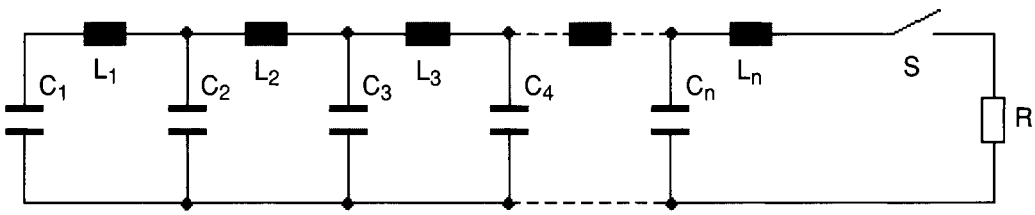


Figure B-1. Homogeneous pulse forming network without losses.

1-Matrix:

$$E = \begin{pmatrix} 1 & 0 & 0 & \dots & 0 & 0 & 0 \\ -1 & 1 & 0 & \dots & 0 & 0 & 0 \\ 0 & -1 & 1 & \dots & 0 & 0 & 0 \\ \vdots & \vdots & \vdots & \ddots & \vdots & \vdots & \vdots \\ 0 & 0 & 0 & \dots & -1 & 1 & 0 \\ 0 & 0 & 0 & \dots & 0 & -1 & 1 \end{pmatrix}$$

Matrix of inductances:

$$L = \begin{pmatrix} L_1 & 0 & 0 & \dots & 0 & 0 \\ 0 & L_2 & 0 & \dots & 0 & 0 \\ 0 & 0 & L_3 & \dots & 0 & 0 \\ \vdots & \vdots & \vdots & \ddots & \vdots & \vdots \\ 0 & 0 & 0 & \dots & 0 & 0 \\ 0 & 0 & 0 & \dots & 0 & L_n \end{pmatrix}$$

Matrix of Zeroes ($n \times n$-matrix):

$$O = \begin{pmatrix} 0 & 0 & 0 & \dots & 0 & 0 \\ 0 & 0 & 0 & \dots & 0 & 0 \\ 0 & 0 & 0 & \dots & 0 & 0 \\ \vdots & \vdots & \vdots & \ddots & \vdots & \vdots \\ 0 & 0 & 0 & \dots & 0 & 0 \\ 0 & 0 & 0 & \dots & 0 & 0 \end{pmatrix}$$

Matrix of resistances:

$$W = \begin{pmatrix} 0 & 0 & 0 & \dots & 0 & 0 \\ 0 & 0 & 0 & \dots & 0 & 0 \\ 0 & 0 & 0 & \dots & 0 & 0 \\ \vdots & \vdots & \vdots & \ddots & \vdots & \vdots \\ 0 & 0 & 0 & \dots & 0 & 0 \\ 0 & 0 & 0 & \dots & 0 & -R \end{pmatrix}$$

Vector of capacitance voltages and initial capacitance voltages:

$$U\,[\mathrm{t}] = \begin{pmatrix} \mathrm{u}_1\,[\mathrm{t}] \\ \mathrm{u}_2\,[\mathrm{t}] \\ \mathrm{u}_3\,[\mathrm{t}] \\ \vdots \\ \mathrm{u}_{n-1}\,[\mathrm{t}] \\ \mathrm{u}_n\,[\mathrm{t}] \end{pmatrix}, \quad u_0 = \begin{pmatrix} \mathrm{u}_0 \\ \mathrm{u}_0 \\ \mathrm{u}_0 \\ \vdots \\ \mathrm{u}_0 \\ \mathrm{u}_0 \end{pmatrix}$$

Vector of inductance currents and initial inductance currents:

$$I\,[t] = \begin{pmatrix} \mathrm{i}_1\,[\mathrm{t}] \\ \mathrm{i}_2\,[\mathrm{t}] \\ \mathrm{i}_3\,[\mathrm{t}] \\ \vdots \\ \mathrm{i}_{n-1}\,[\mathrm{t}] \\ \mathrm{i}_n\,[\mathrm{t}] \end{pmatrix}, \quad i_0 = \begin{pmatrix} 0 \\ 0 \\ 0 \\ \vdots \\ 0 \\ 0 \end{pmatrix}$$

Applying these matrices and vectors the differential equation system is defined with:

$$\frac{d}{dt}\mathbf{v}[t] = \mathcal{A}^{-1} \cdot \mathcal{B} \cdot \mathbf{v}[t], \mathbf{v}[0] = \mathbf{v}_0$$

with the block matrices

$$\mathcal{A} = \begin{pmatrix} \mathcal{C} & 0 \\ 0 & \mathcal{L} \end{pmatrix}$$

and

$$\mathcal{B} = \begin{pmatrix} 0 & -\mathcal{E} \\ \mathcal{E}^T & \mathcal{W} \end{pmatrix}$$

and with the block vectors

$$\mathbf{v}[t] = \begin{pmatrix} u[t] \\ i[t] \end{pmatrix}$$

and

$$\mathbf{v}_0 = \begin{pmatrix} u_0 \\ i_0 \end{pmatrix}$$

ACKNOWLEDGMENT

The author of this chapter would like to express his thanks to Dr. Larry Altgilbers who significantly improved the readability of the text.

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CHAPTER 3

FUNDAMENTAL ASPECTS OF MICROBIAL MEMBRANE ELECTROPORATION

Rafael Pagán and Pilar Mañas

1. INTRODUCTION

Application of pulsed electric fields of high intensity and duration from microseconds to milliseconds may cause temporary or permanent permeabilization of cell membranes. The generally accepted term describing this phenomenon is "electroporation" or "electropermeabilization." Permeabilization of cell membranes is the basis of different applications of PEF for the food industry such as food pasteurization or extraction of intracellular components.

The effects of PEF on biomembranes have been thoroughly studied since the use of PEF has attracted great interest in several scientific areas such as cell biology, biotechnology, medicine, or food technology (Zimmermann, 1986; Palaniappan and Sastry, 1990; Ho and Mittal, 1996; Prassanna and Panda, 1997). PEF can cause electrofusion or electroporation in cells (Chang, 1992). The electrofusion process consists of the fusion or coalescence of two or more cells into one large cell. This process has proved to be a relevant tool in genetic engineering because it provides selectivity and efficient control of the fusion process, prediction of the fusion conditions, and high yields of viable hybrids. On the other hand, electroporation consists of the formation of pores on the membrane of cells and organelles. Depending on the PEF intensity, electroporation may induce the formation of transient or permanent pores causing a reversible or an irreversible electroporation, respectively (Zimmermann, 1986; Rols *et al.*, 1990; Tsong, 1991; Weaver and Chizmadzhev, 1996). The occurrence of transient pores, which reseal after removing the electrical field, ensures the survival of the electrically stimulated recipient cells. At these conditions, electroporation is a valuable tool for injecting exogenous molecules such as drugs, proteins, RNA, or DNA into cells without causing deterioration of cellular functions. On the contrary, when PEF is applied at higher intensity, electroporation is so intense that electropermeabilization might be irreversible or even cell membrane and other structures might be disintegrated. Under these circumstances, PEF would cause permanent cell membrane damage or cell lysis which is the basis for the success of PEF treatments as a novel food processing method for the food industry.

PEF is one of the most relevant nonthermal food preservation processes because of its potential to inactivate microorganisms without altering sensorial and nutritional properties of foods (Barbosa-Cánovas *et al.*, 1999). However, PEF technology is not yet being used to preserve food commercially

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since several aspects are still not clear. The design of effective PEF treatments that assure the safety and stability of foods requires, among others, knowledge of the way PEF treatments kill microorganisms. A better understanding of the mechanism of microbial inactivation by PEF would help in defining PEF treatments that could form alternatives to traditional heat preservation. It would also allow the discovery of a biological basis for the mathematical models describing microbial inactivation by PEF treatments. These findings could explain the kinetics of microbial inactivation under the influence of different factors, and help to find out the causes of possible deviations. Additionally, a thorough knowledge of the factors that affect microbial PEF resistance will be required in order to extend the range of products that can be processed by PEF treatments.

Different components of interest for the food industry are located into plant cells. These components are extracted using different solid–liquid extraction methods. The efficiency of the extraction process is mainly influenced by the degree of cell membrane disintegration. PEF offers a way of disintegrating the cell membranes with a lower energy consumption than current methods such as heating. It has been demonstrated that a PEF pretreatment increase the extraction of sugar beet, potato starch intracellular pigments, or apple juice (Knorr and Angersbach, 1998; Bazhal *et al.*, 2001; Eshtiaghi and Knorr, 2002; Chalermchat *et al.*, 2003). PEF has also been suggested as a pretreatment step to facilitate the drying and osmotic dehydration of fruits and vegetables (Taiwo *et al.*, 2002). Extraction or diffusion of intracellular plant components is also a topic of interest of this book (see Chapter 6).

Whereas electropermeabilization has been thoroughly studied in biomembranes, mainly from eukaryote cells, its occurrence and relation to microbial inactivation by PEF is not well understood. This chapter aims to summarize the different theories on electroporation of eukaryote cell membranes and to present recent discoveries on electroporation of cell membranes of prokaryote cells in order to contribute to a better knowledge of the mechanism of microbial inactivation by PEF.

2. ELECTROPORATION OF BIOMEMBRANES

Basically, electroporation is a consequence of a significantly increased transmembrane potential resulting in structural changes in the membranes and formation of aqueous pathways or electropores. The membrane polarization is due to the accumulation of negative and positive charges within the cell at the membrane areas closest to the cathode and anode, respectively. At these areas the potential is too intense and electropores appear (Chang, 1992). The membrane area of electroporation is considered to cover less than 0.1% of the total surface area. Electropores can either reseal or remain open depending on the intensity of the electric field, the pulse number, the pulse width, frequency, and on several other factors related to the cell such as cell size and shape, surface membrane potential, etc. However, the exact mechanism of pore formation, expansion, and subsequent shrinkage and resealing or cell lysis remains controversial.

Several theories (Zimmermann *et al.*, 1974; Zimmermann, 1986; Tsong, 1991; Chang, 1992; Kinosita *et al.*, 1992; Newman *et al.*, 1992), based on the experiments carried out on model systems, such as liposomes or protoplasts, and on large eukaryote cells, such as erythrocytes, chloroplasts, green alga cells, sea urchin eggs, hamsters ovary cells, etc., have been proposed in order to explain the mechanism of the reversible electroporation and/or the electrical membrane breakdown.

One of the most accepted theories is the so-called "electromechanical instability theory" (Zimmermann *et al.*, 1974; Zimmermann, 1986; Ho and Mittal, 1996; Weaver and Chizmadzhev, 1996; Barbosa-Cánovas *et al.*, 1999). This theory considers cell membrane as a capacitor that is filled with a dielectric material of a very low dielectric constant in comparison to inside the cell and the surrounding environment. Because of the difference in dielectric constants, free charges accumulate

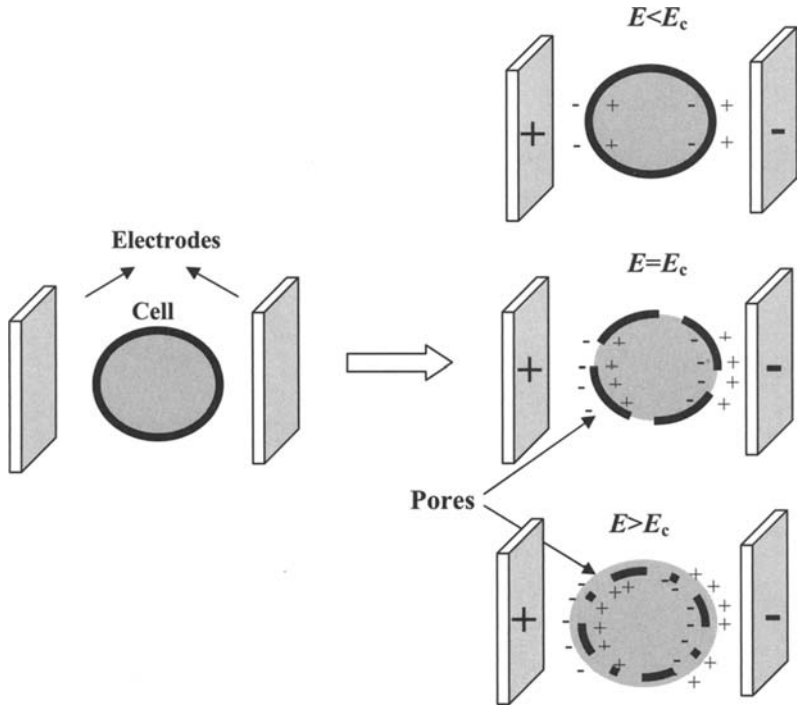

Figure 3.1. Electroporation of cell membrane when exposed to PEF treatments. E: electric field strength; E_c: critical electric field strength.

at both membrane surfaces generating a transmembrane potential of about 10 mV. Maintaining the transmembrane potential is vital for the cell since it forms part, in addition to the difference in proton concentration, of a form of potential energy called protonmotive force which can be used to drive a variety of energy-linked processes. These include the entrance of certain substrates into the cell against a concentration gradient; maintaining the cells turgor; maintaining the proper intracellular pH; turning flagella; driving a reverse flow of electrons through the respiratory chain to reduce NAD when the supply of $NADH_2$ is inadequate; generating ATP, etc. (Neidhardt *et al.*, 1990).

When an external electrical field is applied, transmembrane potential increases because more free charges accumulate at the two membrane surfaces. These charges are opposite and attract each other resulting in membrane compression, and therefore, membrane thickness is reduced. On the other hand, viscoelastic forces oppose the electrocompression of the membrane. However, when the transmembrane potential reaches approximately 1 V, the electrocompressive forces exceed the viscoelastic properties of the membrane and electrical membrane breakdown occurs (Fig. 3.1).

The number and size of pores depend on the electric field strength and the treatment time. The electric field intensity at which membrane breakdown occurs is called threshold or critical electric field. When the electric field applied reaches values close to the critical electric field or when the treatment time is short, the number and size of the generated pores is low. In these conditions, permeabilization of the membrane is reversible since cell membrane restores its structure and functionality when PEF treatment ceases. However, when more intense PEF treatments are applied the number of pores and their size increase resulting in an irreversible permeabilization or mechanical disruption of the cell (Zimmermann, 1986).

In contrast to the electric compressive forces, other theories consider more likely the occurrence of molecular reorientations either on the lipid bilayer or the protein channels as the cause of pore formation.

Electropermeabilization might be a consequence of a dipolar reorientation of the phospholipids within the two monolayers of the membrane under the electric field applied. These conformational changes in membrane structure could result in the loss of its functions as a semipermeable barrier resulting in the inactivation of the cell (Sale and Hamilton, 1968; Tsong, 1991).

According to Tsong (1991) exposure of a biomembrane to an electric field can cause formation of hydrophobic and hydrophilic pores in the lipid fraction. Hydrophilic pores conduct electricity generating localized Joule heating. Thus, the temperature increases resulting in thermal transitions of the lipid bilayer, which might also impair the semipermeable nature of the cell membrane.

Protein channels, pores, and pumps are also present in cell membranes (Tsong, 1991). Their functionality, as it has been described above, is dependent on transmembrane potential. The opening and closing potential of the channels formed by proteins is about 50 mV, which is considerably lower than the critical transmembrane potential. Therefore, when a PEF treatment is applied most of the protein channels are opened. Again, a Joule heating or other electric modifications may occur and protein channels might become irreversibly denatured resulting in the formation of pores. Pore creations and resealing occurring in the protein channels may take place from nanoseconds to hours.

Pore formation might also be due to structural defects in the membrane consisting of spontaneous pores that expand when the electric field exceeds the critical transmembrane potential (Tsong, 1991).

The theories described above explain the formation of pores in the cell membrane. Nevertheless, so far there is no clear evidence on the underlying mechanism of membrane permeabilization at the molecular level. In many eukaryotic cells the cytoplasmic membrane is the only barrier which separates the cytoplasm from the environment, and it is likely that, under these circumstances, the theories proposed are correct. However, microorganisms, including yeasts and bacteria, have additional structures located externally to the cytoplasmic membrane (Neidhardt *et al.*, 1990). Figure 3.2 shows the structure of the envelopes of Gram-positive and Gram-negative bacteria. In Gram-positives, there is a thick cell wall made of various peptidoglycan layers and teichoic acids, which confers rigidity and physical resistance to the cell. In Gram-negatives, the cell wall is thinner, but it is surrounded by an outer membrane. The outer membrane differs from the typical biomembrane since its external leaflet is made of lipopolysaccharides instead of phospholipids, and it confers especial resistance against the entrance of some molecules such as antibiotics, bile salts, and some bacteriocins. In both types of cells, but more especially in Gram-negatives, these particular structures form a space with a different chemical composition from the environment. Also, yeasts have an external cell wall. The influence of these external structures on cell electroporation by PEF, if any, remains unknown.

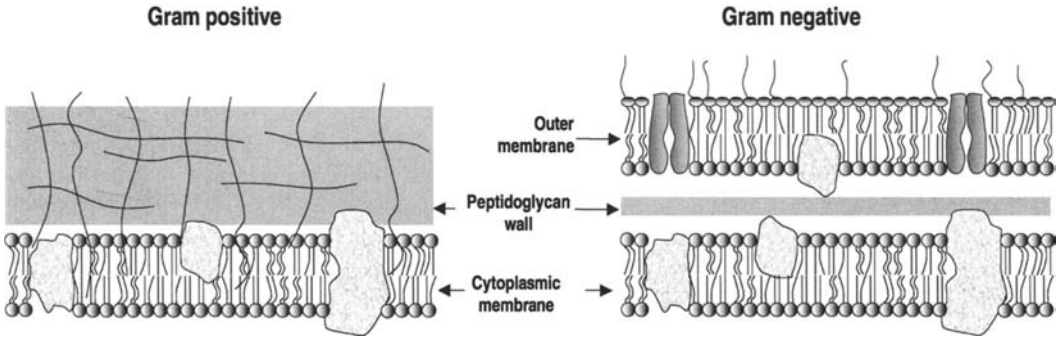

Figure 3.2. Envelopes of Gram-positive and Gram-negative bacteria.

3. ELECTROPORATION OF MICROORGANISMS OF INTEREST IN FOOD PRESERVATION

Either moulds, yeast, and bacteria are microorganisms of interest in food preservation. Most act as spoilage organisms or pathogens, so prevention of their growth or even better their destruction are the major concerns of food preservation technologies. Many attempts have been made in order to discover whether membrane electroporation is the cause of microbial inactivation by PEF.

The first systematic studies on the effect of PEF treatments on the inactivation of microorganisms were reported in the late 1960s (Hamilton and Sale, 1967; Sale and Hamilton, 1967, 1968). Nevertheless, most of their conclusions are still valid today. The authors proved that microbial inactivation was due to the direct effect of PEF treatments rather than to the products of electrolysis or temperature increase. It was finally proposed that PEF treatments caused an irreversible loss of the membrane functions as the semipermeable barrier between the bacterial cell and its environment, and that this was the cause of cell death. Several decades later, the precise mechanism of microbial inactivation by PEF remains controversial.

Most authors have established that PEF exerts its action primarily on the cytoplasmic membrane (Hamilton and Sale, 1967; Sale and Hamilton, 1967, 1968; Tsong, 1991; Ho and Mittal, 1996; Weaver and Chimadzhev, 1996; Barbosa-Cánovas *et al.*, 1999; Wouters *et al.*, 2001, 2002; Heinz *et al.*, 2002; Aronsson *et al.*, 2005). However, it is not so clear whether microbial inactivation is due to the formation of a few reversible small pores, to many irreversible large pores, or to the complete mechanical disruption of the membrane. Moreover, other phenomena associated with alteration of membrane functions or with chemical stresses might also be involved.

Maintenance of the integrity and functionality of cell envelopes is vital for microorganisms. Cell envelopes protect microorganisms from the surrounding environmental conditions and act as a semipermeable barrier. The cell membrane controls the passage of small molecules, nutrients, and end products of metabolic activities, into and out of the cell; extrudes polymeric substances such as extracellular enzymes and cell wall materials; it is the site of many complex activities within the cell, e.g., RNA, protein and cell wall synthesis, electron transport, and oxidative phosphorylation and it plays an important role in the control of DNA synthesis. Basically, the cytoplasmic membrane controls the cellular metabolic activities by maintaining an effective osmotic boundary between the cell interior and the surrounding environment. Any damage in the membrane, which impairs one or more of these activities, could result in cell death.

There are many ways to study whether cell envelopes are intact or not. The most common methods used to determine microbial membrane damage are electron microscopy examinations, measurement of leakage of intracellular material, measurement of osmotic response, measurement of dye exclusion, and measurement of sublethal injury by a selective medium plating technique. Next, the most interesting results obtained by using these techniques are reviewed.

3.1. Electron Microscopy Examination

Because of its higher resolving power, electron microscopy has been used to examine morphological changes in cells, either at cytoplasmic organelles or cell envelopes, after PEF treatments. With regards to membrane damage, the technique allows detecting pores from 1 nm in diameter. However, it should be noted that it would not always allow observing lysed cells since their debris might be removed during the washing and cell concentration steps.

Most studies carried out on microorganisms treated by PEF have shown an increase in surface roughness, craters, elongation, disruption of organelles, separation and condensation of cytoplasmic contents, cell wall breakage, or pore formation (Jayaram and Castle, 1992; Pothakamury *et al.*, 1996, 1997; Harrison *et al.*, 1997; Calderón-Miranda *et al.*, 1999c; Dutreux *et al.*, 2000b; Rowan *et al.*,

2000; Aronsson and Rönner, 2001). It is noticeable that most of the morphological changes have been observed in bacteria rather than in yeast in spite of their lower PEF resistance (Harrison *et al.*, 1997; Aronsson and Rönner, 2001). Nevertheless, the use of this technique has not confirmed any relationship between membrane damage and microbial inactivation by PEF. For instance, Pothakamury *et al.* (1997) described by scanning electron microscopy (SEM) that cells subjected to a PEF treatment lost smoothness and uniformity, and some cells were shrunken. Using transmission electron microscopy (TEM) the authors observed that PEF-treated bacteria exhibited thinner or ruptured cell walls and the cytoplasmic content leaked out of the cells. These spectacular morphological changes were mostly observed at the most intense PEF treatment conditions. However, the extension of the changes was not noticeable at low intensity PEF treatments in spite of the fact that most cells were also inactivated. Also, Harrison *et al.* (1997) and Aronsson and Rönner (2001) observed that the frequency of morphologically damaged cells did not correspond to the log reductions of viability. This lack of relation cannot be taken as proof of PEF treatments not causing structural disorganization of the membrane. However, these results allow confirming that the complete disorganization of the cell membrane is not the major mode of microbial inactivation but is a secondary event. In the case membrane damage is involved microbial inactivation might be due to more subtle changes such as the formation of transient pores or to smaller irreversible electropores.

3.2. Leakage of Intracellular Material

Already in 1967, Hamilton and Sale demonstrated the release of the periplasmic β-galactosidase enzyme in a permease-negative mutant of *Escherichia coli* treated by PEF. The most commonly used techniques to evaluate the presence of intracellular material outside the cell are the measurement of UV-absorbing material such us nucleic acids and proteins at 260 and 280 nm, and the presence of adenosine triphosphate (ATP) by using a luceferin–luceferase assay. Several authors (Hamilton and Sale, 1967; Grahl and Markl, 1996; Simpson *et al.*, 1999; Wouters *et al.*, 2001; Aronsson *et al.*, 2005) have observed the leakage of these intracellular components from PEF-treated cells confirming that membrane electroporation occurs. However, it has not been possible to find a relationship between the amount of intracellular components outside the cell and the number of inactivated cells. Simpson *et al.* (1999) observed a significant increase in leakage of UV-absorbing material at a field strength which was nonlethal for both *Listeria monocytogenes* and *Salmonella enterica* serovar Typhimurium. Also, Aronsson *et al.* (2005) observed a similar amount of intracellular compounds outside the cell in *Escherichia coli* and *Listeria innocua* cells which showed a very different PEF sensitivity. These authors also reported large amounts of released ATP and UV-absorbing material in PEF-treated *Saccharomyces cerevisiae* cells, whereas the level of inactivation was relatively low.

Therefore, these results confirm the occurrence of electroporation and, since the leakage of intracellular material occurs in surviving cells, it also confirms the presence of reversible or repairable pores. However, it does not discard the participation of other phenomena related to microbial inactivation.

3.3. Loss of Osmotic Response

This technique evaluates membrane integrity alterations through the loss of the ability of the cell to plasmolize. When a cell with an undamaged membrane is suspended in a hypertonic medium, such as a strong salt solution, water diffuses from the cytoplasm to the external medium. This initial passive response causes a strong condensation of the cytoplasmic material, which can be measured through the increase in optical density of cell suspensions (Korber *et al.*, 1996). On the contrary, in cells whose membrane is altered, salt and water diffuse freely and no response is detected.

Hamilton and Sale (1967) were the first authors reporting that PEF-treated *E. coli* cells partially lost the ability to plasmolize in a hypertonic medium. Our preliminary results have also confirmed the occurrence of this phenomenon in PEF-treated *Escherichia coli* and *Salm.* Typhimurium cells. These results also confirm the existence of membrane damage and do not discard the occurrence of other phenomena related to microbial inactivation.

3.4. Permeabilization to Nonpermeant Dyes

The measurement of the increased uptake of fluorescent dyes that do not normally penetrate the membrane of healthy cells is a direct and sensitive technique to evaluate membrane integrity. The most common molecule used to assess membrane permeabilization is the fluorescent probe propidium iodide (PI) (Kaneshiro *et al.*, 1993). PI is a strongly hydrophilic, small molecule (660 Da) which is a nucleotide-binding probe unable to enter intact cells. The advantages of PI staining are its high sensitivity and the potential to obtain quantitative results by using a spectrofluorimeter, to observe stained cells by using an epifluorescent microscope and to analyze individual cells in combination with flow cytometry or image analysis. Nevertheless, in some cases results obtained are not easy to explain since total fluorescence emitted depends also on other physiological facts besides membrane integrity, such as the availability of binding sites within the DNA and RNA molecules.

PI has been the most common fluorescent probe to assess membrane electroporation of PEF-treated bacteria and yeast (Weaver *et al.*, 1988; Wouters *et al.*, 2001, 2002; Unal *et al.*, 2002; Ulmer *et al.*, 2002; García *et al.*, 2006a; Aronsson *et al.*, 2005). Results described by Wouters *et al.* (2001), Aronsson *et al.* (2005), Unal *et al.* (2002), and García *et al.* 2003 have allowed to confirm the occurrence of irreversible pores and their correlation with microbial inactivation, at least within the two first Log_{10} cycles of inactivated cells of bacteria such as *Escherichia coli*, *Salm.* Typhimurium, *Salmonella enterica* serovar Senftenberg, *Listeria innocua*, *Listeria monocytogenes*, *Lactobacillus plantarum*, *Lactobacillus fermentum*, or *Lactobacillus leichmanni.*

Membrane permeabilization confirmed by the cell uptake of PI after PEF treatment has been observed to be dependent on different process parameters, microbial characteristics, and product parameters. Table 3.1 summarizes the most relevant factors affecting microbial PEF resistance. Those factors which have been found to affect membrane electroporation assessed by cell uptake of PI are underlined.

3.4.1. Effect of Process Parameters: Electric Field Strength, Treatment Time, and Specific Energy

Electric field strength and treatment time are the two main process parameters influencing microbial inactivation by PEF. As described by Unal *et al.* (2002), increases in field strength decrease the count of survivors and proportionally increase the fluorescence intensity (Wouters *et al.*, 2001;

Table 3.1. Factors affecting microbial inactivation by pulsed electric fields (PEF).

Process parameters	Microbial characteristics	Product parameters
Electric field strength	Intrinsic resistance	Conductivity
Treatment time	Cell size and shape	pH
Pulse characteristics	Growth conditions	Water activity
Frequency	Cell concentration	Composition
Temperature	Recovery conditions	
Specific energy		

Underlined appear investigated factors affecting membrane permeabilization.

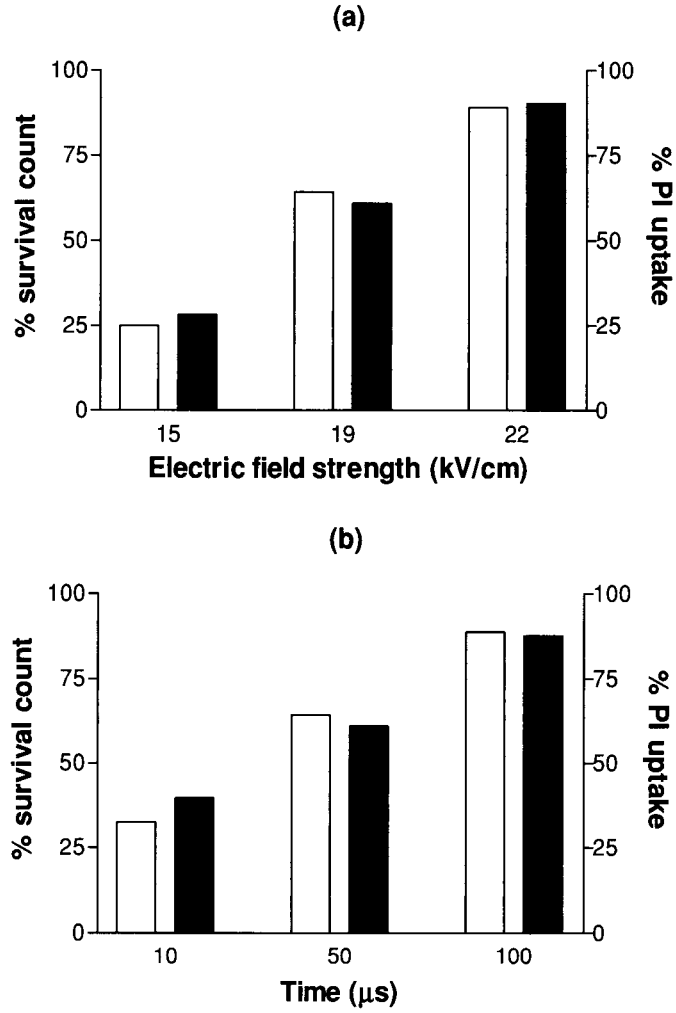

Figure 3.3. Relationship between membrane permeabilization and *Salmonella enterica* serovar Senftenberg 775W cell inactivation depending on the electrical field strength and the treatment time. Percentage of fluorescent and dead cells after PEF treatments in citrate–phosphate buffer of pH 7 at 15, 19, and 25 kV/cm for 50 μs (a) and at 19 kV/cm for 10, 50, and 100 μs (b). Survivors (white bars); fluorescence (black bars).

García *et al.*, 2006a). An increase in the number of electric pulses results in a larger number of permeabilized cells. It has also been found a linear relationship between the degree of permeabilization and the treatment time (Wouters *et al.*, 2001; García *et al.*, 2006a). The relationship between cell death and membrane permeabilization after PEF treatments at different electric field strengths and treatment times is illustrated in Fig. 3.3.

Heinz *et al.* (1999) have proposed the specific energy (J/mL) as a new control parameter for PEF processing instead of treatment time. Specific energy would be a better control parameter, especially when exponential decay pulses are applied, as it involves different factors such as electrical field strength, pulse width and resistance of the treatment chamber, and the temperature of the treatment medium. Specific energy also has been found to determine the degree of membrane permeabilization. An increase in the energy input as a result of applying more electric pulses resulted in a larger number of permeabilized *Lactobacillus plantarum* cells (Wouters *et al.*, 2001).

Results obtained about the influence of process parameters on the occurrence of irreversible electroporation clearly suggest that membrane permeabilization is directly involved in the inactivation of vegetative bacterial cells.

3.4.2. Effect of Type of Microorganism

Microbial inactivation by PEF also depends on the intrinsic resistance of microorganisms, species, and strains (Wouters and Smelt, 1997; MacGregor *et al.*, 2000; Wouters *et al.*, 2002; Lado and Yousef, 2003). In general, Gram-positive are more resistant than Gram-negative bacteria (Sale and Hamilton, 1967; Hülsheger *et al.*, 1983; Mazurek *et al.*, 1995; Aronsson and Rönner, 2001), and bacteria are more resistant than yeast (Sale and Hamilton, 1967; Qin *et al.*, 1998). Membrane permeabilization confirmed by the uptake of PI after PEF has also been observed to depend on the microorganism investigated. Apart from some exceptions, the relationship between irreversible permeabilization and inactivation has been confirmed in both Gram-positive and Gram-negative bacteria (Wouters *et al.*, 2001; Unal *et al.*, 2002; García *et al.*, 2006a; Aronsson *et al.*, 2005). In fact, as described by Unal *et al.* (2002), the fluorescence staining technique might potentially be used to determine the relative sensitivity of bacteria to PEF.

On the contrary, Aronsson *et al.* (2005) did not observe a linear relationship between PEF permeabilization and inactivation of *Saccharomyces cerevisiae* cells. They observed a rapid increase in PI uptake after apparently nonlethal PEF treatments which suggests that the ability to survive permeabilization is not correlated to PEF resistance. These results together with those previously commented about morphological changes observed by TEM examinations and the higher amounts of leaked intracellular components in relation to the level of inactivation suggest that other mechanisms rather than or in addition to membrane permeabilization seem to be involved in PEF inactivation of yeast.

The intrinsic resistance of microorganisms seems to be related to their cell size and shape (Kehez *et al.*, 1996; Qin *et al.*, 1998; Heinz *et al.*, 2002). *Listeria monocytogenes* is the smallest vegetative cell bacteria investigated and has shown the highest resistance to PEF treatments. Moreover, the major sensitivity of yeast to PEF treatments is believed to be due to their higher cell size. The influence of the cell size on the inactivating effect of PEF treatments is related to the transmembrane potential created by an external electric field. The smaller the cell size, the smaller the membrane potential induced by the action of the electrical field and a higher microbial resistance to PEF treatments is reached (Zimmermann *et al.*, 1974; Hülsheger *et al.*, 1983). Wouters *et al.* (2001) have demonstrated that cells of *Lactobacillus plantarum*, sorted by flow cytometry on the basis of different sizes and shapes, showed differences in membrane permeabilization. Larger cells were more easily permeabilized than smaller cells; however, with increasing time or energy input of the PEF treatment, the difference in membrane permeabilization gradually disappeared.

García *et al.*, 2005c have shown that, when comparing the PEF resistance of eight bacterial strains under the same experimental conditions, none of the general assumptions about the influence of microbial characteristics such as cell size, shape, and type of cell envelopes on the bacterial PEF resistance exerted the expected influence. For example, when treated at pH 7.0, as illustrated in Fig. 3.4a, *St. aureus* was more PEF sensitive than that of the Gram-negatives *Salm. enterica* Senftenberg or *E. coli* O157:H7 despite being a Gram-positive. Moreover, *Y. enterocolitica*, which is one of the smallest bacterium investigated, was more PEF sensitive than larger cells like, for instance, *Lb. plantarum.* Also, the shape of the bacteria did not exert the expected influence. For instance, the rod-shaped *E. coli* cells were as sensitive as *St. aureus* or *Y. enterocolitica,* which are spherical and cocobacilli cells, respectively. When treated at pH 4.0, as seen in Fig. 3.4b, the situation was not different. For instance, *Lb. plantarum*, which is a large cell, was the most resistant

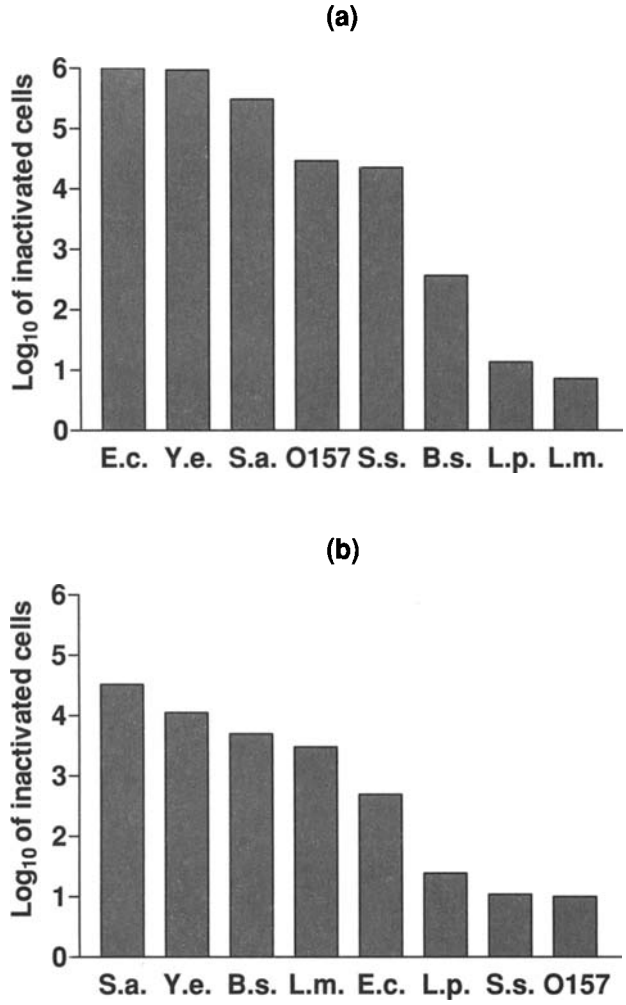

Figure 3.4. Log_{10} cycles of inactivation of *B. subtilis subsp. niger* (B. s.), *Lb. plantarum* (L. p.), *L. monocytogenes* (L. m.), *St. aureus* (S. a.), *E. coli* (E. c.), *E. coli* O157:H7 (O157), *Salm.* Senftenberg 775W (S. s.), and *Y. enterocolitica* (Y. e.) after a PEF treatment at 25 kV/cm for 200 exponential decay pulses in citrate–phosphate buffer of pH 7.0 (a) and pH 4.0 (b).

Gram-positive bacterial strain at pH 4.0. *E. coli* O157:H7 and *Salm.* Senftenberg 775W were the most PEF resistant strains, and *L. monocytogenes,* despite being a Gram-positive and the smallest cell tested, was more PEF sensitive than *Lb. plantarum* or *E. coli*. In conclusion, Gram-positive bacterial cells were not necessarily more PEF resistant than Gram-negatives, neither larger nor sphere-shaped cells were more PEF susceptible than smaller or rod-shaped cells, respectively. The influence of these microbial characteristics on PEF inactivation was dependent on the pH of the treatment medium. The treatment medium pH also determined differences in membrane permeabilization assessed by PI uptake among the microorganisms investigated. García *et al.* (2006a) have shown that loss of viability might correlate either with the occurrence of irreversible pores, when *L. monocytogenes* and *Lb. plantarum* cells were PEF-treated at pH 7.0, or with the formation of reversible and irreversible pores during PEF when *Salm.* Senftenberg 775W and *E. coli* were PEF-treated at the same pH. However, when cells were PEF-treated at pH 4.0, loss of viability was correlated with a permanent loss of membrane integrity in *L. monocytogenes* cells, and the degree of permeabilization was higher

than the percentage of inactivated cells in *Lb. plantarum* and much lower in *Salm.* Senftenberg 775W and *E. coli.*

Regarding growth conditions such as growth phase, growth medium composition, growth temperature, or the cell exposure to different environmental stress conditions very little is known.

Generally, it has been demonstrated that bacterial cells in the exponential growth phase are more sensitive to PEF treatment than stationary growing cells (Hülsheger *et al.*, 1983; Gásková *et al.*, 1996; Pothakamury *et al.*, 1996; Wouters *et al.*, 1999; Álvarez *et al.*, 2000). However, the influence of the cell age was not very important and the difference between the survival fractions after a PEF treatment on the most and the least resistant cells was just two log cycles (Pothakamury *et al.*, 1996). The reason for the smaller PEF resistance of exponentially growing cells seems to be the greater electromechanical instability of microorganisms due to either the continuous cell division at this growth phase or the larger cell size (Jacob *et al.*, 1981). However, Wouters *et al.* (2001) described a similar level of membrane permeabilization for both exponential and stationary growth phase cells. These results suggest once more that other phenomena in addition to permanent membrane permeabilization are involved in the mechanisms of inactivation.

PEF resistance is also influenced by microbial growth temperature. Álvarez *et al.* (2003) showed that *Listeria monocytogenes* incubated at 35°C was more resistant to PEF treatments than cells incubated at 4°C. It is known that growth temperature induces changes in the cell membrane or cell wall composition, such as variations in fatty acids chain length, in a ratio of saturated to unsaturated fatty acids, or the presence of cyclopropane fatty acids in cell envelopes. Membrane fluidity is significantly related to the lipid composition. Bacterial membranes show the ability to adapt to changes in growth temperature by maintaining membrane fluidity at the proper value in order to keep membrane functionality (Tsuchiya *et al.*, 1987). As the electromechanical instability of the cell envelopes is directly involved in microbial PEF resistance these changes in the composition of cell envelopes might be responsible for the differences in the PEF resistance observed. Further research is needed to clarify these aspects and particularly it would be of great interest to evaluate differences in membrane permeabilization among microorganisms grown at different temperatures using the PI exclusion technique.

There are no reports of any detailed investigation about the effect of cell exposure to different environmental stress conditions on the microbial resistance and membrane permeabilization after PEF. For instance, it has been described that microbial resistance to heat can dramatically increase after exposure to different environmental stress conditions including heating at sublethal temperatures, presence in the medium of chemical compounds such as ethanol, antibiotics and amino acid restrictors shock, and acidic shock, etc. (Neidhardt *et al.*, 1984; Farber and Pagotto, 1992; Pagán *et al.*, 1997; Rowan, 1999). Heat shock is the most well known stress condition and its occurrence has been widely described in food processing. It is believed that a heat shock triggers a physiological response (Schlesinger, 1986) that leads to the synthesis of special proteins known as heat shock proteins. Most of these new proteins are synthesized by any stress condition and seem to play a role in the protection of microorganisms against any physicochemical agent. Because this is a universal mechanism, described in most cells under different conditions, it would be very valuable to study whether sublethal PEF treatments increase microbial resistance to lethal PEF, or whether heat shock proteins, synthesized during any kind of stress conditions, affect microbial resistance to PEF treatments by protecting cells against PEF damage, by avoiding membrane permeabilization or allowing the electropermeabilized membrane to reseal. More research is needed in order to describe the kind of stresses PEF treatment can induce in microorganisms and the effect of heat shock proteins on microbial PEF resistance. The occurrence of stress conditions should be taken into account when designing combined methods since cells might be more PEF resistant than expected.

3.4.3. Effect of Product Characteristics

With regards to product parameters influencing electroporation by PEF, only the conductivity and the pH of the medium have been examined.

The effect of the conductivity on membrane permeabilization, as described on microbial PEF resistance, is unclear. Heinz *et al.* (2002) have described a direct effect of conductivity on the degree of membrane permeabilization by PEF treatment, but this effect is negligible if the duration of the pulse is longer than 1 μs. On the other hand, Wouters *et al.* (2001) have reported a higher membrane permeabilization degree of *Lactobacillus plantarum* cells suspended in a phosphate buffer having a conductivity of 4 mS/cm than of 15 mS/cm after PEF treatment at 25 kV/cm and with the same energy input. However, at the same electric field strength, less membrane permeabilization was necessary to obtain a certain level of inactivation at 15 mS/cm than at 4 mS/cm.

On the other hand, results obtained so far have shown that the effect of the pH of the treatment medium on microbial PEF resistance and on membrane permeabilization is sometimes greater than that due to the genotype. According to these results, Gram-positive bacteria are not necessarily more PEF resistant that Gram-negative. For instance, even though *Salm.* Senftenberg was more sensitive than *Listeria monocytogenes* when PEF treated in pH 7 (at 25 kV/cm), it showed a much higher resistance when treated at pH 4 (García *et al.*, 2005a). The evaluation of membrane permeabilization with PI showed that whereas a linear relationship between the percentage of fluorescent and inactivated cells could be observed either when *Listeria monocytogenes* was PEF-treated at pH 7 or 4 or *Salm.* Senftenberg at pH 7, less that 30% of *Salm.* Senftenberg cells were fluorescent in relation to more than 90% of inactivated cells when treated at pH 4. The same behavior was also observed in other Gram-negative bacteria such as *Escherichia coli* or *Salm.* Typhimurium (García *et al.*, 2006a). Figure 3.5 illustrates this phenomenon with *Escherichia coli* cells.

These results would suggest that permanent membrane permeabilization does not occur under every treatment condition. However, it should be noted that the PI exclusion technique does not allow assessing the occurrence of small pores (<660 Da) and therefore subtle membrane permeabilization might also occur not being noticeable by using this methodology. Since the mechanism of inactivation by PEF seems to be related to the effects on the cell envelopes it seems reasonable to speculate that the differences in the structure and the composition between the cell envelopes of Gram-positive and Gram-negative bacteria could be responsible for this different microbial response observed depending on the treatment medium pH.

An interesting and almost nonexplored aspect of PEF treatments is the occurrence of reversible pores in microorganisms. Reversible pores might include pores which immediately reseal after PEF treatment, also known as transient pores, and repairable pores. These might be repaired by the cells after treatment, only under favorable conditions in the phenomenon called sublethal injury. Nevertheless, we will refer to reversible pores, as most authors do, as those immediately resealed after cessation of the electric field, and repairable pores as those that account for sublethal injury.

Pagán and Mackey (2000) reported the use of the fluorescent dye PI added to the treatment medium as a marker of permanent or nonpermanent loss of membrane integrity during high hydrostatic pressure treatments. Based on the same approach, García *et al.* (2006a) have shown that when PI was present in the suspending medium during PEF treatment, the degree of staining of *Listeria monocytogenes* cell population was approximately twofold greater than when it was added after PEF treatment for 100 μs at 19, 22, or 25 kV/cm. These results would indicate that a percentage of cells were able to reseal their pores just after PEF treatment and survive. Ulmer *et al.* (2002) also observed partial reversibility of membrane permeabilization just only when cells of *Lactobacillus plantarum* were inactivated below critical values of 13 kV/cm, above these values cell damage was irreversible.

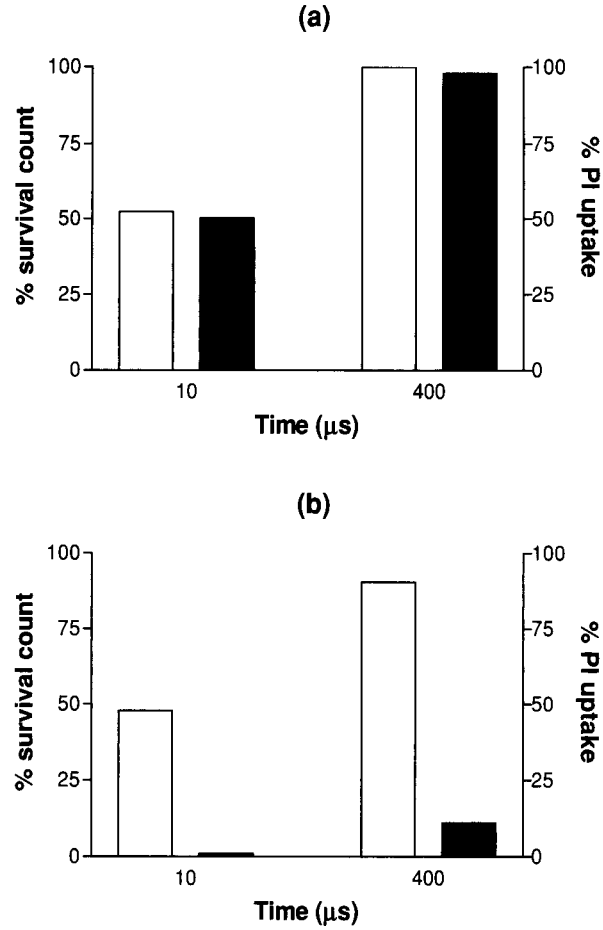

Figure 3.5. Relationship between membrane permeabilization and *Escherichia coli* cell inactivation depending on the treatment medium pH. Percentage of fluorescent and dead cells after PEF treatments at 19 kV/cm for 10 and 400 μs either in citrate–phosphate buffer of pH 7 (a) or 4 (b). Survivors (white bars); fluorescence (black bars).

The higher sensitivity of some Gram-positive bacteria to acidic pHs under PEF treatment seems to be related to the partially reversible membrane permeabilization. The low pH of the treatment medium would affect the cytoplasm therefore disturbing the homeostasis of the cells. Moreover, unfavorable acidic conditions within the cytoplasm might prevent cells from repairing sublethal injuries hampering the reversion of the repairable pores (Wouters *et al.*, 2001). García *et al.* (2006a) have shown how *Listeria monocytogenes* cells in pH 7 buffer exhibited less inactivation compared to cells treated in pH 4. The presence of PI in the suspending medium during PEF treatment yielded a similar degree of staining. Therefore, these results suggest that the magnitude of membrane permeabilizing during the treatment would be similar independently of the differences in PEF resistance shown. Whereas at neutral pHs damaged cells would repair sublethal injuries, cells at pH 4 would not be able to repair the damages and would die. Following the same methodology, García *et al.* (2006a) also demonstrated that the PI exclusion technique did not allow confirming these results in Gram-negative bacteria since, as mentioned above, these were not permeable to PI when PEF treated at pH 4, even when PI was added to the treatment medium before PEF treatment. These results would suggest that in case reversible pores occur in Gram-negative bacteria treated at pH 4, the pores would be smaller than those irreversible pores detected when treated at pH 7. They also support the view

that bacterial inactivation by PEF is not a simple phenomenon directly related to permanent pore formation in the cytoplasmic membrane, and the presence of an external periplasmic space delimited by the outer membrane might possibly modify charge distribution and therefore interfere with the electroporation process.

The fact that reversible pores have been detected is a very relevant aspect to be taken into account from a practical point of view because it means that PEF might act synergistically with many other hurdles, proving to be valuable in developing appropriate combination processes. Combined processes using PEF treatment might look for the conditions to avoid resealing of the pores and the possibility of combining with the addition of food preservatives (Gásková *et al.*, 1996; Liu *et al.*, 1997; Calderón-Miranda *et al.*, 1999a,b; Dutreux *et al.*, 2000a; Terebiznik *et al.*, 2000; Pol *et al.*, 2000). Permeable cells under PEF treatments may facilitate the entry of antimicrobial substances such as organic acids, nisin, or lysozyme, increasing their bactericidal action. Dutreux *et al.* (2000a) suggested that these findings open up the prospect of a treatment for killing high PEF resistant Gram-positive organisms at low temperature. The capability of permeabilizing cells suggests PEF treatments might even improve the action spectrum of natural antimicrobials. The possibility of combining PEF treatments and organic acids at neutral pHs might also be considered since the more abundant organic acid form at neutral pHs, the dissociated molecule, which do not normally penetrate cell membranes, might enter the cell.

3.5. Occurrence of Sublethal Injury

Membrane damage measured using a selective medium plating technique includes the loss of both membrane integrity and functionality. The method consists of plating survivors after treatments into two culture media: a nonselective one, which allows cells to repair sublethal damages and recover; and a selective one, in which survivors are not capable of repairing their damages and finally die (Mackey, 2000). Membrane damage detected following this methodology is commonly called "sublethal injury." As illustrated in Fig. 3.6, sublethally injured cells are estimated by the difference in the number of survivors obtained after plating treated cells in both nonselective and selective

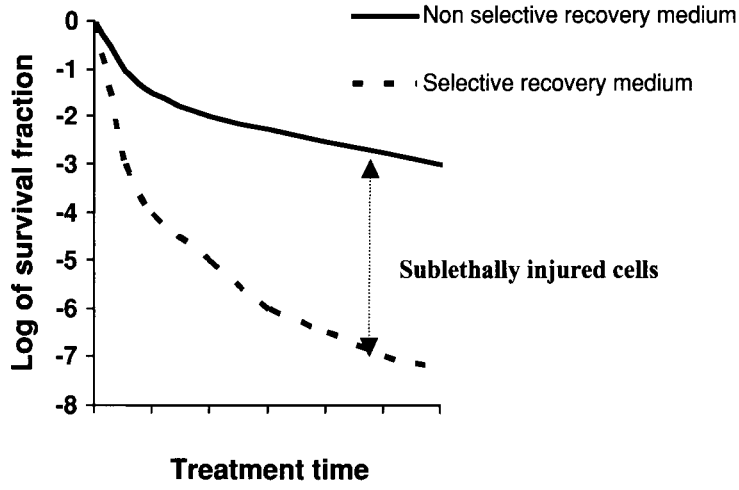

Figure 3.6. Theoretical survival curves obtained after plating PEF-treated cells in both nonselective and selective recovery media.

media. The most common selective media used to detect damage to the cytoplasmic membrane is agar with sodium chloride added (Mackey, 2000).

This technique has some disadvantages since it is an indirect way of evaluating membrane damage, it is not possible to directly visualize the damages, and results would refer to either loss of membrane integrity or functionality. However, it offers some advantages in comparison to the other techniques commented above. First of all, it offers much higher sensitivity since it is possible to study the behavior of up to 6–8 Log cycles within the population rather than just 1–2 Log cycles, which represent only 90–99% of the total population. This is fairly important because the last surviving cells are of the most relevance to food preservation since they are implied in the occurrence of tails, i.e., when treatment time is extended in order to attain a greater microbial inactivation and it is observed that the survival fraction decreases more slowly after an initial first period at a greater inactivation rate. In fact a few surviving cells, proven the environmental conditions are adequate, rapidly multiply resulting in infective concentrations. On the other hand, the technique offers additional information about loss of membrane functionality as a semipermeable barrier which would imply an impairment of homeostatic or barrier functions associated with cell envelopes. Therefore, it shows up the optimal treatment conditions to combine PEF with additional stresses which may prevent cell repair and survival.

The occurrence of sublethal injury in PEF-treated cells is a matter of controversy. Although the detection of reversible pores under PEF, as described above, would suggest so, most published investigations (Simpson *et al.*, 1999; Russell *et al.*, 2000; Dutreux *et al.*, 2000a,b; Ravishankar *et al.*, 2002; Ulmer *et al.*, 2002; Wuytack *et al.*, 2003; Aronsson *et al.*, 2004; Evrendilek *et al.*, 2004) have not in fact detected it using the selective plating medium technique. Hence, it has been generally accepted that bacterial inactivation by PEF is an "all-or-nothing" effect. Only Damar *et al.* (2002) detected a scarce percentage of sublethally injured cells, less than 45% of surviving cells, when *Escherichia coli* and *Staphylococcus aureus* were PEF treated at 20 kV/cm in peptone solution. Also, Liang *et al.* (2002) or Unal *et al.* (2001) and Ravishankar *et al.* (2002) have observed sublethally injured cells, but only when PEF were combined with ozone or heat, respectively. In this case, it is not clear whether sublethal injury is due to PEF, or a consequence of heat and ozone, which might be responsible for the occurrence of sublethal injuries per se.

On the contrary, some results obtained by our research group (García *et al.*, 2003, 2005a,b) have demonstrated that PEF-treated *Escherichia coli, Escherichia coli* O157:H7, *Pseudomonas aeruginosa*, *Yersinia enterocolitica*, *Salm.* Senftenberg 775W, *Salm.* Typhimurium, *Listeria monocytogenes*, and *Bacillus subtilis* cell populations were sublethally injured in a large proportion depending on the treatment conditions. For example, more than 99.9% of survivors of *Escherichia coli* were sublethally injured when cells were treated at acidic pH for 400 μs at 19 kV/cm. Also, more than 99.9% of survivors of *Listeria monocytogenes* were sublethally injured when cells were treated at pH 7 for 400 μs at 25 kV/cm.

García *et al.* (2005a) have demonstrated that the discrepancies in published data may arise from the fact that the occurrence of sublethal membrane damage by PEF treatments depends on the pH of the suspending medium and on the bacterial species. In this way, six Gram-negative bacteria showed a higher resistance to PEF at acidic pH (as compared to neutral pH) that was correlated to the capability to repair the cytoplasmic membrane, which was extensively damaged. For *Listeria monocytogenes* and *Bacillus subtilis* the opposite was true, the greater resistance to PEF, the occurrence of membrane damage and the subsequent repair ability was detected at neutral pH. At acidic pH the cells became more sensitive and irreversibly damaged. Figure 3.7 shows that more than 99.9% of survivors of *Listeria monocytogenes* and *Escherichia coli* O157:H7 cells were injured after a PEF treatment in citrate–phosphate buffer of pH 7.0 and 4.0, respectively.

The growth of sublethally injured cells in nonselective media indicates that, in some cases, damages inflicted by PEF are repairable. García *et al.* (2006b) have shown that these damages are

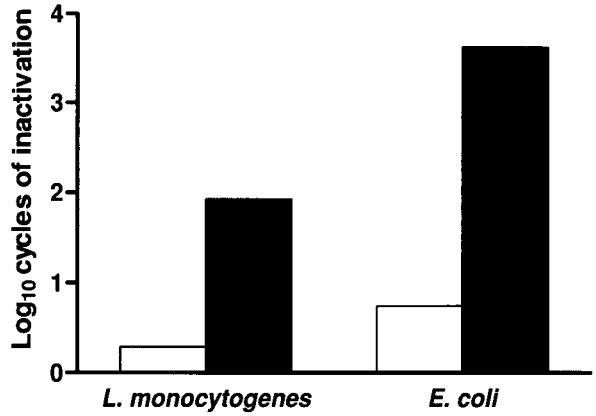

Figure 3.7. Occurrence of sublethal injured depending on the microorganism and the treatment medium pH. Log_{10} cycles of inactivation of *Listeria monocytogenes* and *Escherichia coli* O157:H7 cells after PEF treatments at 25 kV/cm for 200 μs or 19 kV/cm for 100 μs, in citrate–phosphate buffer of pH 7.0 and 4.0, respectively, and recovered in the nonselective (white bars) and the selective (black bars) medium.

repaired and cells start to multiply in less than 3 h when incubated in an appropriate nutritive medium at the optimum growth temperature. Two different types of sublethally injured cells were detected when *Escherichia coli* cells were PEF-treated at pH 4. Whereas a small proportion (<5%) was repaired after PEF in less than 2 min, the repair of the remaining 95% injured cells lasted 2 h and was dependent on biosynthetic requirements. The addition of inhibitors such as chloramphenicol, cerulenin, penicillin G, rifampicin, and sodium azide to the repair medium showed that the repair of such sublethally injured cells required energy and lipid synthesis, and was not dependent on protein, peptidoglycan, or RNA synthesis (Fig. 3.8). Since in prokaryote cells lipids are only located in their membranes, the requirement of the lipid synthesis for the repair of the PEF-damaged cells clearly

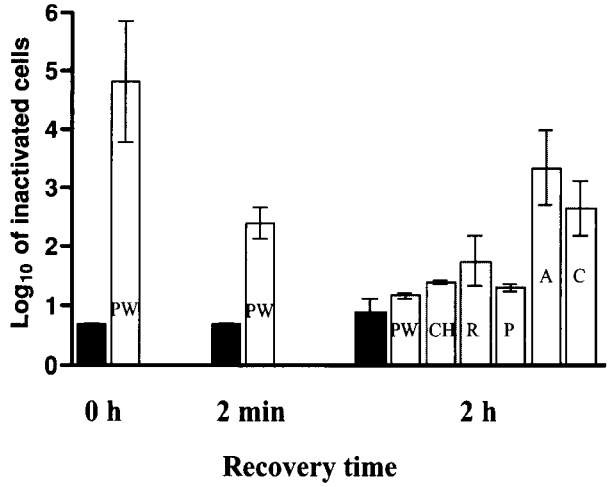

Figure 3.8. Effect of inhibitors of specific metabolic processes on repair of membrane damage after PEF. Survival fraction of *Escherichia coli* after a PEF treatment at 25 kV/cm for 200 exponential decay pulses in citrate–phosphate buffer of pH 4.0, and a subsequent incubation for 2 h at room temperature in peptone water (0.1%) alone (PW) or with chloramphenicol (CL), rifampicine (R), penicillin G (P), sodium azide (A), and cerulenin (C) added and recovered in the nonselective (black bars) and the selective medium (white bars). UT: untreated. Data are means ± standard deviations (errors bars).

reveals that the cell envelopes are one of the vital structures affected by PEF. Again, these results allow confirming that the damages inflicted in cell envelopes, which might include loss of membrane integrity and functionality, are repairable or irrepairable. Whereas repairable membrane injuries would allow the survival of microorganisms under appropriate recovery conditions, irrepairable damages would lead to microbial PEF inactivation.

These results show a clear concordance with those previously discussed in relation to PI uptake. For instance, Gram-positive bacteria such as *Listeria monocytogenes* or *Bacillus subtilis*, which had shown reversible pores by using the PI exclusion technique when treated in citrate–phosphate buffer at pH 7, also showed sublethally injured cells under these treatment conditions. On the contrary, the same microorganisms PEF-treated in citrate–phosphate buffer at pH 4, did show neither reversible pores nor sublethally injured cells. In the case of the Gram-negative bacteria tested such as *Escherichia coli* or *Salm.* Senftenberg when treated in citrate–phosphate buffer at pH 4, results obtained by the selective medium plating technique would suggest that repairable membrane permeabilization would also occur. However, reversible pores would be small enough avoiding PI uptake.

As described above about membrane permeabilization, sublethal injury confirmed by the selective medium plating technique would also depend on process parameters, microbial characteristics, and product parameters. As described previously, the occurrence of sublethal injury depended on the intrinsic microbial PEF resistance and the pH of the treatment medium. When sublethal injury was detected, the proportion of sublethal injured cells increased with the intensity of the treatment time: the longer the treatment time and the higher the electric field applied, the greater the proportion of sublethally injured cells (García *et al.*, 2005a). Regarding the effect of cell exposure to different environmental stress conditions, our preliminary results have shown that microbial PEF resistance of *Listeria monocytogenes* does not change when subjecting to a heat shock previous to the PEF treatment; however, sublethal injury practically disappears. These results would indicate that environmental stresses such as a heat shock may increase microbial PEF resistance since it protects cells against PEF-damage or allows membrane damage to be repaired.

On the other hand, it is remarkable that PEF-sublethally injured cells stored under acidic conditions lost viability during storage time (García *et al.*, 2003, 2005a,b). Figure 3.9 shows the number of survivors of *Salm.* Senftenberg 775W after PEF and a subsequent incubation under acid conditions for 2 h. The sensitivity of these cells seemed to be related to the occurrence of sublethal injury since the number of survivors recovered in the selective medium and the number of survivors after the

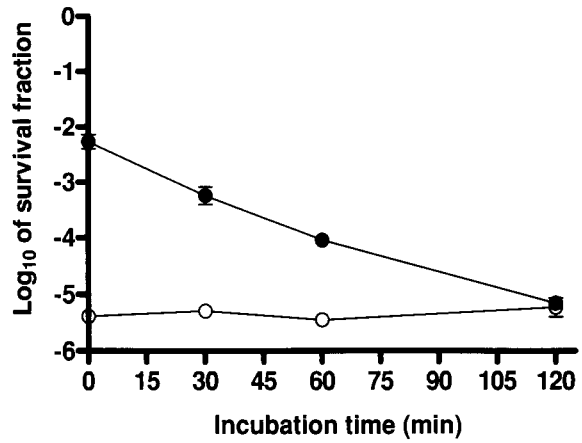

Figure 3.9. Survivors of *Salm.* Senftenberg 775W after a PEF treatment at 25 kV/cm for 400 μs in citrate–phosphate buffer at pH 4.0 and a subsequent incubation at pH 4.0, and recovered in a nonselective (•) and a selective medium (○).

subsequent incubation under acid conditions for 2 h was practically the same. This means, from a practical point of view, that if adequate posttreatment holding conditions are selected, the intensity of treatments could be diminished without affecting the microbial quality of the product. Alternatively, a higher degree of safety could be attained. In this sense, García *et al.* (2005b) observed that a new proportion of initially non-sublethally injured cells after PEF treatment was additionally injured as a consequence of the storage in the acidic conditions under refrigeration. This indicated that PEF treatments would be capable of inflicting some specific extra damage on cells, not observable immediately after PEF by the selective medium plating technique that would make the cells sensitive to a new damage inflicted by the storage in acidic conditions.

All the techniques described have contributed to confirm the occurrence and relation of the electroporation to microbial inactivation by PEF. Some techniques such as TEM examinations or the measurement of leakage of intracellular material have allowed obtaining visual or direct evidence of the phenomenon; others, such as the measurement of dye exclusion and of sublethal injury by the selective medium plating technique have allowed to find out correlation between electroporation and microbial inactivation. However, the analysis of the 90% of the population by the measurement of the dye exclusion or the leakage of the intracellular material together with the evaluation of sublethal injury by the selective medium technique allow obtaining a clearer picture of the phenomena affecting the majority of the population.

It should be noted that the selective medium plating technique, despite it is an indirect way of evaluating membrane damage based in both the loss of membrane integrity and functionality, offers much higher sensitivity than the others being possible to evaluate up to 99.99999% of the total population rather than just to 90 or 99%. This is of the most importance in food preservation since a few surviving cells may rapidly multiply resulting in infective concentrations. Moreover, the fact that a small resistant population fraction exists, being responsible for the occurrence of tails in survival curves under PEF, would suggest that other mechanisms rather than electroporation might be involved in PEF inactivation. However, the concordance of the results obtained by the PI exclusion technique and the measurement of sublethal injury by the selective medium plating technique obtained by our research group would suggest that electroporation is also the cause of the inactivation of these more resistant cells.

The occurrence of sublethal injury in the membrane provides new useful data that contribute to the understanding of mechanisms of electroporation in bacterial cells. It would also contribute to clarify the environmental circumstances under which PEF might act synergistically with other hurdles for food preservation. Further work is in progress.

4. CONCLUDING REMARKS

It is well known that PEF causes the formation of pores in biomembranes, including those of microorganisms of interest in food preservation. Damage to the cell membrane has proved to be one of the critical events leading to death of PEF-treated bacterial cells. Nevertheless, other phenomena associated with alteration of membrane functions or with chemical stresses cannot be discarded.

Although results are scarce, and in some occasions, contradictory, bacterial inactivation seems to be due to the occurrence of electropores rather than to the complete mechanical disruption of the membrane. Pores formed may be either permanent or reversible depending on whether they remain open or are resealed when the electric field ceases. Reversible pores might include pores which immediately reseal after PEF treatment, also known as transient pores, and repairable pores. These might be repaired by the cells after treatment, only under favorable conditions.

The occurrence of permanent or irreversible pores seems to lead to cell death. A linear relationship between the percentage of irreversibly permeabilized bacterial membranes and inactivated cells has been observed by several authors when varying the treatment conditions and bacterial species tested. These results would clearly suggest that irreversible membrane permeabilization is directly involved in the inactivation of vegetative bacterial cells. Such a relationship does not exist for yeast, suggesting that alternative mechanisms might be involved in yeast inactivation by PEF.

The occurrence of reversible pores has been observed in bacterial cells depending on factors such as the intrinsic microbial resistance, process, and product parameters. However, it has not been found any relationship between the presence of reversible pores and loss of viability. Moreover, under appropriate recovery conditions reversibly permeabilized cells have shown the ability of repairing their damages and outgrowing. Conversely, under unfavorable environmental conditions such as acidic pH, reversibly permeabilized microorganisms are inactivated.

Finally, it should be noted that these studies on the electroporation of microorganisms under PEF are of the most interest for the food preservation industry since the presence of reversible pores and the occurrence of sublethal injury will prove valuable in developing appropriate combination processes using PEF treatments. The use of several hurdles in combination or in a successive manner may act additively or synergistically and difficult the survival of spoilage and food poisoning microorganisms and may allow achieving the desired level of microbial inactivation while retaining the essential organoleptic properties and nutritive value of the food.

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PART II

EFFECTS OF PULSED ELECTRIC FIELDS

CHAPTER 4

MICROBIAL INACTIVATION BY PULSED ELECTRIC FIELDS

Ignacio Álvarez, Santiago Condón, and Javier Raso

1. INTRODUCTION

Foods are contaminated by microorganisms during harvesting, processing, and handling operations. Depending on the food composition and environmental factors some of these microorganisms will grow. Microbial growth and metabolisms will induce quality changes such as pH modification, off-odors, gas, or slime-formation that lead to food spoilage (Huis in't Veld, 1996). In addition to spoilage microorganisms, pathogenic microorganisms are frequently found in foods. While microbial food spoilage is a huge economical problem, food-borne illnesses are an enormous public health concern worldwide with severe direct and indirect economic consequences (Baird-Parker, 2000). In spite of all efforts conducted by the food industry and the food safety authorities, the number of reported outbreaks of food-borne illnesses caused by pathogenic microorganisms is increasing (Meng and Doyle, 2002).

The most effective way to preserve the food quality and to assure microbial safety is to prevent the contamination of raw material and foods at the primary production step and during processing. However, the large number of possible sources of microbial contamination makes microbial control by preventing the access of microorganisms enormously difficult.

The objective of food preservation technologies used by the food industry is to control microorganisms once they are contaminating foods. Food preservation technologies are based on the prevention of microbial growth or on the microbial inactivation.

In many cases, foods are preserved by inhibiting microbial activity through those factors that most effectively influence the growth and survival of microorganisms such as temperature, water activity, addition of preservatives, pH, and modified atmosphere. In this case, the microorganisms will not be destroyed and will still be metabolically active and viable if transferred to favorable conditions. As estimates of the infection dose of some pathogenic microorganisms are very low, growth of these microorganisms in foods is not necessary to cause infection (Blackburn and McClure, 2002). Therefore the application of a treatment aiming at microbial destruction is of primary importance to produce safe foods.

Heating is the method of bacterial destruction more frequently used in the food industry. Foods that have been submitted to an adequate thermal pasteurization are free of vegetative pathogens. On the other hand, thermal sterilization renders foods free of microorganisms that are capable of reproducing in the food under normal nonrefrigerated conditions and free of viable microorganisms,

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including bacterial spores, of public health significance. Thermal processing is a very effective technology for microbial inactivation; however, excessive heat treatment may cause undesirable effects on foods such as protein denaturation, nonenzymatic browning, and loss of vitamins and volatile flavor compounds (Arnoldi, 2002). As a result, heat processing is not a suitable treatment for minimally processed, fresh-like, nutritious products that the consumer presently demands (Zwietering, 2002). In order to reduce the negative effects of heat treatments in foods, alternative technologies capable of inactivating microorganisms at temperatures below those used during thermal processing are being demanded by the food industry (Devlieghere *et al.*, 2004).

Pulsed electric field technology (PEF) is viewed as one of the most promising nonthermal methods for inactivating microorganisms in foods. Electric fields in the range of 5–50 kV/cm generated by the application of short high voltage pulses (μs) between two electrodes cause microbial inactivation at temperatures below those used in thermal processing. The precise mechanisms by which microorganisms are inactivated by pulsed electric fields are not well understood; however, it is generally accepted that PEF leads to the permeabilization of microbial membranes (Wouters *et al.*, 2001). Inactivation of vegetative cells of bacteria and yeast by PEF has widely been demonstrated. However, the few studies conducted on the inactivation of bacterial spores by PEF describe these structures as resistant to PEF treatments (Pagán *et al.*, 1998; Pol *et al.*, 2001a). Therefore, it is likely that initial applications of PEF processing will be aimed at replacing thermal pasteurization as a means of killing vegetative microorganisms rather than sterilization. Not only do PEF treatments have to improve food quality, but they must also promote an equivalent or, preferably an enhancement of safety levels when compared with other preservation technologies. Therefore, the objective of PEF pasteurization must accomplish the inactivation of all pathogenic microorganisms as well as a large proportion of the spoilage ones with a minimal alteration of the food properties.

In order to use PEF technology as a pasteurization process it is necessary to estimate its efficacy against pathogenic and spoilage food-borne microorganisms. To obtain this objective there is a need to accumulate knowledge on the critical factors affecting microbial inactivation, to describe the PEF inactivation kinetics and to understand the mechanisms involved in microbial PEF inactivation. These studies are necessary to identify the most PEF-resistant pathogens of concern for each specific food product and to establish optimized processes that are applicable under a wide range of conditions.

The present chapter reviews the current state of the art in microbial inactivation by PEF. After discussing critical factors determining microbial inactivation by PEF and mathematical kinetic models used for describing PEF death, the most successful combinations of PEF with other preservation techniques for enhancing the safety of minimally processed foods are presented. The chapter concludes with some aspects that need further investigation for the development of PEF processes to supply safe food products of high organoleptic and nutritional quality.

2. CRITICAL FACTORS DETERMINING MICROBIAL INACTIVATION BY PULSED ELECTRIC FIELDS

The efficiency of a given processing technology for microbial inactivation is dependent on a number of factors. Factors such as electric field strength, pressure intensity, ultrasound amplitude, etc., which are related to the processing conditions, are unique to each technology. Other factors influencing microbial inactivation such as those related to microbial characteristics (growth phase, strain of the microorganism, etc.) or treating medium (pH, a_w, etc.), are independent of the technology itself. However, the effect of these factors on microbial resistance depends on the processing technology. On the other hand, the most resistant pathogen of concern is different for each technology.

For example, *Salmonella* serovar Senftenberg, a very heat-resistant vegetative cell, is more sensitive to HHP and PEF than other heat sensitive *Salmonella* serovars (Metrick *et al.*, 1989; Álvarez *et al.*, 2003a). Therefore, the influence of critical factors affecting microbial inactivation has to be established for each specific processing technology.

Factors that influence microbial inactivation by PEF are critical to the outcome of the process. This in conjunction with the great differences on the treatment conditions and on the characteristics of the apparatus employed makes it difficult to compare the results obtained among distinct laboratories, and in some cases contradictory conclusions have been pointed out. Sometimes essential details (electrical conductivity, temperature of the treatment medium, sublethal damage, etc.) could not have been considered in the final lethal effect of PEF treatments. In this section, most of the factors influencing the microbial PEF resistance are going to be treated in order to clarify their effect.

The critical factors determining microbial inactivation by PEF can essentially be classified as processing factors, treatment medium characteristics, and microbial characteristics. An adequate knowledge of them and of their influence is essential in order to reach reliable conclusions from ulterior investigations related to the studies of PEF inactivation kinetics and/or mechanism of action of PEF treatments.

2.1. Processing Factors

The main process parameters that determine PEF treatments are electric field strength, shape and width of the pulse, treatment time, frequency, specific energy, and temperature. Among them, electric field strength, treatment time, and/or pulse energy are the basic control parameters of PEF processes. Their intensity determines the final lethal effect on the microbial population. Width and frequency of the pulses contribute to define the process time. In some cases, their influence on the microbial inactivation can be affected by the temperature of the treatment medium.

2.1.1. Electric Field Strength

Field strength is one of the most determining factors of the microbial PEF lethality. When the influence of this parameter on microbial inactivation is analyzed, both its intensity and its distribution inside the treatment chamber have to be considered.

Over a threshold field strength, called critical electric field strength (E_c), PEF microbial sensitivity enhances by increasing the intensity of the field strength. Based on this, E_c has been used to characterize the PEF resistance of different microorganisms (Hülsheger *et al.*, 1981, 1983; Castro *et al.*, 1993; Gásková *et al.*, 1996; Grahl and Märkl, 1996; de Jong and van Heesch, 1998). However, E_c only has sense while it is defined for a certain treatment time. Thus, as it can be observed in Fig. 4.1, E_c for *Listeria monocytogenes* could vary from 5 to 22 kV/cm depending on the treatment time.

Other authors have defined the critical field strength as the minimal field strength that induces the creation of membrane pores (Neumann and Rosenheck, 1973; Zimmermann *et al.*, 1974; Neumann, 1989; Heinz *et al.*, 2001; Bazhal *et al.*, 2003). This parameter could be of more interest to characterize microbial PEF resistance, since it can be estimated on the basis of the shape and size of the microorganisms, and also since an exponential relationship could be observed between the number of permeabilized and inactivated cells (Wouters *et al.*, 2001). However, some discrepancies have been determined between the estimated and real E_c for some microorganisms. Permeabilization of cell membranes could also be associated to other parameters such as stochastic spatial orientation of microorganisms which impacts on the efficiency of the treatment as it is the case with the diversity

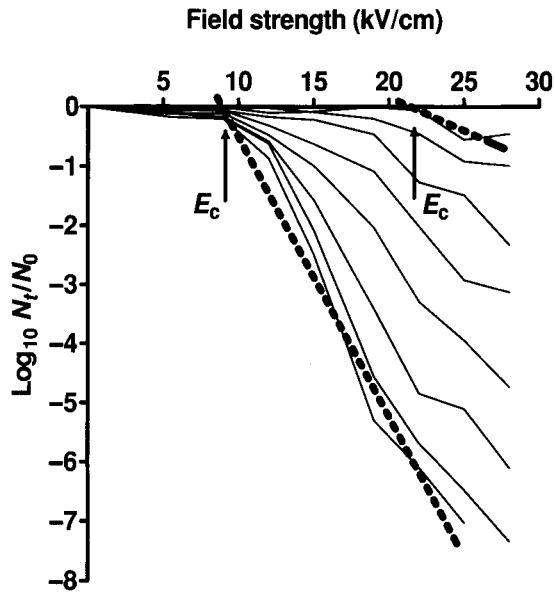

Figure 4.1. Influence of the electric field strength on the PEF inactivation of *E. coli* at constant treatment times (thin lines). The thick lines represent the regression line of the straight part of the curve at a certain treatment time (40 and 2000 μs). Arrows indicate the E_c after 40 and 2000 μs. Treatment conditions: McIlvaine buffer pH 7.0, 2 mS/cm; 2 μs square wave pulses; frequency: 1 Hz.

in size (Heinz *et al.*, 2001). The relationship between the microbial inactivation and the electric field strength at values over E_c will be treated in Section 3 of this chapter.

The distribution of the electric field strength inside the treatment chamber is the other parameter that has to be considered at this point. It has to be homogeneously distributed in order to have a perfect control and feasibility of the actual field applied. Besides, heterogeneous field strengths could induce the existence of arcing areas due to very high field strengths or zones in which microorganisms are undertreated. Distribution of the field strength depends on the characteristics of the treatment chamber, basically shape of electrodes, gap of the chamber, gas impurities in electric materials, etc. Therefore, the design of PEF treatment chambers should be based on mathematical simulations in order to determine the distribution of the field strength (Qin *et al.*, 1995; Qiu *et al.*, 1998; Fiala *et al.*, 2001; Góngora-Nieto *et al.*, 2003).

2.1.2. Pulse Shape

An important aspect that differentiates between PEF processing and other microbial inactivation technologies is that the PEF treatment is delivered by pulsing. The pulses commonly used in PEF treatments are usually either exponential or square wave pulses (Jeyamkondan *et al.*, 1999). In terms of pulse polarity, these pulses can be monopolar or bipolar. Bipolar pulses consist of a one positive and one negative pulse. Most published reports state that square wave pulses of the same electric field strength and energy are more effective in terms of microbial inactivation than exponential ones with the same characteristics. However, these conclusions are based on studies that have been conducted or treated only at one electric field strength (12 kV/cm) and the improvement resulted in only a 60% more inactivation of *Saccharomyces cerevisiae* (Zhang *et al.*, 1994a). Figure 4.2 shows that when comparing treatments of the same specific energy and electric field strength, the lethal efficiency of

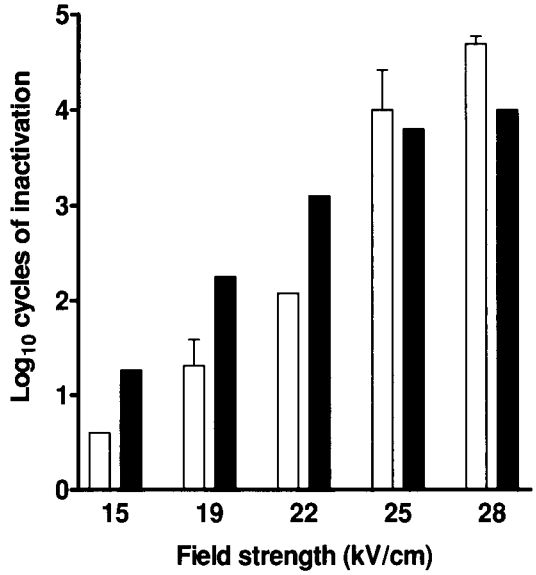

Figure 4.2. Influence of the electric field strength and pulse shape on the PEF inactivation of *Listeria monocytogenes* at the same specific energy (200 kJ/kg). Treatment conditions: square wave pulses (■), exponential decay pulses (□); McIlvaine buffer pH 7.0, 2 mS/cm; frequency: 1 Hz.

exponential and square wave pulses of the same specific energy depends on the electric field strength of the pulses.

Basic research on microbial inactivation by PEF has generally been conducted with square wave pulses because the definition of the electric field strength and treatment time is more accurately when these pulses are delivered. However, generally pulse generators that are capable of satisfying industrial requirements deliver exponential decay pulses. Therefore, an equivalence between both pulse shapes would be very useful to transfer laboratory data to practical applications.

Bipolar pulses were not either significantly more efficient than monopolar ones from a practical point of view. Either application of exponential of square waveform bipolar pulses also resulted in an only 50–60% more inactivation of several microorganisms than did monopolar pulses did. Based on this, improvement on microbial inactivation efficiency does not justify the application of bipolar pulses; however, it has been reported that these pulses reduce the deposition of solid in the electrodes of the treatment chamber and decrease food electrolysis (Qin *et al.*, 1994).

2.1.3. Pulse Width

There is some controversy with respect to the influence of the pulse width on the PEF microbial lethality. Some authors have indicated that after the same treatment time, inactivation tested in several microorganisms was independent of the pulse width (Hülsheger *et al.*, 1981; Raso *et al.*, 2000; Álvarez *et al.*, 2003b,c). However, others have obtained a higher inactivation with longer pulses (Wouters *et al.*, 1999; Aronsson *et al.*, 2001). These results were obtained with treatments in which the final temperature exceeded 40°C in some cases, and the longer the pulse applied, the higher the final temperature. This temperature rise, even being low, could increment the PEF lethal effect. Thus, Álvarez *et al.* (2003b) observed a higher inactivation of *L. monocytogenes* with longer pulses when treatment temperature exceeded 32°C but not at lower temperatures.

On the other hand, for pulses of the same specific energy, shorter pulses applied at higher field strengths were more effective on the inactivation of several microorganisms than wider pulses applied at lower field strengths (Schoenbach *et al.*, 1997; Heinz *et al.*, 1999; Vernhes *et al.*, 2002). Furthermore, shorter pulses (1–2 μs) offer other advantages such as lower Joule heating effect and reduction in the migration of compounds from the electrodes to the treatment medium or in the formation of chemical species (Sale and Hamilton, 1967; Sale and Hamilton; 1968, Mizuno and Hori, 1988; Lubicki and Jayaram, 1997; Morren *et al.*, 2003; Evrendilek *et al.*, 2004). Ho and Mittal (2000) reported that pulses wider than 20 μs could induce electrolytic phenomena originating chemical species that could result lethal for microorganisms.

2.1.4. Treatment Time

Treatment time could be defined as the effective time during which range microorganisms are subjected to the field strength. It depends on the number of pulses and the width of the pulses applied. This parameter and the electric field strength are the main factors determining the lethal effect of PEF treatments (Sale and Hamilton, 1967; Jayaram *et al.*, 1991; Barsotti and Cheftel, 1999; Wouters *et al.*, 2001). The mathematical relationship between microbial inactivation and treatment time will be treated in Section 3.

2.1.5. Frequency

Studies on microbial inactivation by PEF have been conducted at frequencies ranged from 1 to 500 Hz. If the same number of pulses is applied, microbial inactivation is generally independent of the number of pulses applied per second (Hülsheger *et al.*, 1981; Jeantet *et al.*, 1999; Raso *et al.*, 2000; Álvarez *et al.*, 2003b,c). By increasing the pulse frequency the processing time decreases. However, the temperature of the treatment medium increases with frequency (Raso *et al.*, 2000), enabling to achieve temperatures that could affect food properties.

2.1.6. Specific Energy

PEF microbial inactivation increases with the energy applied per mass unit (specific energy) (Mizuno and Hori, 1988; Grahl *et al.*, 1992; Heinz *et al.*, 2001; Álvarez *et al.*, 2003b).

The use of the specific energy has been proposed as a control parameter of PEF processes instead of the treatment time (Heinz *et al.*, 2001). Different arguments justify the use of this parameter instead of treatment time, especially when exponential decay pulses, in which the measurement of the treatment time is not precise, are applied. On the other hand, the specific energy is calculated as an integrated parameter that considers the resistance of the treatment chamber, which depends on its dimensions and conductivity of the treatment medium, in addition to the electric field strength and treatment time. Although PEF microbial inactivation increases with the energy applied (Mizuno and Hori, 1988; Grahl *et al.*, 1992; Heinz *et al.*, 2001; Álvarez *et al.*,2003b), at the same specific energy level treatments at higher electric field strength are more lethal (Fig. 4.3). Therefore, specific energy cannot be used as a unique parameter to characterize a pulse electric field treatment. Electric field strength of the treatment should be reported as well.

Heinz *et al.* (2001) compared the total specific energy and the electric field strength necessary to inactivate 2 $\log_{10}$ cycles of the microbial population of different microorganisms obtained by different authors. By using both parameters, it was possible to provide an estimation of the efficacy of pulsed electric field treatments for the preservation of food in a wide range of field strengths (0–45 kV/cm). This study demonstrated that PEF treatments at higher electric field strengths were more suitable because they permitted to obtain a given level of inactivation with shorter treatment

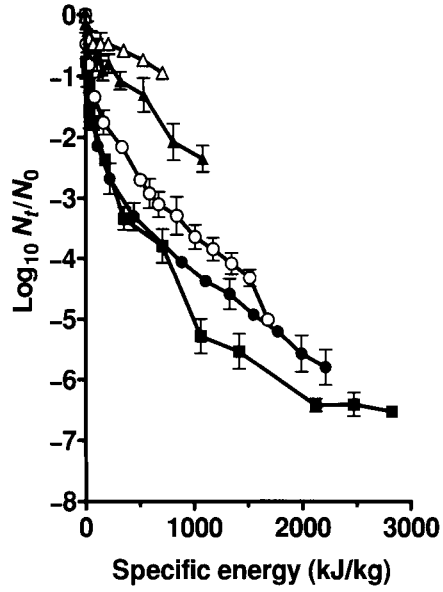

Figure 4.3. Influence of the electric field strength and the specific energy on the PEF inactivation of *Salmonella* serovar Senftenberg. Treatment conditions: 12 kV/cm (△), 15 kV/cm (▲), 19 kV/cm (○), 22 kV/cm (●), and 28 kV/cm (■); McIlvaine buffer pH 7.0, 2 mS/cm; 2 μs square wave pulses; frequency: 1 Hz.

times and lower energetic requirements. However, above a given field strength (threshold), further increments in the electric field strength scarcely saved energy to obtain a certain inactivation level. Similar results were obtained by Álvarez *et al.* (2003c) and Wouters *et al.* (1999) at different levels of inactivation for *Yersinia enterocolitica* and *Listeria innocua*, respectively.

2.1.7. Temperature

The philosophy of the PEF technology is to inactivate microorganisms at nonlethal temperatures in order to avoid thermal effects on the food properties. However, PEF lethal effect increases with temperature of the treatment medium synergistically (Fig. 4.4). This increment has been observed at both nonlethal (Zhang *et al.*, 1994c; Pothakamury *et al.*, 1996; Aronsson and Ronner, 2001; Hodgins *et al.*, 2002; Smith *et al.*, 2002; Heinz *et al.*, 2003; Fleischman *et al.*, 2004) and lethal temperatures (Jayaram *et al.*, 1991; Reina *et al.*, 1998; Liang *et al.*, 2002; Heinz *et al.*, 2003; Hermawan *et al.*, 2004; Sepúlveda *et al.*, 2004, 2005).

Coster and Zimmermann (1975) indicated that the increment on the rate of inactivation with temperature could be due to a diminution of the minimum transmembrane potential necessary to induce the dielectric disruption of cell membranes. This diminution could be due to the temperature-related phase transition of the phospholipids from gel to liquid-crystalline (Stanley, 1991). Heinz *et al.* (2001) observed that the theoretical critical field strength for *Escherichia coli* (8 kV/cm), calculated on the basis of the shape and size of the microorganism, was significantly reduced (5 kV/cm) when this microorganism was treated at 42°C.

2.2. Microbial Characteristics

Microbial inactivation by PEF has been found to depend on microbial characteristics such as the type of microorganism, cell size and shape, and growth conditions.

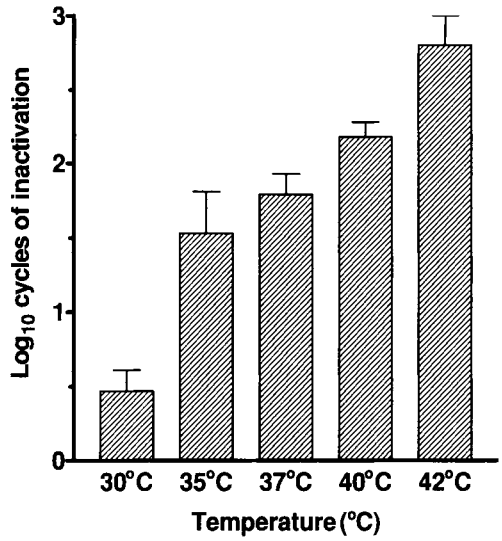

Figure 4.4. Influence of the inlet temperature on the PEF inactivation of *E. coli* at 21 kV/cm and 84 kJ/kg. Treatment conditions: Ringer solution 3.2 mS/cm; exponential decay pulses; frequency: 400 Hz max.; flow: 2.2 L/h.

2.2.1. Type of Microorganism

It has been demonstrated that PEF treatments inactivate bacterial vegetative cells, moulds, and yeasts (Raso *et al.*, 1998a,b; Aronsson *et al.*, 2001; Wouters *et al.*, 2001; Cserhalmi *et al.*, 2002), but bacterial spores are resistant to PEF treatments. In general, it has been reported that yeasts are the most PEF sensitive microorganisms, and Gram-positive are more resistant than Gram-negative bacteria (Sale and Hamilton, 1967; Hülsheger *et al.*, 1983; Qin *et al.*, 1991, 1998; Zhang *et al.*, 1994c; Wouters and Smelt, 1997). However, as it will be discussed later on, this statement is an aspect that has to be reconsidered because intrinsic microbial resistance is very variable.

Although Márquez *et al.* (1997) inactivated 3.4 and 5 $\log_{10}$ cycles of the population of *Bacillus subtilis* and *Bacillus cereus* spores, respectively, after treatments at 50 kV/cm, the rest of investigations indicated that PEF did not affect the viability of bacterial spores (Knorr *et al.*, 1994; Grahl and Märkl, 1996; Pagán *et al.*, 1998; Raso *et al.*, 1998a,c; van Heesch *et al.*, 2000; Katsuki *et al.*, 2000; Cserhalmi *et al.*, 2002). The presence of immature spores in the spore suspension could be the cause of the effect observed by Márquez *et al.* (1997).

Basically, the different structure of the envelopes from spores and vegetative cells would explain their differences on PEF resistance. Bacterial spores are resistant to PEF because the spore coats and mainly the cortex, that enclose the cytoplasmatic membrane, probably prevent the permeabilization effect of PEF. It has been indicated that protoplast of bacterial spores has to be released from their envelopes in order to inactivate them once germinated (Hamilton and Sale, 1967). With respect to nonbacterial spores, ascospores, and conidiospores of moulds and yeast were sensitive to PEF; however, ascospores of *Neosartoria fischeri* were resistant to these treatments (Raso *et al.*, 1998a,b).

2.2.2. Cell Size and Shape

Intrinsic microbial resistance also seems to be strongly related to cell size and cell shape (Qin *et al.*, 1991, 1998; Kehez *et al.*, 1996). These factors would be responsible not only for the different

PEF sensitivity of yeast against bacteria but also for the one among strains of the same microorganism (Lado and Yousef, 2003).

The influence of the cell size and shape on the lethal effect of PEF has been related to the transmembrane potential created by external electric field strengths. The smaller the cell size, the lower the value of the induced membrane potential induced by an external field and the higher is the microbial resistance to the treatment (Zimmermann *et al.*, 1974; Hülsheger *et al.*, 1983). For cells with spherical shape an estimate for this membrane potential can be obtained by solving the Maxwell's equations in spherical coordinates assuming several simplifying restrictions (Neumann, 1996):

$$V_M = fRE,$$

where V_M is the transmembrane potential induced by an external field strength E; f is a characteristic factor of spherical shapes; and R is the cell radius. For cells with nonspherical shape, the transmembrane potential can also be calculated by solving the Maxwell's equations in ellipsoidal coordinates (Zimmermann *et al.*, 1974).

Obviously, cell size and cell shape, as well as the varying morphological and biochemical properties of cells, are responsible for the particular behavior of microorganisms. However, other elements could determine the intrinsic PEF microbial resistance since large microorganisms like yeasts have shown higher PEF resistance in comparison to smaller microorganisms (see kinetics section). It can be speculated whether the stochastic spatial orientation of microorganisms inside of an electric field impacts on the efficiency of the treatment as it is the case with the diversity in size (Heinz *et al.*, 2001).

2.2.3. Culture Conditions

The influences of the growth phase and the growth temperature on the PEF resistance have been the culture conditions most investigated. Studies indicate that microorganisms at the exponential phase are more PEF sensitive than those at the stationary phase (Jacob *et al.*, 1981; Hülsheger *et al.*, 1983; Tatebe *et al.*, 1995; Gásková *et al.*, 1996; Pothakamury *et al.*, 1996; Barbosa-Cánovas *et al.*, 1998; Wouters *et al.*, 1999, 2001; Álvarez *et al.*, 2002; Rodrigo *et al.*, 2003). This higher microbial sensitivity could be related to the higher size of cells in the exponential phase. In a growing culture cells are observed to elongate and then form a partition that eventually separates the cell into two daughter cells. For yeast, it has been hypothesized that during the exponential phase the area between the mother and daughter cells is more susceptible to electric field effects.

Culture temperature also influences microbial resistance to PEF. Reported data indicate that cells grown at temperatures lower than the optimal one are more sensitive to PEF treatments than those grown at the optimal temperature (Álvarez *et al.*, 2002; Ohshima *et al.*, 2002; Russell 2002; Álvarez *et al.*, 2003c). However, these differences in resistance never exceed 1 $\log_{10}$ cycle. Lipid composition variations in cell envelopes induced by modifications on the growth temperatures could be the origin of the distinct PEF sensitivity. At low growth temperatures, the degree of fatty acid instaurations of the phospholipids of the cell membrane raises which could increase the fluidity of the bacterial cell membrane (Tsuchiya *et al.*, 1987; Püttmann *et al.*, 1993). This higher membrane fluidity could reduce the cell stability to PEF treatments, decreasing the microbial PEF resistance.

2.3. Treatment Medium Characteristics

Pulsed electric field treatments have been applied to a range of different products (buffers, fruit juices, milk, dry herbs, beer, liquid egg white, liquid whole egg, "horchata de chufa"—tiger nut milk

or earth almond milk). The effect of PEF treatments on the inactivation of microorganisms in solid products or containing particles (agar, gellan gum gel, emulsions, beef burgers) has received less attention (Zhang *et al.*, 1994b; Keith *et al.*, 1997, 1998; Dutreux *et al.*, 2000b; Mañas *et al.*, 2001; Bolton *et al.*, 2002; Ravishankar *et al.*, 2002).

Food products are complex matrixes whose physical and chemical characteristics strongly influence the PEF sensitivity of microorganisms present in them. Electrical conductivity, pH, water activity, and treatment medium composition are the main factors so far investigated.

2.3.1. Electrical Conductivity

The influence of the conductivity of the treatment medium on the microbial inactivation by PEF has been investigated by different authors (Jayaram *et al.*, 1993; Vega-Mercado *et al.*, 1996; Sensoy *et al.*, 1997; Wouters *et al.*, 1999; Álvarez *et al.*, 2000, 2002, 2003c; Dutreux *et al.*, 2000b; Wouters *et al.*, 2001). Most studies conclude that conductivity affects microbial inactivation by PEF. However, it is unclear whether the effect of the conductivity is due to the influence of this parameter on the electric field strength and pulse width, or whether the conductivity influences the effect of the electric field strength on cell membranes. For a constant input voltage, the higher the conductivity of the treatment medium, the lower the actual field strength. Therefore, to obtain the same field strength, the higher the conductivity of the medium, the higher the input voltages which have to be selected. Moreover, this parameter also modifies the pulse shape and width mainly in exponential decay pulses. In these cases in which pulse width can be modified by the electrical conductivity, it could be more suitable to use the specific energy instead of the treatment time to study the influence of this parameter. When energy is considered it has to be pointed out that for the same field strength, the higher the conductivity, and the greater the pulse energy. Thus, the higher inactivation observed in media of lower conductivity after the same field strength and energy applied could be explained by the higher number of pulses applied. Wouters *et al.* (2001) determined that to achieve the same *Lactobacillus plantarum* membrane permeabilization it was necessary to apply more energy levels in a 15 mS/cm medium than in another of 4 mS/cm, after the same treatment time applied at 25 kV/cm. On the other hand, the conductivity of the treatment medium also determines the heating increment due to the application of PEF. The influence on the temperature could also explain the effect of the conductivity on the PEF microbial inactivation. Álvarez *et al.* (2000, 2003b,c) determined that the electrical conductivity of the treatment medium (up to 4 mS/cm) did not influence the microbial sensitivity of *Salmonella* serovar Senftenberg, *L. monocytogenes*, and *Y. enterocolitica* when the same field strength and specific energy were applied and the final temperature was below 30°C.

2.3.2. pH

The pH of the treatment medium is one of the parameters that has generated most controversy among literature. Some authors have observed that microorganisms were more PEF sensitive in acidic media (Wouters *et al.*, 1999; Álvarez *et al.*, 2002; Geveke and Kozempel, 2003; Aronsson *et al.*, 2005), others have indicated that microbial resistance was lower at neutral pH (Jeantet *et al.*, 1999; Álvarez *et al.*, 2000; García *et al.*, 2003; Geveke and Kozempel, 2003), and nondependence of the pH on microbial PEF inactivation has also been reported (Sale and Hamilton, 1967; Hülsheger *et al.*, 1981; Heinz and Knorr, 2000; Ravishankar *et al.*, 2002; Smith *et al.*, 2002; Álvarez *et al.*, 2003c) (Fig. 4.5).

The pH influence on microbial inactivation seems to be correlated with the type of microorganisms. García *et al.* (2005) observed that under the same experimental conditions six Gram-negative microorganisms were more resistant at acidic pH; however, the resistance of the two Gram-positive

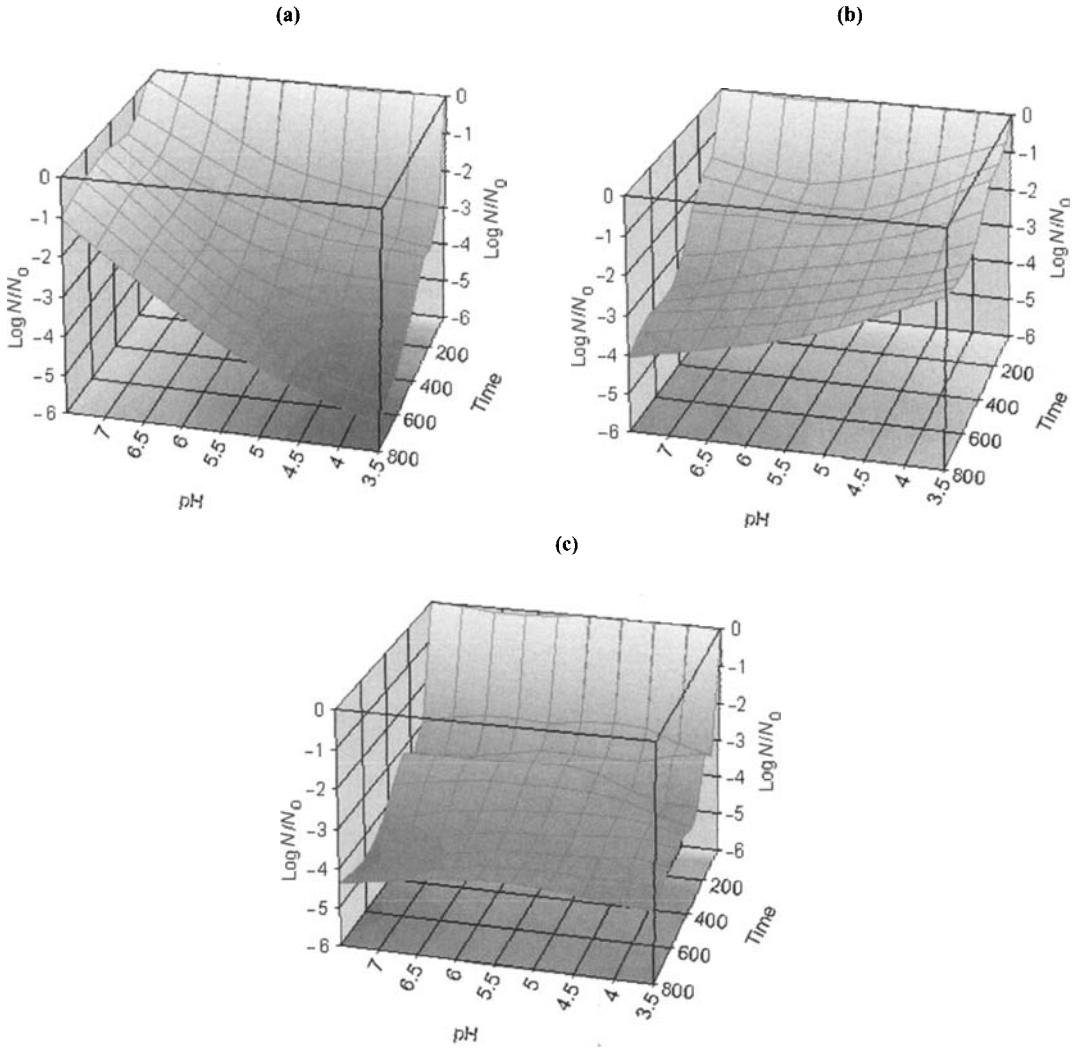

Figure 4.5. Influence of the pH of the treatment medium on the PEF inactivation of *L. monocytogenes* (a), *Salmonella* serovar Senftenberg (b), and *Y. enterocolitica* (c). Treatment conditions: 25 kV/cm (*L. monocytogenes*), 22 kV/cm (*Salmonella* serovar Senftenberg, and *Y. enterocolitica*); McIlvaine buffer of different pH; 2 mS/cm; 2 μs square wave pulses; frequency: 1 Hz.

microorganisms investigated decreased by decreasing the pH. The mechanism that completely explains these differences has not been elucidated yet. It is assumed that the higher sensitivity in acidic media could be related to a change in the cell capability to maintain a transmembrane pH gradient due to membrane electroporation. Loss of membrane continuity would be expected to impair pH homeostasis, which could modify the intracellular pH affecting main components of the cell (DNA, RNA, enzymes, etc.) (Vega-Mercado *et al.*, 1996). This mechanism would explain the higher sensitivity of Gram-positive bacteria in acidic media, but not the behavior shown by Gram-negative. For this bacterial group, the cell envelopes could react as a protective barrier or determine a more stable membrane configuration in acidic media which induces to a higher PEF resistance. On the other hand, it has been observed the existence of sublethal injury on the population of Gram-negative bacteria after PEF treatments when treated in acidic media (Garcia *et al.*, 2005). Therefore, the recovery

capability of pH-induce-sublethal-injured cells could explain the higher resistance of Gram-negative bacteria in acidic media.

2.3.3. Water Activity

The influence of the a_w on microbial inactivation by PEF has also been investigated. It has been reported that a decrease in the a_w increased the microbial PEF resistance of several bacteria and yeasts (Álvarez *et al.*, 2000, 2002, 2003c; Min and Zhang, 2000; Aronsson and Ronner, 2001). When microorganisms are transferred to an environment with low a_w, water exits from the cell and it results on a reduction of cell volume which would increase PEF resistance. Also, cell shrinkage could probably cause a thickening in the cell membrane, followed by a reduction of the membrane permeability and fluidity (Neidhardt *et al.*, 1990). This lower fluidity could also influence the higher resistance to PEF at reduced a_w.

As it happens with heat treatments (Corry, 1974; Smith *et al.*, 1982; Lee *et al.*, 1989), the effect of the reduction of the a_w on the microbial PEF inactivation also depended on the solute added to the medium (Fig. 4.6). Due to that the molecular weight of glycerol is lower than the one of sucrose, 10% (w/v) of glycerol and 50% (w/v) of sucrose were required in order to reduce a_w up to 0.93. As it is observed in the figure, both microorganisms were more sensitive to PEF when 10% glycerol was added to the treatment medium. This higher sensitivity could be explained by the fact that glycerol can pass through cell membranes by passive diffusion. This would compensate the difference of osmotic pressure between intra- and extracellular media, avoiding cell plasmolysis and cell shrinkage, and moreover causing an increase of the cell size. This size change would reduce the intensity of the field strength necessary to create membrane pores, and therefore, for the same field strength, the microbial inactivation would be higher. When glycerol was added to the same concentration as sucrose (50%), the microbial resistance increased. For this concentration, the compensating effect on the osmotic pressure due to the entrance of glycerol into the cell would not be effective, leading to cell shrinkage and the consequent increase of PEF resistance.

2.3.4. Composition of the Treatment Medium

Although several investigations have been carried out related to the influence of the composition of the treatment medium on the microbial PEF sensitivity, the different treatment conditions and media used by distinct authors make it difficult to obtain definitive conclusions to this respect.

Hülsheger *et al.* (1981) observed that the PEF resistance of *E. coli* was higher in media in which composition Ca^{++} and Mg^{++} ions were present. This higher resistance is probably due to the stabilization effect of divalent cations on cell membranes (Toko and Yamafuji, 1981). These authors also observed that treatment media in which composition chloride is included, chlorine can be formed due to the application of pulsed electric fields and this component acted as a bactericide agent.

Grahl and Märkl (1996) determined that *E. coli* resistance increased with the fat concentration of milk. However, other authors have observed that PEF sensitivity of this microorganism and also that of *B. cereus* were nondependent on the fat or protein content when buffers were used as treatment media (Reina *et al.*, 1998; Mañas *et al.*, 2001; Pol *et al.*, 2001a).

Some investigations have been done in solid media. Zhang *et al.* (1994b) inactivated 5 $\log_{10}$ cycles of the microbial population of *E. coli*, *S. aureus*, and *S. cerevisiae* inoculated in potato dextrose agar with treatments of 40 kV/cm. A maximum of 3 $\log_{10}$ reduction of *E. coli* O157:H7 was achieved by five square pulses of 30 kV/cm in water-based gellan gum gel (Ravishankar *et al.*, 2002). Dutreux *et al.* (2000b) and Mañas *et al.* (2001) inactivated 5 and more than 2.5 $\log_{10}$ cycles of the population

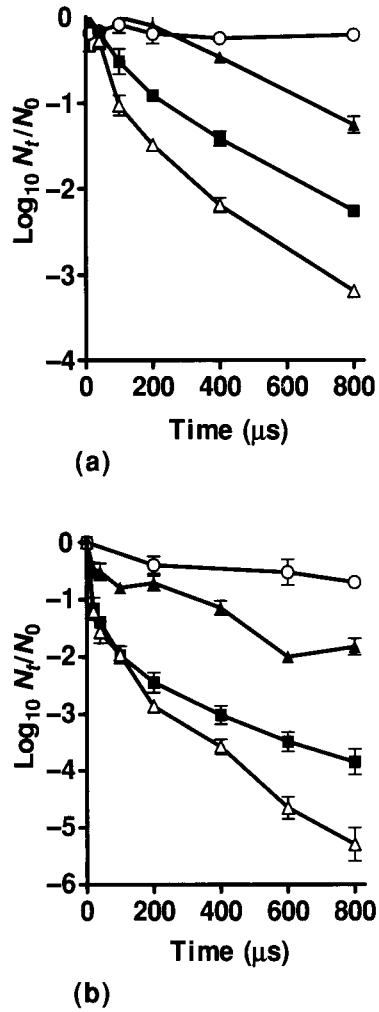

Figure 4.6. Influence of the a_w of the treatment medium on the *Salmonella* serovar senftenberg (a) and *L. monocytogenes* (b) PEF inactivation. Treatment conditions: McIlvaine buffer pH 7.0 and >0.99 (■), $a_w = 0.93$ and 50% sucrose (○), $a_w = 0.93$ and 10% glycerol (△), $a_w = 0.80$ and 50% glycerol (▲); field strength: 22 kV/cm, 2.2 kJ/kg/pulse (*Salmonella*), 28 kV/cm, 3.6 kJ/kg/pulse (*L. monocytogenes*); 2 μs square wave pulses; frequency: 1 Hz; conductivity: 2 mS/cm.

of *E. coli* adhered to latex pearls and inoculated in a fish egg suspension, after treatments of 41 and 33 kV/cm, respectively. However, the PEF lethal effect in other solid substrates has been much lower. Keith *et al.* (1998) only reduced 0.6 $\log_{10}$ cycles of the total aerobic microbial population present in flour after PEF treatments at 20 kV/cm. Similar results were obtained in onion powder, sweet basil, beef trimmings, or beef burgers (Keith *et al.*, 1997; Bolton *et al.*, 2002).

As it happens with other inactivation techniques like heat, high hydrostatic pressures, ionizing radiation, or ultrasonic waves, microbial inactivation by PEF depends on many factors. Much research has been conducted in order to know the influence of these factors on microbial PEF resistance. However, the substantial variety in PEF equipment operating in different laboratories as well as the several protocols used to generate data make comparison of results and achievement of definitive conclusions rather challenging. As it is very difficult to standardized experimental procedures used in different laboratories, Wouters *et al.* (2001) listed the critical treatment conditions

that should be reported in studies and that would be particularly beneficial for comparison of results.

3. MODELING MICROBIAL INACTIVATION BY PULSED ELECTRIC FIELDS

For safe product design of PEF-treated foods, a defined reduction of target microorganisms is required. Quantitative data are essential to demonstrate that the PEF process can deliver the defined level of microbial inactivation and that the end-product meets the specifications for safety and stability. The development of mathematical models that can predict the death of pathogens and spoilage microorganisms by PEF is a very useful tool to design safe and effective processes. Predictive microbiology is an emerging multidisciplinary area of food microbiology whose aim is to develop and apply mathematical models in order to predict the responses of microorganisms to process parameters and environmental variables (McDonal and Sun, 1999).

To develop a predictive model it is necessary to follow several steps (McMeekin and Ross, 2002). The first step is to describe the microbial evolution as function of time (primary models). Secondary models characterize parameters appearing in primary model approaches as function of process parameters and environmental conditions. Finally, primary and secondary models are combined to predict the microbial evolution as a function of process parameters and environmental conditions.

3.1. Collecting Data for Modeling Microbial Inactivation by PEF

Data collection is the first stage of the microbial modeling process. Over the years various methods have been developed for testing microbial resistance to a lethal agent. The two most widely used methods include: end point methods and methods based on death curves. The end point methods are based on the determination of the inactivation produced by the lethal agent for a given treatment (e.g., 20 kV/cm for 50 μs). However, in order to obtain reliable data to describe the inactivation kinetics of microorganisms, multiple data points need to be examined. Analysis of only one end point as employed in most PEF published experiments will not provide sufficient information to correctly model the inactivation of microorganisms during the PEF treatment (Aronsson and Rönner, 2001; Lado and Yousef, 2003; Evrendilek and Zhang, 2005). End point methods are more useful for validation studies in which individual death data points obtained in foods are compared with their predictions by the model.

Modeling microbial inactivation requires death or survival curves that describe the decrease in microbial concentration with time of lethal treatment. The elaboration of the survival curve in which the logarithm of survivors is plotted against the time of the inactivation process requires acquisition of multiple data points along the time for a given treatment intensity. According to Legan *et al.* (2002), ideally this involves 10–12 points over 6–7 $\log_{10}$ reduction in population size, which implies an inoculation level of at least 10^8–10^9 cfu/mL. As PEF survival curves generally did not follow a linear inactivation, the placement of the points can be more important than the number in order to identify regions of rapid change in curvature.

Mathematical models that predict PEF microbial inactivation should be based on consistent laboratorial data. These data should be repeatable, reproducible, and free from methodological artifacts that could influence their value. To achieve this, rigorous approach must be adopted in specifying all aspects of methodology for data collection. In order to obtain quality PEF data and to improve the precision of results, the major sources of variation must be identified. For PEF microbial

Table 4.1. Experimental artifacts that can influence the kinetics of PEF microbial inactivation.

Artifact source	Causes
Strain and culture conditions	Lack of standardization on the inoculum preparation
	Mixtures of cells at different stages of growth
	Mixtures of strains with different resistance
PEF treatment	Inaccurate measurement of treatment parameters
	Nonuniform distribution of the electric field strength in the treatment chamber
	Changes in the electric field strength during the treatment
	Changes of sample temperature during treatment
Treatment medium	Inaccurate measurement of treatment medium properties
	Generation of electrochemical reaction products
Recovery conditions	Use of selective media
	Short incubation times
	Holding of the microorganisms in the treatment medium before enumeration

inactivation, the potential variability may derive from a number of sources, including strain and culture conditions, PEF treatment, treatment medium, and recovery conditions (Table 4.1).

3.1.1. Strain and Culture Conditions

An important aspect in determining PEF inactivation of microorganisms is the type of strain and the culture conditions used. Microbial resistance to PEF is inherent to the species and strain (Wouters *et al.*, 2001; Álvarez *et al.*, 2003a; Lado and Yousef, 2003). Models may be developed by using a strain of a pathogen or spoilage microorganism or using strain cocktails. In experiments to generate death data, it is preferable to use single strains because strains mixed in a cocktail can lead to multiphasic death curves that are difficult to interpret (Smelt *et al.*, 2002). Ideally, if a single strain is used, the most resistant strains of the target microorganisms should be selected for evaluation and optimization of PEF processes. For validation experiments, using cocktails of representative strains could be more effective than single strains.

The culture history in terms of growth and storage conditions can significantly affect the microbial PEF resistance (Pothakamury *et al.*, 1996; Álvarez *et al.*, 2002; Lado and Yousef, 2003). Therefore, to generate death data preparation of the inoculum must be standardized to minimize its influence on variability between repeated experiments either from day to day or from test period to test period.

Debate continues between the mechanistic theory, which assumes that microorganisms in a population have identical resistance to a lethal agent, and the vitalistic theory, which assumes that there is a distribution of microbial sensitivity to the inactivation process (Stewart *et al.*, 2002). Mathematical models based on a distribution of resistances must be supported by an innate variation within the microbial population. A variability of sensitivities to the PEF treatment within a population can be caused by methodological artifacts such as a contamination of the inoculum with several populations of different species or strains, or an inoculum that consists of a mixing of cells in different phase of growth. Heterogeneity in the microbial population can change the shape of the survival curve and lead to erroneous conclusions on the kinetics of microbial inactivation (Cerf, 1977).

3.1.2. PEF Treatment

The electric field strength and the treatment time are critical process parameters to define a PEF treatment. To obtain good PEF inactivation data these parameters should be monitored continuously.

Actual electric field strength and pulse width should be measured with a high voltage probe connected to an oscilloscope. This probe should be located as close as possible to the treatment chamber to allow the treatment parameters to be determined without errors. Equipments that deliver square waveform pulses are more appropriate than those that deliver exponential waveform ones for generation of PEF death data. Square wave pulses allow the actual electric field and pulse width to be measured more accurately. This is due to the fact that the electric field strength is constant during the duration of the pulse and the pulse width is the actual time at which the voltage is applied between the electrodes of the treatment chamber.

Probably the most important methodological artifact on the estimation of the microbial resistance to PEF is the heterogeneity of the treatment intensity (Fiala *et al.*, 2001). This heterogeneity can be caused by a nonuniform distribution of the electric field strength in the treatment chamber, by changes in the electric field strength during the own treatment or by the presence of dead space in the treatment chamber. Local electric field strength variation in the treatment chamber should be avoided because subpopulation of cells subjected to a lesser electric field strength will appear to be more PEF resistant and tailing will occur in the survival curves (Mañas *et al.*, 2001). Therefore studies of kinetics of microbial inactivation by PEF require a previous investigation of the distribution of the electric field strength in the treatment chamber that generally is most uniform in static that in continuous chambers (Jeyamkondan *et al.*, 1999).

During PEF treatments some heat is generated in the treatment medium. Increases in the temperature of the treatment medium can cause variations in the electric field strength during the own PEF treatment. They may also overestimate the effectiveness of the treatment due to the sensitizing effect of temperature on PEF microbial resistance (Pothakamury *et al.*, 1996; Wouters *et al.*, 1999; Heinz *et al.*, 2003). Therefore for death kinetics data acquisition heating effects should be minimized by delivering short pulses at low frequencies.

3.1.3. Treatment Medium

Predictive microbiology assumes that the behavior of microorganisms in model systems such as broth cultures or buffers predicts the microorganisms behavior in foods with corresponding levels of environmental factors (Whiting, 1995). In order to be able to use the inactivation data to develop models which predict the PEF inactivation in foods, physical and chemical characteristics of the model systems in which microorganisms are treated should be known and controlled during the treatment. As it has been previously indicated, microbial inactivation by PEF has been found to depend on the pH and a_w of treatment medium (Álvarez *et al.*, 2000; Aronsson and Ronner, 2001). However, the influence of other factors that may be important such as the acidulant used to adjust the pH, the humectant used to control a_w, or the type and concentration of ions used to modify the conductivity is still unclear. Therefore, further studies are necessary to know the influence on PEF inactivation of other physicochemical parameters in order to extend the models developed in model systems to different foods.

During the PEF treatment electrochemical reactions can occur and they may result in partial electrolysis of the solution, corrosion of the electrode, and introduction of particles of the electrode material in the liquid (Morren *et al.*, 2003). These phenomena should be minimized because they could provoke microbial inactivation and overestimate microbial inactivation by PEF. Several studies have demonstrated that microbial inactivation by PEF was not due to the presence of toxic components when carbon (Sale and Hamilton, 1967) or stainless steel electrode were used (Jacob *et al.*, 1981; Wouters *et al.*, 1999). However, Reyns *et al.* (2004) observed the presence of electrochemical reaction products with bactericidal properties when electroporation cuvettes, whose electrodes were made of aluminum, were used as treatment chamber. Therefore, suitable

electrode materials and treatment medium should be selected in order to minimize electrochemical reactions.

3.1.4. Recovery Conditions

One of the most important factors in estimating the microbial resistance to a lethal agent is the quantification of the survivors to the treatment. The conditions under which microorganisms are incubated after the PEF treatment and the incubation time can affect the measured resistance of cells.

It was initially thought that PEF did not cause sublethal injury and the number of microorganisms recovered in selective media was the same than those recovered in nonselective media (Simpson *et al.*, 1999). However, later on it has been demonstrated that PEF-treated cells were sensitive to the presence of NaCl in the recovery media or to a subsequent storage in a medium of pH 4 after a PEF treatment in the same acid medium (García *et al.*, 2003).

Although recovery in anaerobic conditions or in low oxygen concentrations may have interest with regard to the margin of safety for foods that are packed under vacuum or modified atmospheres, the influence of atmosphere composition on the measured PEF resistance has not been investigated.

When deciding experimental approaches to the generation of data directed to model microbial inactivation by PEF it is important to use procedures that recover the greatest number of cells. The use of selective media, short incubation times or the holding of the microorganism in the treatment medium before enumeration may overestimate the effectiveness of the treatment and hence the margin of safety that has been achieved.

3.2. Primary Models for Describing Microbial Inactivation by Pulsed Electric Fields

Primary models are mathematical expressions that describe the changes in microbial numbers with time. Generally, primary models for modeling microbial inactivation are based on the description of the survival curve. Plots of the $\log_{10}$ of survivors against the time for constant electric field strength have been the most used approach to study the microbial resistance to PEF. However, other approaches have been used, such as plots in which the $\log_{10}$ of survivors is plotted against the $\log_{10}$ of the treatment time or in which the number of survivors is plotted in logarithmic scale against the electric field strength for a constant treatment time (Hülsheger *et al.*, 1981, 1983; Grahl and Märkl, 1996).

In thermal and nonthermal inactivation of microorganisms four types of survival curves are generally observed: linear curves, curves with a shoulder (concave downward), curves with a tail (concave upward), and sigmoidal curves (Xiong *et al.*, 1999). Linear and concave upward are the most commonly observed survival curves in inactivation of microbial cells by PEF.

Some authors have found a linear relationship between the $\log_{10}$ of survivors and the treatment time at constant electric field strength (Mizuno and Hori, 1988; Martín-Belloso *et al.*, 1997a,b; Sensoy *et al.*, 1997; Reina *et al.*, 1998; Heinz *et al.*, 1999). This linear relationship has also been reported when both the number of survivors and the treatment time have been plotted in logarithmic scale (Hülsheger *et al.*, 1981; Qin *et al.*, 1995; Grahl and Märkl, 1996; Pothakamury *et al.*, 1996). However, in general these linear relationships have been observed in studies where the inactivation was not extended more than 4 $\log_{10}$ cycles. If the treatment time is prolonged to get more inactivation, concave upward survival curves are reached (Jayaram *et al.*, 1992, 1993; Raso *et al.*, 2000; Ohshima *et al.*, 2002; Álvarez *et al.*, 2003a,b,c,d; Rodrigo *et al.*, 2003). These curves are also called survival curves with a slope tailing. These graphs are characterized by a fast inactivation in the first treatment steps and then the survivor number slowly decreases while the treatment becomes longer. There are several explanations for this kind of tailing. If there is a variability of PEF resistances

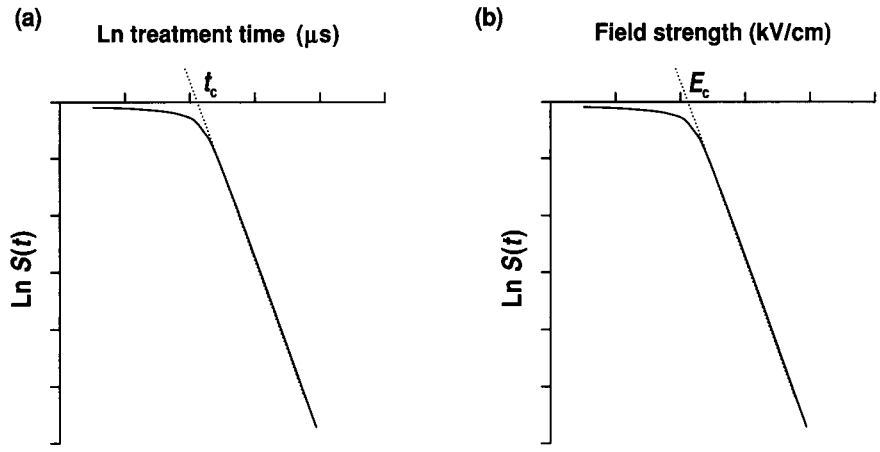

Figure 4.7. Hülsheger *et al.* (1981) model. Relationship between the treatment time and the survival fraction (a) and relationship between the electric field strength and the survival fraction (b).

within a population of cells, the survival curve is the cumulative form of a temporal distribution of lethal effects and the tailing corresponds to the death of the more resistant ones. A mix of two fractions or subpopulations of different PEF resistance can also cause concave upward survival curves. The first portion of the curve mainly describes the death of the least resistant microorganisms and the second portion describes the death of more resistant ones. The variability of resistances within the population or the existence of two subpopulations could be innate to the population or be caused by an adaptation of the microorganisms to the treatment which leads to significantly increase their resistance to PEF. According to the mechanisms of microbial inactivation by PEF, the innate distributions of resistances could be caused by individual variations in the cell size, cell morphology, or composition and structure of the microbial membranes within the microbial population (Lebovka and Vorobiev, 2004). However, these changes in size, morphology, or membrane structure could be induced by the electric field applied during the own PEF treatment. Concave upward survival curves could be also caused by protection resulting from the contents of dead cells, which shield the remaining survivors, or resulting from a microbial aggregation during the PEF treatment.

Several approaches have been proposed to model microbial inactivation by PEF. The first mathematical model to describe inactivation of microbial cells by PEF was developed by Hülsheger *et al.* (1981). This model supposes the existence of two linear relationships, one of them between the natural logarithm of the survivor fraction and the natural logarithm of the treatment time (Fig. 4.7a) and the other between the natural logarithm of the survivor fraction and the electric field strength (Fig. 4.7b). The corresponding equations were expressed as

$$Ln\, S = -b_t \ln\left(\frac{t}{t_c}\right) \tag{4.1}$$

$$Ln\, S = -b_E \ln\left(\frac{E}{E_c}\right), \tag{4.2}$$

where S is the survival fraction; b_t and b_E are constants; t is the treatment time; E is the electric field strength; and t_c and E_c are the critical treatment time and the critical electric field strength, respectively. The t_c is the intercept of the regression line that describes the relationship between the natural logarithm of the treatment time and the natural logarithm of the survival fraction and the abscissa for the 100% of survivors (Fig. 4.7a). The E_c is the intercept of the regression line that

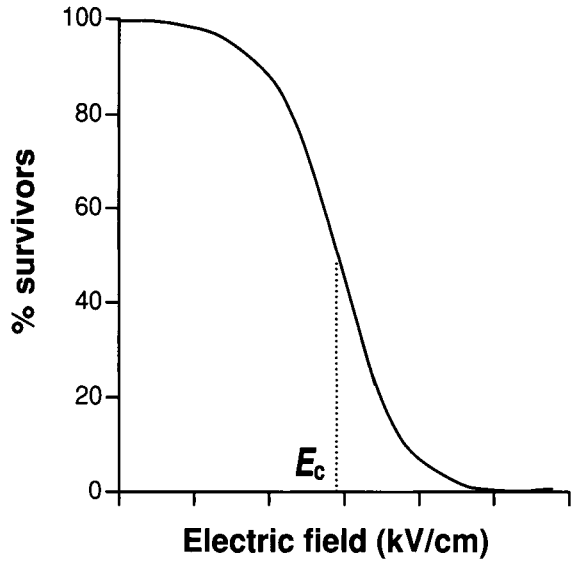

Figure 4.8. Peleg model based on the Fermi equation.

describes the relationship between the electric field strength and the natural logarithm of the survivor fraction and the abscissa that corresponds for the 100% of survivors.

Hülsheger *et al.* (1981) observed that the E_c and t_c values depended on the treatment time and the electric field strength, respectively. However, after a certain treatment time and electric field strength, those values could be considered constant. Taking this into account, the survivor fraction could be expressed according to the following expression:

$$s(E,t) = \left(\frac{t}{t_c}\right)^{(-(E-E_c)/k)}, \tag{4.3}$$

where t_c and E_c are the threshold values of the treatment time and field strength, respectively, and k is a constant factor characteristic for each microorganism.

The peculiarity of Hülsheger's model is that it allows calculating a critical treatment time (t_c) and a critical electric field strength (E_c) over which treatments start being lethal for the microorganisms. These values are characteristic for each microorganism and are useful to analyze the microbial behavior against PEF treatments.

Peleg (1995) used a sigmoid function based on the Fermi equation to describe the relationship between the percentage of survivors and the electric field strength for a given number of pulses (n) (Eq. (4.4)) (Fig. 4.8). The parameters characteristic of this model are the critical electric field strength (E_c), which corresponds to the inflection point of the curve and represents the electric field strength necessary to inactivate the 50% of the population, and a_c which represents the slope of the sigmoid curve in E_c.

$$s(E,n) = \frac{100}{1 + e^{(E-E_c(n))/a_c(n)}} \tag{4.4}$$

Both Hülsheger's and Peleg's models describe accurately the microbial inactivation by PEF and have potential use to predict microbial inactivation (Grahl and Märkl, 1996; Sensoy *et al.*, 1997). However, generally these models have been used to model inactivation data that cover few $\log_{10}$ cycles. Consequently, these models may not be sensitive to describe the PEF inactivation at the

Table 4.2. Mathematical equations used for different authors to describe concave upward PEF microbial survival curves.

Model	Mathematical equation	Where	Reference
Cole *et al.* (1993)	$\text{Log}_{10}\, S(t) = \frac{\alpha + (\omega - \alpha)}{1 + e^{(4\sigma(\tau - \log_{10} t))/(\omega - \alpha)}}$	α: upper asymptote (log cfu/mL); ω: lower asymptote (log cfu/mL); σ: maximum slope of the inactivation curve; τ: log time at which the maximum slope is reached; t: time (μs); S: survival fraction	Raso *et al.* (2000)
Peleg and Penchina (2000)	$\text{Log}_{10}\, S(t) = -a \ln(1 + ct)$	a and c: constants; t: time (μs); S: survival fraction	Álvarez *et al.* (2003e)
Pruitt and Kamau (1993)	$S(t) = pe^{-k_1 t} + (1 - p)e^{-k_2 t}$	p: fraction of survivors in population 1 (PEF sensitive); $1 - p$: fraction of survivors in population 2 (PEF resistant); k_1: specific death rate of subpopulation 1; k_2: specific death rate of subpopulation 2; t: time (μs); S: survival fraction	Álvarez *et al.* (2003e)
Augustin *et al.* (1998)	$S(t) = (1 + e^{(t-m)/s^2})^{-1}$	m: Log_{10} time to destroy the 50% of the population (μs); s: parameter proportional to the standard deviation of the PEF resistance ($\mu s^{0.5}$); t: Log_{10} of the treatment time (μs); S: survival fraction	Álvarez *et al.* (2003d)
Weibull (Peleg and Cole, 1998)	$\text{Log}_{10}\, S(t) = -bt^n$	b: scale parameter; n: shape parameter; t: time (μs); S: survival fraction	Rodrigo *et al.* (2003)
Weibull (Mafart *et al.*, 2002)	$\text{Log}_{10}\, S(t) = -\left(\frac{t}{\delta}\right)^p$	δ: scale parameter; p: shape parameter; t: time (μs); S: survival fraction	Gómez *et al.* (2005b)
Weibull (van Boekel, 2002)	$\text{Log}_{10}\, S(t) = -\left(\frac{1}{2.303}\right)\left(\frac{t}{\alpha}\right)^\beta$	α: scale parameter; β: shape parameter; t: time (μs); S: survival fraction	Álvarez *et al.* (2003a,b,c,d)

inactivation levels of interest for industrial application. It is widely recognized that extrapolation beyond the data range used to develop the model is inappropriate and yields potentially erroneous estimations.

Although a linear relationship between the $\log_{10}$ of the survivors and the treatment time has been reported, the traditional first-order reaction kinetics and D values (decimal reduction time) have not been used extensively in calculations of the PEF microbial resistance (Rodrigo *et al.*, 2001).

Several equations have been proposed for describing concave upward survival curves corresponding to microbial inactivation by PEF (Table 4.2). This type of survival curves has been modeled by purely empirical equations (Cole *et al.*, 1993; Peleg and Penchina, 2000), by a two-fraction model that supposes the existence of two subpopulations of different PEF resistance (Pruitt and Kamau, 1993) and by models based on the existence of distribution of resistances within the microbial population (Augustin *et al.*, 1998; Peleg and Cole, 1998; Mafart *et al.*, 2002; van Boekel,

2002). Among these models, the model based on the Weibull distribution is gaining popularity due to its simplicity and flexibility. This model uses two parameters to describe the survival curves and can describe concave upward, concave downward, and linear survival curves. It has been successfully used to model microbial death by different inactivation methods such as heat (Peleg and Cole, 1998; Fernández *et al.*, 1999; van Boekel, 2002; Mafart *et al.*, 2002), high hydrostatic pressures (Chen and Hoover, 2003), chemicals (Virto *et al.*, 2005), or PEF (Rodrigo *et al.*, 2001; Álvarez *et al.*, 2003a,b,c,d; Rodrigo *et al.*, 2003). This model assumes that microorganisms in a population have different resistances, and a survival curve is just the cumulative form of a distribution of lethal events.

The Weibull model estimates the microbial inactivation through two parameters that are called the scale parameter (b, δ, and α; Table 4.2) and the shape parameter (n, p, and β; Table 4.2). The mathematical equation of the cumulative form of the Weibull distribution initially proposed by Peleg and Cole (1998) (Table 4.2) was modified by Mafart *et al.* (2002) (Table 4.2) so that the equation had a parameter with the dimensions of time. This parameter in the equation proposed by Mafart *et al.* (2002) (δ value) represents a characteristic time at which the survival function $\text{Log}_{10}\, S(t) = -1$. Similarly, to the equation proposed by Mafart *et al.* (2002), in the equation proposed by van Boekel (2002) the α parameter has dimensions of time and in this equation represents a characteristic time at which the survival function $\text{Log}_{10} S(t) = -0.434$ (i.e., $S(t) = \exp(-1)$). On the other hand, the shape parameter represents the form of the curve: a shape parameter <1 corresponds to a concave upward curve, a shape parameter >1 corresponds to a concave downward curve; and a shape parameter $= 1$ corresponds to a linear curve. Opposite to the scale parameter the value of the shape parameter is independent of the mathematical equation based on the Weibull distribution used to fit the survival curve.

Figure 4.9 shows how the Weibull model fits very reasonably the concave upward survival curves that cover more than 4 $\log_{10}$ cycles corresponding to the inactivation of a yeast, a Gram-negative bacteria and a Gram-positive bacteria by PEF treatments at different electric field strengths. Table 4.3 shows parameters of the Weibull model obtained by fitting the equation proposed by van Boekel (2002) to the survival curves which correspond to the PEF inactivation of eight different microorganisms by electric field strengths ranging from 9 to 28 kV/cm. The root mean square error (RMSE) and the determination coefficient (R^2) between the experimental and predicted values show that the model was effective in modeling PEF inactivation of Gram-positive (*L. monocytogenes* and *Enterococcus faecium*) and Gram-negative (*Y. enterocolitica*, *E. coli*, and *Salmonella* serovars Enteritidis, Senftenberg, and Typhimurium) bacteria and yeast (*S. cerevisiae*). Overall, the survival curves fitted with the Weibull equation yielded shape factors that were <1 indicating that all the survival curves were concave upward and scale factors decreased when the electric field strength increased.

3.3. Secondary Models for Describing Microbial Inactivation Pulsed Electric Fields

The parameters of the Weibull model presented in Table 4.2 determine the way in which microbial population change with time under a specific electric field strength. The secondary level models describe how the primary level parameters vary with changes in the treatment intensity and environmental conditions. For the Weibull model these parameters are the shape and the scale factors.

Analysis of the influence of the electric field strength on the shape factor shows that in seven of the eight microorganisms investigated this value does not depend on electric field strength in a systematic way. For each microorganism, nonsignificant differences were found in the shape values (β) among the treatments at different electric field strengths (ANOVA, $P < 0.05$). For *Y. enterocolitica*, the β value decreased with the electric field strength following a second-order polynomial relationship.

When for a given microorganism, nonsignificant differences in the shape values among treatments are observed, the survival curves may be refitted with the shape value sets at their mean

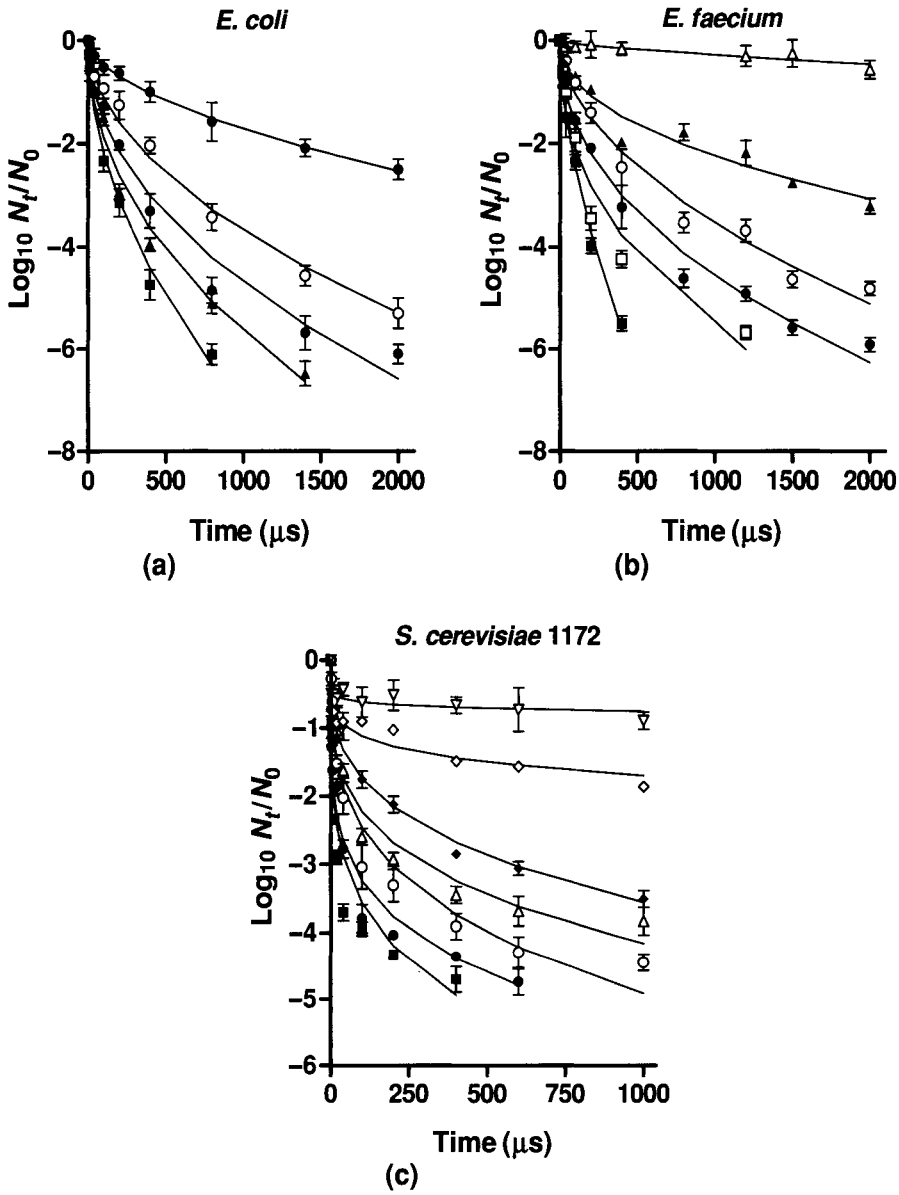

Figure 4.9. Fit of the mathematical model based on the Weibull distribution to the survival curves corresponding to the PEF inactivation of *E. coli*, *E. faecium*, and *S. cerevisiae*: 2.5 kV/cm (▽), 5.5 kV/cm (◇), 9 kV/cm (◆), 12 kV/cm (△), 15 kV/cm (▲), 19 kV/cm (○), 22 kV/cm (●), 25 kV/cm (□) y 28 kV/cm (■); McIlvaine buffer pH 7.0, 2 mS/cm; 2 μs square wave pulses; frequency: 1 Hz.

value in order to simplify the Weibull model by reducing the number of parameters from two to one (Mafart *et al.*, 2002; Álvarez *et al.*, 2003b,d). If the shape parameter is common for treatments at different electric field strengths, the prediction of the microbial inactivation by PEF treatments requires the development of only one secondary model that describes the relationship between the scale parameter and the electric field strength. In the case of *Y. enterocolitica*, as the shape parameter is field strength dependent, prediction of microbial inactivation requires the development of two secondary models: one to describe the relationship between the shape parameter and the electric field

Table 4.3. α and β values estimated from the fitting of the mathematical model based on the Weibull distribution to the survival curves corresponding to the PEF inactivation of different microorganisms at different electric field strengths.

Microorganism	kV/cm	α (μs) (CL 95%)	β (CL 95%)	R^2	RMSE
E. faecium	15	27.139 (−0.348 – 54.630)	0.455 (0.333 – 0.576)	0.960	0.205
	19	19.649 (3.691 – 35.610)	0.533 (0.429 – 0.637)	0.982	0.245
	22	5.291 (−0.826 – 11.410)	0.449 (0.352 – 0.545)	0.976	0.334
	25	2.390 (−2.137 – 6.917)	0.423 (0.278 – 0.567)	0.959	0.401
	28	5.678 (1.599 – 9.759)	0.602 (0.490 – 0.714)	0.993	0.164
L. monocytogenes	15	2101.0 (1697.0 – 2505.0)	0.847 (0.561 – 1.132)	0.982	0.029
	19	843.8 (712.5 – 975.2)	0.881 (0.670 – 1.092)	0.975	0.067
	22	439.7 (357.9 – 521.5)	0.883 (0.748 – 1.018)	0.99	0.076
	25	111.4 (67.5 – 155.3)	0.621 (0.521 – 0.721)	0.988	0.132
	28	76.4 (39.9 – 112.8)	0.755 (0.633 – 0.877)	0.995	0.197
S. cerevisiae	9	0.114 (0.131 – 2.098)	0.309 (0.223 – 0.396)	0.981	0.15
	12	0.241 (−0.105 – 0.587)	0.271 (0.218 – 0.325)	0.968	0.229
	19	0.307 (−0.213 – 0.826)	0.300 (0.229 – 0.371)	0.958	0.33
	22	0.010 (−0.007 – 0.027)	0.218 (0.181 – 0.255)	0.976	0.244
	28	0.014 (−0.027 – 0.054)	0.237 (0.163 – 0.310)	0.921	0.476
Y. enterocolitica	15	1.672 (−0.195 – 0.354)	0.326 (0.268 – 0.384)	0.986	0.246
	19	0.441 (−0.234 – 0.112)	0.287 (0.230 – 0.345)	0.989	0.26
	22	0.040 (−0.025 – 0.105)	0.226 (0.188 – 0.264)	0.986	0.267
	25	0.022 (−0.011 – 0.054)	0.220 (0.188 – 0.253)	0.989	0.249
	28	0.012 (−0.029 – 0.053)	0.214 (0.135 – 0.292)	0.985	0.377
E. coli	15	87.026 (70.530 – 103.500)	0.562 (0.523 – 0.601)	0.998	0.04
	19	25.291 (14.930 – 35.650)	0.579 (0.519 – 0.639)	0.995	0.129
	22	7.576 (−1.239 – 16.390)	0.488 (0.375 – 0.600)	0.978	0.353
	25	4.907 (0.672 – 9.142)	0.483 (0.401 – 0.564)	0.989	0.224
	28	4.490 (0.520 – 8.460)	0.517 (0.419 – 0.614)	0.99	0.206
S. enteritidis	15	0.589 (−0.213 – 1.390)	0.305 (0.248 – 0.362)	0.982	0.221
	19	0.172 (−0.161 – 0.504)	0.280 (0.216 – 0.344)	0.973	0.305
	22	0.099 (−0.064 – 0.261)	0.274 (0.224 – 0.324)	0.982	0.268
	25	0.059 (−0.028 – 0.145)	0.272 (0.229 – 0.314)	0.987	0.255
	28	0.056 (−0.053 – 0.166)	0.287 (0.227 – 0.348)	0.981	0.316
S. senftenberg 775W	12	304.633 (125.90 – 483.40)	0.362 (0.214 – 0.511)	0.93	0.076
	15	117.763 (72.56 – 163.00)	0.590 (0.395 – 0.785)	0.98	0.102
	19	11.368 (4.366 – 18.370)	0.460 (0.400 – 0.521)	0.984	0.19
	22	1.111 (−0.165 – 2.387)	0.338 (0.280 – 0.396)	0.982	0.228
	28	0.756 (0.184 – 1.328)	0.360 (0.322 – 0.399)	0.99	0.228
S. typhimurium	15	0.150 (−0.150 – 0.451)	0.244 (0.184 – 0.304)	0.973	0.177
	19	0.106 (−0.032 – 0.244)	0.253 (0.216 – 0.290)	0.99	0.16
	22	0.086 (−0.098 – 0.270)	0.203 (0.164 – 0.242)	0.983	0.206
	25	0.084 (−0.111 – 0.279)	0.270 (0.202 – 0.339)	0.967	0.371
	28	0.014 (−0.020 – 0.048)	0.233 (0.182 – 0.284)	0.976	0.32

strength, and another to describe the relationship between the scale parameter and the electric field strength.

The effect of the electric field strength on the scale parameter obtained by refitting the survival curves with the simplified Weibull model can be described via an exponential relationship. This model is analogous to the relationship between the D_t value and the temperature in the TDT curve (van Boekel, 2002). Therefore similarly to the classical method for describing the heat resistance

Table 4.4. Shape (β) value, Z_{PEF} value, and relationship between the scale (α) value and the electric field strength for different microorganisms.

Microorganism	Relationship $\alpha - E$	β	Z_{PEF} (kV/cm)
E. faecium	$\text{Log}_{10} - \alpha = -0.086E - 2.8$[a]	0.492	11.628
L. monocytogenes	$\text{Log}_{10} - \alpha = -0.106E + 4.9$[a]	0.798	9.469
S. cerevisiae	$\text{Log}_{10} - \alpha = -0.056E - 0.071$[a]	0.253	17.857
Y. enterocolitica	$\text{Log}_{10} - \alpha = 0.006E^2 - 0.45E + 5.6$[a]	$\beta = 0.001E^2 - 0.034E + 0.727$	—
E. coli	$\text{Log}_{10} - \alpha = -0.060E + 2.37$[b]	0.526	16.667
S. enteritidis	$\text{Log}_{10} - \alpha = -0.061E + 0.4$[a]	0.283	16.393
S. senftenberg	$\text{Log}_{10} - \alpha = -0.058E + 2.0$[b]	0.437	17.170
S. typhimurium	$\text{Log}_{10} - \alpha = -0.067E + 0.15$[a]	0.241	14.925

[a]Electric field range: 15–28 kV/cm; [b]Electric field range: 19–28 kV/cm.

of bacteria based on two parameters (D and z) the PEF resistance can be characterized by three parameters: shape, scale, and Z_{PEF} (Table 4.4) (Álvarez *et al.*, 2003b). The Z_{PEF} value corresponds to the increase in electric field strength that would produce a reduction in the scale value by a factor 10. In the case of *Y. enterocolitica* in which the shape value was electric field dependent, the relationship between the β value and the electric field was described by a quadratic polynomial relationship (Álvarez *et al.*, 2003c). This kind of relationship could imply that after a given electric field strength value, further electric field strength increases would not result in a faster death.

Table 4.4 shows that in the range of the electric field strengths studied the Z_{PEF} value was around 10 kV/cm for Gram-positive bacteria and 17 kV/cm for Gram-negative bacteria This could indicate that if this is a general behavior, it should be possible to set a Z_{PEF} value characteristic for Gram-negative and another for Gram-positive bacteria. This greatly simplified the Weibull model which could help to its application in the food industry. However, to confirm this extreme, further studies on the kinetics of PEF inactivation of other microorganisms are required.

When microbial inactivation is modeled by the traditional firstorder kinetics, the D value can be used directly to compare the resistance of different microorganisms because the death rate is constant and independent of the treatment time. However, when nonlinear survival curves are modeled by the Weibull model, the scale parameter by itself is not a measurement of the death rate or the PEF sensitivity of the microorganism. According to the equation of the Weibull model survival fraction depends not only on the scale parameter but also on the shape parameter that is different for each microorganism (Table 4.4). In this case, to compare microbial resistance, it is necessary to establish a comparison criterion such as the inactivation for different microorganisms achieved by a given treatment condition or the treatment time to obtain a given level of microbial inactivation at a given treatment intensity. In order to compare the PEF resistance of microorganisms of Table 4.4, Fig. 4.10 shows the time necessary to inactivate 5 $\log_{10}$ cycles of the microbial population in the range of electric field strengths investigated. Several conclusions on PEF microbial resistance can be drawn from this figure. *L. monocytogenes* was the most PEF resistant microorganism; the 5 $\log_{10}$ cycles of inactivation were only obtained at 28 kV/cm. On the other hand, *Salmonella* serovar Enteritidis was the most sensitive one; the 5 $\log_{10}$ cycles of inactivation were obtained even at 15 kV/cm. As *L. monocytogenes* is a pathogenic microorganism, it can be considered as one of the key pathogens of concern for establishing processing parameters for food processing by PEF. On the other hand, it was initially thought that yeast was more PEF sensitive than bacteria and Gram-negative bacteria were more PEF sensitive than Gram-positive ones. These conclusions were generally obtained from studies of PEF resistance by end point methods. These results based on modeling techniques have shown that microorganisms vary greatly in their resistance to PEF. They have also shown that the

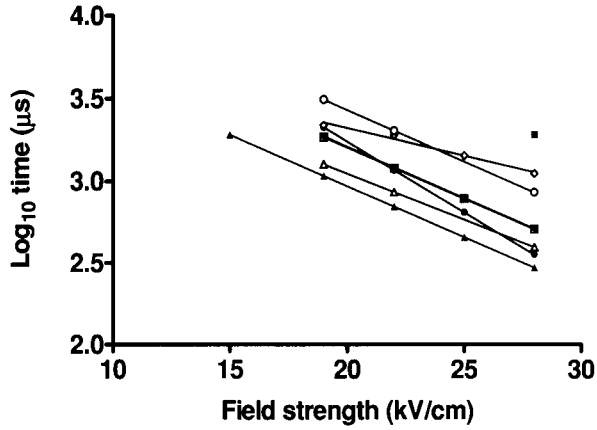

Figure 4.10. Time to inactivate 5 Log_{10} the population of several microorganisms at different electric field strengths. Estimations have been obtained from the van Boekel's mathematical equation based on the Weibull distribution (first model) by including the relationship between the α value and the field strength (second model) and the β value characteristic of each microorganism. (■) *L. monocytogenes*; (□) *E. coli*, (●) *E. faecium*, (○) *S. senftenberg*, (▲) *S. enteritidis*, (△) *S. cerevisiae*, (◆) *S. typhimurium*, and (◇) *Y. enterocolitica*.

yeast *S. cerevisiae* is more resistant than some bacteria such as *Salmonella* serovar Enteritidis and the Gram-positive bacterium *E. faecium* is more sensitive than several Gram-negative bacteria.

The Weibull model has also been effective in modeling the influence of environmental factors that affect PEF microbial inactivation. A mathematical equation based on the Weibull parameters described the joint effect of the electric field strength and pH on PEF inactivation of *Lactobacillus plantarum* and *L. monocytogenes* (Gómez *et al.*, 2005a,b). In both microorganisms, the shape factor depended on the pH of the treatment medium, but for a given pH, the electric field strength did not have a significant effect on the shape parameter. For both microorganisms, the relationship between the shape factor (β) and the pH of the treatment medium was modeled by an empirical equation that is based on the Gompertz equation:

$$\beta = A + Ce^{-e^{(-B(\mathrm{pH}-M))}},$$

where A, B, C, and M are constants.

The following multiple regression equations, developed in these studies, predicted the scale parameters (α) of *L. plantarum* and *L. monocytogenes* for any combinations of electric field strength (E = 15–28 kV/cm) and pH (3.5–7.0):

$$Log_{10}\alpha = 4.82 - 0.37E + 0.00056E^2 + 0.63\mathrm{pH}^2 \text{ (}L.\ monocytogenes\text{)}$$
$$Log_{10}\alpha = 6.32 - 0.12E - 1.09\mathrm{pH} + 0.13\mathrm{pH}^2 \text{ (}L.\ plantarum\text{)}.$$

The models developed were validated in orange and apple juice for *L. plantarum* and in apple juice for *L. monocytogenes*. They provided a valid prediction of the inactivation of both microorganisms in these foods and where there were discrepancies the models were conservative (i.e., provided a prediction that was longer than the actual time to achieve the inactivation).

Several models can be used to describe survival curves corresponding to microbial inactivation by PEF. Selection of the best model is to some degree subjective and the choice may be simply pragmatic based on model validation (Legan *et al.*, 2002). However, other relevant criteria have to be considered to choose the best one. Models should be as simple as possible by describing the experimental data using the smallest possible number of parameters; they should also be based on

mechanisms of inactivation; and should properly accommodate the effect of experimental conditions in order to develop secondary models that describe the influence of different factors on microbial inactivation. The Weibull model is a simple primary model that accurately describes the kinetics of microbial inactivation by PEF even when survival curves cover more than 5 $\log_{10}$ reductions. Although the Weibull model is based on the biological assumption of the existence of a distribution of microorganisms of different resistance, this model is empirical rather than mechanistic because it does not take into account specific mechanisms of inactivation apart from the assumption of the heterogeneity in the population. Therefore, this model does not allow extrapolation. Extrapolation is only possible when adequate knowledge of the physiology of microbial cell population and of the mechanisms of inactivation are available (Smelt *et al.*, 2002). The hypothesis of the distribution of microbial sensitivity to PEF should be demonstrated in order to introduce mechanistic elements in the Weibull model.

4. COMBINATION TREATMENTS

Although the efficacy of PEF to inactivate vegetative microorganisms has been widely demonstrated, in some cases it is necessary to apply very intense PEF treatments to obtain substantial microbial inactivation that ensure food safety and stability. The application of these intense treatments at industrial scale has several technical limitations and may affect food properties. On the other hand, bacterial spores and most enzymes are resistant to PEF treatments. Therefore, the use of this technology for food preservation is limited to foods where enzymatic reactions do not affect food quality and spore germination is inhibited (van Loey and Hendrickx, 2002; Raso and Barbosa-Cánovas, 2003). In order to increase the lethality of PEF treatments or to prevent the deteriorative agents after the PEF treatment, different combinations of PEF with other preservation techniques have been investigated in recent years (Raso and Barbosa-Cánovas, 2003). This approach, known as "hurdle technology" has already been applied successfully using traditional techniques of food preservation (Leistner and Gorris, 1995).

The application of *PEF in combination with moderate temperatures* that do not affect food properties has a great practical interest because it introduces the possibility to pasteurize liquid foods at lower PEF treatment intensities (Heinz *et al.*, 2003). For the most PEF resistant microorganisms, obtaining a level of inactivation that ensures food safety and stability requires to apply treatments of long duration or at very high electric field strengths. Several technical and economical limitations exist in order to scale up the treatment chamber to extend the processing time or to deliver electric field strengths above 30 kV/cm (Heinz *et al.*, 2001).

In order to enhance the lethal effect in addition to applying a moderate heat treatment during the PEF process, the efficacy of applying a heat treatment after a PEF treatment has also been investigated. Lado *et al.* (2004) observed that PEF sensitized *L. monocytogenes* to heat and concluded that this effect is a consequence of the alteration of the expression of molecular chaperones by PEF; however, a previous PEF treatment did not cause significant modification on the heat resistance of *E. coli* O157:H7 (Evrendilek and Zhang, 2003).

Low pH reduces microbial thermal resistance and inhibits germination of surviving spores; therefore, acidification of foods is one of the most used approaches in order to reduce the undesirable effects of thermal processing. *PEF in combination with low pH* has provided mixed results as it has been exposed in the previous sections. While some microorganisms are more PEF sensitive at acidic pH, others are more PEF sensitive at neutral pH, and the PEF resistance of others is not affected by the pH of the treatment medium (Wouters *et al.*, 2001). However, it has been demonstrated that microorganisms which are more PEF resistant in acid media become sublethally injured. For

example, a PEF treatment that caused only a limited inactivation of *E. coli* O157:H7 in apple juice resulted in a considerable decrease in the number of survivors during subsequent storage at 4°C for 48 h in the same apple juice (García *et al.*, 2005). Therefore, although microbial PEF sensitivity does not always increase by lowering the pH of the treatment medium, the key of this combination is the inhibitory effect of low pH on germination of bacteria spores, on the growth of non-acid-tolerant microorganisms and the inactivating action of low pH on PEF sublethally injured microorganisms.

PEF in combination with antimicrobials (nisin, lysozyme, pediocin AcH, organic acids such as benzoic and sorbic acid, ozone, hop, carvacrol) has proven to be effective in order to obtain a substantial inactivation of spoilage and pathogenic microorganisms. The bacteriocin nisin has been the antimicrobial most studied in combination with PEF. It has been observed that the microbial inactivation is enhanced when bacteria are incubated with nisin before PEF treatment, when nisin is present during the PEF treatment or when nisin is added after PEF treatment for both Gram-positive and Gram-negative bacteria (Kalchayanand *et al.*, 1994; Calderón-Miranda *et al.*, 1999a,b,c; Dutreux *et al.*, 2000a). When added simultaneously with nisin, lysozyme or the essential oil carvacrol enhanced the bactericidal effect of the combination on *Salmonella* serovar Typhimurium and vegetative cells of *Bacillus cereus*, respectively (Pol *et al.*, 2001b; Smith *et al.*, 2002).

The efficacy of a PEF treatment on *E. coli* O157:H7 increased in presence of benzoic acid or sorbic acid (Liu *et al.*, 1996). However, this enhancement was observed at pH 3.4 but not at pH 6.4. Therefore, the combination was only effective at high concentrations of the undissociated fraction of the acids.

Combinations of antimicrobials with PEF enhance microbial inactivation and extend the spectrum of action of some antimicrobials with advantages in product stability and safety. However, the effect of antimicrobials on the survivability of microorganisms after PEF treatment has not been investigated.

As in thermal pasteurization, *PEF in combination with refrigeration temperatures* is an effective approach to extend the shelf life of PEF-treated products. Some agents responsible for food deterioration such as bacterial spores and enzymes are resistant to PEF treatments. As refrigeration scarcely changes food properties, this hurdle is one of the most used for their control. Foods processed by PEF have not been commercialized at the moment, but several studies at pilot plant scale have demonstrated that these treatments can extend the shelf life and improve the microbial safety of food products. The best results in these investigations have been obtained when PEF-treated foods were stored under refrigeration.

The studies carried out indicate that the combination of PEF with other preservation factors seems to be a successful approach for nonthermal preservation of foods with a low impact in food quality while assuring food safety and stability.

5. CONCLUSIONS

Maintaining high levels of food safety and quality is essential for a continued consumer confidence in the food industry. There is an increasing consumer demand for foods that contain lower levels of preservatives and less severe processing. These requirements sometimes are not compatible with an improvement in microbial stability and safety. In order to reduce the food-borne microbiologic hazard and to extend the shelf life of minimally processed foods, alternative nonthermal technologies for microbial inactivation have recently received substantial attention in the food industry.

PEF has the potential of inactivating microorganism at temperatures that avoid the harmful effects of heat in organoleptic properties and nutrient value of liquid foods. As for thermal processing, establishment of process parameters for PEF processing has to be based on the knowledge of the

factors that affect microbial resistance, on mathematical description of the inactivation kinetics of the most PEF-resistant pathogens of concern for each specific food and on the understanding of the mechanisms of microbial inactivation.

Many investigations have been focused on identifying critical factors that influence the PEF microbial resistance and presently most of them are well known. Although models used to describe PEF inactivation are mainly empirical they are quite acceptable. However, more research is necessary to identify the key pathogens of concern and to propose surrogates as an alternative to pathogens for validation studies in PEF industrial scale facilities. A deeper knowledge of the mechanisms of microbial inactivation by PEF will contribute to the development of more mechanistic models and to the design of combined treatments to enhance the safety and stability of minimally processed foods of the future.

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CHAPTER 5

EFFECT OF PEF ON ENZYMES AND FOOD CONSTITUENTS

Pilar Mañas and Antonio Vercet

1. INTRODUCTION

Food quality is of key importance in food preservation, even more in the context of novel technologies. The search for alternative methods to preserve foods is driven by the trends in consumption patterns. Consumers nowadays demand healthier, fresher, and more natural foods, with high sensory and nutritional qualities, and at the same time with the highest degree of safety. Pulsed electric field technology aims to offer the consumer these high-quality foods.

Processing may cause detrimental changes in food constituents, such as proteins, with the consequent possible modifications in sensory and nutritional characteristics. PEF technology has been presented as advantageous in comparison to, for instance, heat treatments, because it kills microorganisms while better maintaining the original color, flavor, texture, and nutritional value of the unprocessed food. However, whereas a considerable amount of research papers have been published on the microbial aspects of food preservation by PEF, a lesser amount of information is available about the effect of this technology on food constituents and overall quality and acceptability.

In this chapter, the effect of PEF technology on food constituents will be reviewed. Especial emphasis is dedicated to the potential of PEF to inactivate enzymes which may be responsible of product spoilage during storage. Also, the available information about quality attributes of various PEF-treated products currently under study is presented. Finally, limitations of current studies and research needs for the future are analyzed.

2. EFFECT OF PEF ON ENZYME ACTIVITY

Enzymes are capable of a specific manipulation of all the molecules found in foods, and, therefore a wide variety of enzymes are used as supplements for several food processing operations. However, there are certain enzymes whose activities result in a deterioration of food quality, that have to be controlled in order to maintain quality and extend food shelf life. The effect of PEF on both types of enzymes has been studied, and data available is described next. Treatment conditions and media are detailed in Table 5.1.

Alkaline phosphatase: Alkaline phosphatase is a milk endogenous enzyme that is routinely used to assess the correct application of pasteurization treatments. There are several isoenzymes but

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Table 5.1. Effect on pulsed electric fields on enzymes.

Enzyme	Treatment conditions	Residual activity (%)	Treatment medium	Reference
Alkaline phosphatase	18.8 kV/cm; 400 μs pulse width; 70 pulses; batch system	40	Raw milk	Castro *et al.* (1994)
	21.5 kV/cm; batch system; exponential decay	100	Raw milk	Grahl and Märkl (1996)
	80 kV/cm; 2 μs pulse width; 30 pulses; batch system; exponential decay	95	Buffer pH 9.8	Ho *et al.* (1997)
	6.7–20 kV/cm; 2 μs pulse width; 200 pulses; batch system; square wave	100	Milk	Van Loey *et al.* (2002)
Glucose oxidase	50 kV/cm; 2 μs pulse width; 30 pulses; batch system; exponential decay	25	Buffer pH 5. 1	Ho *et al.* (1997)
Peroxidase				
From soybean	73 kV/cm; 2 μs pulse width; 30 pulses; batch system; exponential decay	73	Buffer pH 6.0	Ho *et al.* (1997)
From milk	21.5 kV/cm; batch system; exponential decay	100	Raw milk	Grahl and Märkl (1996)
From milk	19 kV/cm; 5 μs pulse width; 100 pulses batch system	100	Raw milk	Van Loey *et al.* (2002)
From horseradish	5–25 kV/cm; 1.5 μs pulse width; 207–1242 pulses; exponential decay	65.3–83.3	Buffer	Zhong *et al.* (2005)
Polyphenoloxidase				
From mushroom	50 kV/cm; 2 μs pulse width; 30 pulses; batch system; exponential decay	60	Buffer pH 6.5	Ho *et al.* (1997)
From apple	24 kV/cm; 20 μs pulse width; 300 pulses; batch system; bipolar exponential decay	3	Buffer pH 6.5	Giner *et al.* (1997)
From peach	24 kV/cm; 20 μs pulse width; 400 pulses; batch system; bipolar exponential decay	48	Not reported	Giner *et al.* (1999a)
From pear	22 kV/cm; 20 μs pulse width; 300 pulses; batch system; bipolar exponential decay	30	Not reported	Giner *et al.* (1999b)
From mushroom	10–20–30 kV/cm; 5–40 μs pulse width; 1–1000 pulses; batch system	79–100	Water; Buffer different pHs	Van Loey *et al.* (2002)
From apple	7–31 kV/cm; 1–40 μs pulse width; 1–1000 pulses; batch system	90–100	Buffer different pHs; apple juice	Van Loey *et al.* (2002)
Lipoxygenase				
From soybean	10–20–30 kV/cm; 5–40 μs pulse width; 1–1000 pulses; batch system	90–100	Distilled water	Van Loey *et al.* (2002)
From pea	2.5–20 kV/cm; 1 μs pulse width; 100–400 pulses; batch system	100	Pea juice	Van Loey *et al.* (2002)

(*continued*)

Table 5.1. (*Continued*)

Enzyme	Treatment conditions	Residual activity (%)	Treatment medium	Reference
Lipase				
From milk	21.5 kV/cm; batch system; exponential decay	40	Raw milk	Grahl and Märkl (1996)
From wheat germ	87 kV/cm; 2 μs pulse width; 30 pulses; batch system; exponential decay	15	Distilled water	Ho *et al.* (1997)
From *Ps. fluorescens*	27.4–37.3 kV/cm; 4 μs pulse width; 100 pulses; batch and continuous systems; exponential decay	38–87	Simulated milk ultrafiltrate	Bendicho *et al.* (2002a)
Lysozyme	87 kV/cm; 2 μs pulse width; 30 pulses; batch system; exponential decay	>100	Buffer pH 6.2	Ho *et al.* (1997)
α-amylase	80 kV/cm; 2 μs pulse width; 30 pulses; batch system; exponential decay	15	Buffer pH 7.0	Ho *et al.* (1997)
Lactate dehydrogenase	31.6 kV/cm; 0.96 μs pulse width; 200 pulses; batch system; exponential decay	100	Buffer pH 7.2	Barsotti *et al.* (2002)
Pectinmethylesterase				
From orange	35 kV/cm; 1.4 μs pulse width; treatment time 59 μs; continuous system; 60°C	12	Orange juice	Yeom *et al.* (2000a)
From orange	35 kV/cm; 1 μs pulse width; 1000 pulses; batch system	90–100	Orange juice	Van Loey *et al.* (2002)
From tomato	24 kV/cm; 20 μs pulse width; 400 pulses; batch system; exponential decay	6	NaCl solution	Giner *et al.* (2000)
From tomato	10–20–30 kV/cm; 5–40 μs pulse width; 1–1000 pulses; batch system	90–100	Distilled water	Van Loey *et al.* (2002)
Proteases				
Plasmin	30–45 kV/cm; 2 μs pulse width; 50 pulses; continuous system	10	Simulated milk ultrafiltrate	Vega-Mercado *et al.* (1995a)
Ps. fluorescens M3/6	14–61.6 kV/cm; 16–98 pulses	40; >100; 100 (depending on treatment medium)	Skim milk; casein solution	Vega-Mercado *et al.* (1995b, 1997)
Bacillus subtilis	16.4–27.4 kV/cm; 4 μs pulse width; 80 pulses; batch and continuous systems; exponential decay	87; 110–115; 100 (depending on treatment medium)	Simulated skimmed milk ultrafiltrate; milk	Bendicho *et al.* (2005)
Papain	20–50 kV/cm; 4 μs pulse width; 500 pulses; continuous system; square wave	100; 15 (after 24 h storage)	EDTA solution	Yeom *et al.* (1999)

the most abundant is constituted by two monomers and each dimmer is able to link five molecules of zinc (Belitz *et al.*, 2004). Inactivation of alkaline phosphatase by PEF has been studied because milk pasteurization is one of the possibilities to implement PEF to the food industry. Castro *et al.* (1994) investigated the effect of PEF in milk with different fat contents. PEF treatments applied were 70 pulses of 400 μs and field strengths varying between 18 and 22 kV/cm. These treatments were able to reduce enzyme activity to a 40% of the original. On the contrary, Grahl and Märkl (1996), Ho *et al.* (1997), and Van Loey *et al.* (2002) did not observe a significant enzyme inactivation in either milk or aqueous solutions. This difference could be due to the different electrical parameters (the main difference was pulse width) or to a poor control of temperature that could lead to underestimate the effect of temperature on alkaline phosphatase inactivation, as this enzyme is not particularly heat-resistant. For instance, Van Loey *et al.* (2002) observed that 8000 μs at 10 kV/cm resulted in a 74% inactivation of alkaline phosphatase in raw milk. However, the authors attributed this inactivation to a thermal effect due to an increase in temperature that went up to 70°C.

Oxidases: The effect of PEF treatments on some oxidoreductases has also been studied. One of the main characteristics of this group of enzymes is that their active site normally links a metal molecule that participates directly in the catalysis reaction. For example, polyphenoloxidases have several copper molecules, peroxidases have a heme group, and lipoxygenases contain a nonheme iron placed in its active site. On the other hand, glucose oxidase active center is much more complex, and in this case, a histidine residue seems to be important for redox reactions catalyzed by this enzyme (Belitz *et al.*, 2004). A priori, this kind of enzymes appear sensitive to PEF treatments because of the important implications of redox reactions in their activity, but then once again, results obtained are contradictory.

Glucose oxidase is an enzyme that is used in food industry as a technological tool. Their main uses are the oxidation of glucose to minimize browning and as an antioxidant through its oxygen removal ability. Ho *et al.* (1997) studied the effect of PEF on glucose oxidase obtained from *Aspergillus niger*. After 60 μs at 50 kV/cm (and higher field intensities) a reduction of enzyme activity to the 25% of the original was attained.

Peroxidases have also been studied. Ho *et al.* (1997) studied a peroxidase from soybean in phosphate buffer pH 6.0. Only a moderate reduction (to 70% of the original activity) could be achieved after 200 μs at 73 kV/cm. On the other hand, Grahl and Märkl (1996) studied the effect of PEF on lactoperoxidase in raw milk, and inactivation was negligible. Van Loey *et al.* (2002) obtained the same result: even after energy inputs as high as 500 kJ/kg, no decrease in lactoperoxidase activity was observed. Zhong *et al.* (2005) studied the effect of PEF on horseradish peroxidase. After 1821 μs at 22 kV/cm, only a reduction to the 65% of the original activity was observed. The authors also studied conformational changes using circular dichroism spectra analysis and fluorescence spectrum and noticed some conformational changes (mainly a loss in α-helix content) but without obtaining a clear conclusion.

Polyphenoloxidases (PPO), a group of enzymes, play an important role in quality food of plant origin because they are responsible of enzymatic browning, have also been studied. Ho *et al.* (1997) noticed an inactivation of mushroom PPO up to a 60% of the original activity after 60 μs at 50 kV/cm. Giner *et al.* (1997, 1999a,b) studied the effect of PEF on polyphenoloxidases from pear, peach, and apple. Experimental conditions used in their experiments were the following: exponential pulses in mono and bipolar mode, pulses of 20–100 μs and 3–24 kV/cm. No specification of temperature measurement and control were given although it is mentioned that temperature never exceed 25°C. Results obtained by this research group show an important effect of PEF in the inactivation of PPO. Apple PPO activity was reduced to a 3% of the original activity after 6000 μs at 24 kV/cm. Peach PPO activity could be reduced to a 30% by after 8000 μs at 24 kV/cm. For pear PPO a maximal reduction of 48% was noticed after 6000 μs at 22.3 kV/cm. On the contrary, no inactivation effect

of PEF on PPO was noticed by Van Loey et al. (2002). It has to be mentioned that this study is the most complete and important carried out about the effect of PEF treatments on enzymes relevant to food technology. Indeed, their work with oxidoreductases deserves to be mentioned apart. This research group tested the inactivation of peroxidase, polyphenoloxidase, and lipoxygenase using different field strengths (10, 20, and 30 kV/cm), two different pulses widths (5 and 40 μs), two pulse frequencies (1 and 100 Hz), and a number of pulses varying from 1 to 1000, depending on the field strength applied. Within these experimental conditions, they did not find an inactivation higher than 10%. There was only an exception, corresponding to long processing times applied with a low pulse frequency and a high number of pulses. Under these experimental conditions, an inactivation of 64% for lipoxygenase and 21% for polyphenoloxidase were noticed. In any case, the authors mentioned that the significant inactivation of these enzymes under these conditions was not caused by the high-voltage pulses but by an artifact, namely a small off-state current of the IGBT switch. The intensity of the off-state current varied with the voltage level charged on the capacitor. If treatment times are long, inactivation of these metal-containing enzymes would be due to electrochemical reactions with the electrodes surface rather than to the electric field.

Lysozyme: This enzyme is widely distributed and it is found in egg, many mammal tissues and secretions, in latex exudates of some plants, and in some fungi. It is an *N*-acetylmuramidase that hydrolyzes the cell wall of Gram-positive bacteria and therefore it has found application in food technology as a preservative (Belitz *et al.*, 2004). It consists of a peptide chain with 129 amino acids residues and four disulphide bonds. Ho *et al.* (1997) studied the effect of PEF treatments (60 μs at 87 kV/cm) on this enzyme and reported an activation of lysozyme (an increased activity) after PEF treatments.

Lipase: Grahl and Märkl (1996) studied the effect of PEF on lipase in raw milk and reported an inactivation to the 40% of the original activity. Ho *et al.* (1997) also studied the effect of PEF treatment on lipase from wheat germ. After 60 μs at 87 kV/cm, a reduction to a 15% of original activity could be achieved. Bendicho *et al.* (2002c) studied the effect on a commercial lipase from *Pseudomonas fluorescens*. In this case, lipase could be inactivated to a 38% of the original activity in batch treatments and only to an 87% of the original activity in a continuous system. The authors explain this difference on a basis of the difference in the applied voltage.

When working with enzymes from psychrotrophs, low-temperature-inactivation phenomenon has to be taken into account as well as the possibility of contamination with proteases (Owusu *et al.* 1991). Low-temperature-inactivation consists of a quite curious phenomenon: some of the exogenous enzymes from psychrotrophs (mainly proteases and lipases) inactivate faster at mild temperatures than they do at higher temperatures. There is not a clear explanation but proteolysis and conformational changes that lead to an unstable structure have been used to account for this experimental observation.

α-amylase: Ho *et al.* (1997) studied also an amylase from *Bacillus licheniformis*. This enzyme is used as a technological tool to hydrolyze 1.4, α-glucans. After 60 μs at 80 kV/cm, an inactivation to the 15% of the original activity was noticed.

Lactate dehydrogenase: Barsotti *et al.* (2002) studied the effect of PEF treatments on lactate dehydrogenase, a tetrameric enzyme that it is stabilized by electrostatic interactions. The main reason to study this enzyme was not its importance in food science but its sensitivity to treatments as freezing or high-pressure processing, probably due to a dissociation of the tetrameric structure. After 192 μs at 31.6 kV/cm and 30°C, no lactate dehydrogenase inactivation was observed. According to this result, it can be concluded that high-voltage pulses in the experimental conditions used are not able to break the electrostatic interactions that stabilize the tetramer.

Pectinmethylesterase: Pectinmethylesterase (PME) catalyzes the desterification of pectin molecules. De-esterified pectin molecules are able to interact through calcium bridges leading to a cloud loss and phase separation in juices. Stabilization of cloud in juices requires the inactivation

or inhibition of PME. Yeom *et al.* (2000a) investigated the effect of PEF on PME from Valencia oranges. The maximum inactivation achieved was to the 12% of the original activity after 59 μs at 35 kV/cm. These results disagree with those obtained by Van Loey *et al.* (2002), that reported that after 1000 μs at 35 kV/cm residual PME activity was about 90% of the original. In citrus, several PME isoenzymes, that differ in thermostability, have been found. Most of the problems of cloud stability are caused by the thermoresistant isoenzyme which is only about 2–15% of the total PME activity (Snir *et al.*, 1996), varying with cultivar, degree of ripening, and other factors. Juice cloud stability not only depends on total PME activity, but mainly in the inactivation PME fraction with clarifying capacity. According to this, the effectiveness of PEF treatment in cloud preservation will not be determined only by residual PME activity as will be discussed later. Giner *et al.* (2000) decided to investigate the inactivation on PME from tomato by PEF. A high inactivation was achieved (to a 6.2% of the original activity) after 8000 μs at 24 kV/cm.

Proteases: Most of the enzymes used so far to evaluate the effects of PEF on enzymatic activity have been proteases and the variety of results obtained is tremendous: inactivation, no effect, and even activation. Vega Mercado *et al.* (1995a) studied plasmin, an alkaline protease present in bovine milk. Their results were impressive: plasmin activity decreased to a 10% of the original activity after 100 μs at 30–45 kV/cm at 15°C. The same research group investigated the effect of PEF treatments on an extracellular protease from *Pseudomonas fluorescens* M3/6 (Vega-Mercado *et al.*, 1995b, 1997). With this enzyme results obtained varied depending on the treatment medium. Using TSB, a reduction of 80% of protease activity was achieved; if skimmed milk was used instead of TSB, an increase in proteolytic activity was noticed. When treatments were performed in casein–tris buffer, no protease inactivation was found. This variation in the results reported could perhaps be attributed to an autolysis phenomenon, as it will be discussed later. Yeom *et al.* (1999) investigated the effect of PEF on papain. The activity of papain did not change after PEF treatments, but a change in the behavior during storage depending on the PEF treatment applied was reported. The authors also studied conformational changes and the oxidation of the cysteine residue of the active site and concluded that the inactivation that papain suffers during storage after PEF treatments is due to a loss of the α-helix structure and not to an oxidation of cysteine active site. However, inactivation by proteolysis was not ruled out. Bendicho *et al.* (2005) studied PEF treatments on *Bacillus subtilis* protease. In their investigations, changes in enzymatic activity were only observed when PEF treatment was applied using a continuous flow device with coaxial electrodes. If treatment medium was SMUF (simulated milk ultrafiltrate), a slight inactivation was noticed. On the other hand, if PEF was carried out in milk an enhancement in proteolytic activity was found.

None of the previous works has taken into account autolysis to discuss results. Autolysis is one of the most important mechanisms that could inactivate a proteolytic enzyme and a global view of available data obtained PEF effects on proteases could give some clues about its possible role in the inactivation of proteolytic activity by PEF. First of all, in most of the proteases studied, reported results vary from inactivation to activation of the proteolytic enzyme, depending on treatment medium composition. The presence in PEF treatment medium of proteins could protect enzyme from autolysis acting as substrate for the proteases. Another factor to be taken into account is that, in the first PEF equipments, there was a possibility of a poor control or measurement of temperature. A higher temperature could lead to an increase in autolysis rate. Finally, another possibility is that PEF could originate small conformational changes leading to an enhanced proteolytical activity. Anyway, it is necessary to elucidate this possible mechanism in further investigations.

Summarizing the effects on enzymes: The observed effects of PEF on enzymes by different research groups seem to depend on several factors such as the enzyme, the PEF apparatus, PEF treatment conditions, and medium. One of the main problems is that there is not a standardization of PEF equipment, so the experimental conditions largely vary among the different studies, and in some of them there is a lack of details. This is the main reason explaining why it is very difficult to obtain

conclusions from results obtained at very different conditions of field strength, number of pulses, pulse width and shape, and batch or continuous systems. Another important factor that has to be taken into account is temperature control. Sometimes, the effect of temperature has not been considered and this is quite important if the enzymes are not specially thermoresistant (which is common in the experiments performed with PEF and enzymes) and a critical factor when working with proteases, because of the autolysis. It is possible to estimate a theoretical increase in temperature from the total energy input, assuming that all the electrical energy is dissipated as heat, but heat dissipation toward the environment depends mainly on the thermal properties of the materials used in the chamber, and also on its design. The main PEF parameter that seems to affect the stability of enzymes is pulse duration, even more that field strength.

Although there are some reports about the effect of PEF treatments on enzymes, further research is needed if this emerging technology has to be applied to preserve food. The first problem encountered is that, despite the diversity of results reported, it seems clear that the resistance of enzymes to PEF is higher than that showed by microorganisms. Moreover, whereas the mechanisms of vegetative cellular inactivation by PEF are more or less clear, or at least under study, for enzymes there is still a large number of questions without answers. As enzymes are proteins and some protein structures are stabilized by electrostatic interactions, it seems reasonable that PEF should somehow affect their stability, as will be discussed later. This would also apply to those enzymes that have metals as copper or iron in their active site, and therefore prone to be affected by redox reactions. Although some authors have reported changes in conformation, more research is needed to clarify the mechanisms through which enzymes could be activated or inactivated by PEF. Moreover, clarifying whether enzyme inactivation observed after some PEF treatments is not due to PEF itself, but to a thermal effect or electrochemical reactions is very important to fully exploit PEF technology for food preservation.

Another question still to sort out is whether the inactivation level reached is adequate for food preservation or not. Which is the inactivation level that we have to attain to preserve a food from enzymatic deleterious processes? This is a question that food scientists still have to answer in most cases. There is insufficient knowledge on the relationship between residual activity and quality deterioration. Anyway, contrary to microorganisms, enzymes do not multiply in food so the inactivation level required is lower for enzymes. But the inactivation of enzymes achieved with PEF treatments is not sufficient to control deleterious enzymes because in most of the studies, less than a decimal reduction is achieved. For example, it has been established (Eagerman and Rouse, 1976) that for cloud preservation in orange juice it is necessary to achieve two decimal reductions in PME activity (reduce the PME activity to 1% of the original). For PME inactivation by PEF, in the most intense conditions used and with the most sensitive isoenzyme, only a reduction to 12% of the original activity is noticed. So in this case, the inactivation effect, it is not sufficient to control enzymatic activities.

An additional drawback to implement PEF for controlling enzyme activity in foods is that most of the experiments have been performed in model systems. Their properties are very different from those of food systems as fruit juices, egg, or milk. Medium composition is very important because it determines both the parameters of PEF treatments and also the behavior of enzymes.

All these reasons indicate that still much research effort is needed to clarify the effects of PEF on enzymes under various experimental conditions.

3. EFFECT OF PEF ON FOOD CONSTITUENTS

As it has been reviewed in Chapter 4 of this book, it is well demonstrated that in several conditions, PEF is able to efficiently inactivate vegetative cells of microorganisms. However, there is a relative lack of knowledge about the effect of this emerging technology on food components. Next, the information about the effect of PEF on proteins, fats, vitamins, and pigments is presented and

discussed. However, it is noteworthy that the number of research works is scarce and results obtained are sometimes contradictory. A summary of the information available is presented in Table 5.2.

3.1. Proteins

Protein constituents of foods provide, in addition to nutritive value, a desirable textural quality. These properties are determined by protein structure and behavior. The native form of a protein is held together by a delicate balance of forces: hydrophobic, ionic, and van der Waals interactions, hydrogen and disulfide bonds. Each protein has a particular structure that is also maintained by different type of bonds. Native proteins (and enzymes) have at least, three structural levels that can be affected during food preservation treatments. The primary structure is determined by the specific amino acid sequence of the polypeptide chain. The secondary structure is related to the regular arrangements of the polypeptide chains in α-helix, β-sheet, and turns, and it is mainly maintained by hydrogen bonds. The tertiary structure refers to the arrangement of the secondary structure into globular units or domains. This level is stabilized by different types of bonds (hydrophobic, ionic, and van der Waals interactions, hydrogen and disulfide bonds) depending on the protein. Some proteins may also have an additional structural level, the quaternary structure, which is referred to the association of different monomeric subunits.

For protein denaturation and enzyme inactivation the first step that takes place is normally protein unfolding. This phenomenon is considered as reversible, and consists of a conformational change due to a modification of the balance of forces that maintains the native structure. Sometimes, the protein is able to recover its native structure, but in other cases, this structural change is followed by a rearrangement that leads to an inactive structure. After unfolding, some secondary events, that are highly specific for individual proteins, may take place. These events are either covalent changes, which result in chemically modified proteins, or noncovalent changes (Klibanov, 1983), which may lead to an incorrect folding (if they only affect to one molecule) or to aggregation (if several protein molecules are involved).

Protein structure is very dependent on environmental factors such as pH, presence of denaturating agents, soluble solids, or ionic strength, which can affect the delicate equilibrium that maintains the native structure. How PEF treatment can affect protein structure? It seems reasonable that the application of high-voltage pulsed electric fields could ionize some chemical groups and also break electrostatic interactions inside a polypeptide chain or between two monomeric units of protein. Anyway, the investigations performed till now are often descriptive and do not throw light on the possible mechanisms that could operate in protein modification by pulsed electric fields.

As the potential implementation of PEF in food preservation would be mainly related to liquid foods, it is necessary to study possible changes in proteins of liquid food systems as egg or milk. Available data about the effect of PEF on food proteins is mainly focused on egg white proteins (ovoalbumin and egg white) and β-lactoglobulin, the most abundant protein in cow's milk whey and its primary gelling agent.

Ovoalbumin is the major protein of egg white and also the main determinant of gelling properties of egg and its products. It is a phosphoglycoprotein with four thiol groups that are buried within the structure and that become exposed when the protein unfolds. Fernández-Díaz *et al.* (2000) studied the effect of exponential decay pulses (180 μs at 31.5 kV/cm) on ovoalbumin solutions. Their first observation was that ovoalbumin solutions (2% protein content) submitted to PEF treatments showed an increased reactivity of sulphydril groups. Nevertheless, this was a transient change because if ovoalbumin PEF-treated solutions were kept at 4°C during 30 min, thiol groups became less reactive again. According to this observation, PEF treatments would not induce permanent modifications in ovoalbumin. The authors also analyzed the fourth derivates of the UV spectra of native and PEF-treated ovoalbumin and spectra of both proteins were identical. As this method detects structural

changes in the environment of aromatic amino acid residues, this result suggests that no protein unfolding was induced by PEF treatments. A possible hypothesis given by the authors, which could explain why thiol groups became more reactive without changing the structure, is that PEF treatment increased the ionization of SH to S^-. As the thiol groups of ovoalbumin appear to be relatively close to the protein surface, their enhanced ionization could take place without important changes in the protein structure. Taking into account all the results obtained in this research work, it can be concluded that treatment conditions applied do not induce a significant unfolding or aggregation of ovoalbumin.

There are also some reports on the effect of PEF treatments on egg white. Jeantet *et al.* (1999) measured the surface hydrophobicity of egg white proteins and did not find any increase after PEF treatment, suggesting that no protein denaturation occurs. Fernández-Díaz *et al.* (2000) also found that PEF treatments did not induce significant changes in the gelling properties of dialyzed egg white.

Professor Cheftel's research group also studied the effect of PEF treatments on β-lactoglobulin solutions at different protein concentrations (Barsotti *et al.*, 2002). After the application of PEF treatments (up to 260 μs at 30 kV/cm) to β-lactoglobulin solutions with protein concentrations ranging from 2 to 12% w/v, no changes in the fourth derivate of the UV spectra were observed and no changes in PAGE and PAGE–SDS electrophoretic patterns were detected. PEF treatments on higher protein concentration β-lactoglobulin solutions (16.7%) did not induce changes in turbidity and viscosity. According to these results, it can be concluded that PEF treatments did not cause significant β-lactoglobulin unfolding or aggregation. Also, Ma *et al.* (1998) have reported no changes in the electrophoretic patterns of liquid whole egg treated at a selected electric field strength of 48 kV/cm and a maximum treatment time of 120 μs (temperature below 40°C). Even the most heat-sensitive proteins, such as the globulins responsible for the foaming ability of egg white, were unaffected.

Moreover, Li *et al.* (2005) have investigated the effect of processing on bovine immunoglobulin G secondary structure using circular dichroism spectrometry. PEF treatment at 41.1 kV/cm for 54 μs with bipolar pulses did not cause detectable changes in its secondary structure or its thermal stability.

Recently, a new approach to the application of PEF in food technology has emerged. PEF could be used to modify the relationship between structure/function of proteins. This new idea has its basis on the application of long length exponential pulses (ms instead of μs), less number of pulses, and a lower field strength. Perez and Pilosof (2004) studied the effect of PEF treatments on ovoalbumin and β-lactoglobulin. PEF conditions used were the following: exponential decay pulses of 2 ms, field strength was 12.5 kV/cm, and a number between 1 and 10 pulses. It has to be noted that conditions applied in this work are not sufficient to inactive microorganisms. Results obtained both for ovoalbumin and β-lactoglobulin show that PEF treatment can induce protein denaturation that leads to a partial aggregation. PEF treatment also affects the gelling properties of the proteins: enhancing gelation rate of β-lactoglobulin and decreasing that of egg white. The authors also proposed a model to explain the effects of PEF on proteins that involves several possible mechanisms: (1) a polarization of the protein molecule; (2) a dissociation of the quaternary structure by breaking noncovalent bonds; (3) changes in protein structure that lead to the exhibition of hydrophobic and thiol groups that were previously inside the protein core; and (4) if the duration of electric pulse was high enough, the formation of aggregates. Anyway, all the proposed mechanisms have to be proven and further research is needed to clarify this hypothesis.

Apart from the possible effect of long pulses at low electric field strengths described above, the results obtained so far strongly suggest that PEF could be applied to foods at intensities high enough to reduce microbial load without damaging the protein structure and functional properties.

3.2. Fats and Emulsions

Very few studies have been carried out to study the effect of PEF treatments on fats and fat-based foods. Only scattered data are available about the distribution of oil droplets in

Table 5.2. Effect of PEF treatments on foods.

Food	Treatment conditions	Characteristics studied	Comments	Reference
Milk	40 kV/cm, 40 μs, pulses 2 μs width	Physical and chemical properties (not specified)	Nd[a]	Qin *et al.* (1995)
		Sensory evaluation	No difference with heat-pasteurized milk	
	Nonspecified	Whey protein content	Nd[a]	Grahl and Märkl (1996)
		Vitamin A	Nd[a]	
		Vitamin C	90% loss	
		Sensory evaluation	Nd[a]	
	29 kV/cm, 200 μs, exponential decay pulses, 0.8 μs width	Fat globule size and distribution	Nd[a]	Barsotti *et al.* (2002)
	35 kV/cm, 188 μs, bipolar square wave pulses 2.9 μs width	Protein content	Nd[a]	Michalac *et al.* (2003)
		Total solids	Nd[a]	
		Color	Nd[a]	
		pH	Nd[a]	
		Particle size	Nd[a]	
		Density	Nd[a]	
		Electrical conductivity	Nd[a]	
	18.3–27.1 kV/cm, 400 μs, exponential decay pulses	Vitamin retention	Nd[a], except vit C (maximum loss, 30% approx.)	Bendicho *et al.* (2002b)
Yogurt-based products	30 kV/cm, 32 μs, square monopolar pulses 1.4 μs width + mild heat (60°C, 30 s)	Color (L, a, b)	Nd[a]	Yeom *et al.* (2004)
		pH	Nd[a]	
		°Brix	Nd[a]	
		Sensory evaluation	Nd[a]	
Orange juice	35 kV/cm, 59 μs	Pectinmethylesterase activity	90% decrease	Yeom *et al.* (2000a,b) and Ayhan *et al.* (2001, 2002)
		Vitamin C content	Nd[a]	
		Flavor compounds retention	Depending on the compound (100–130%)	
		Browning index	Nd[a]	
		Color (L, a, b)	Nd[a]	
		Particle size	Smaller	
		°Brix	Nd[a]	
		pH	Nd[a]	
	35 kV/cm, 60–87 μs	Flavor compounds retention	95–99%	Qiu *et al.* (1998)
		Vitamin C retention	96–95%	
	30 kV/cm, 240–480 μs, square waveform pulses, 2 μs width	Flavor compounds retention	97–91%	Jia *et al.* (1999)
	80 kV/cm, 40–60 μs, bipolar pulses, 2–3 μs width	Vitamin C retention	97.5%	Hodgins *et al.* (2002)
		Aroma compounds	Nd[a]	
		Pectinmethylesterase activity	92.7% reduction	
	40 kV/cm, 97 μs, pulses 2.6 μs width; maximum temperature 58°C for 5 s	Vitamin C retention	Nd[a]	Min *et al.* (2003a)
		Flavor compounds retention	98–81%, depending on the compound	
		Color (L, a, b)	Higher L value and hue angle	

(*continued*)

Table 5.2. (*Continued*)

Food	Treatment conditions	Characteristics studied	Comments	Reference
		Sensory evaluation	Same rating in color and appearance, but lower rating in texture, flavor, and overall acceptability than untreated control	
Tomato juice	40 kV/cm, 57 μs, bipolar square waveform pulses, 2 μs width; maximum temperature 53.5°C for 5 s	Flavor compounds retention	93–110%, depending on the compound	Min and Zhang (2003) and Min *et al.* (2003b)
		Ascorbic acid content	Nd[a]	
		Non-enzymatic browning degree	Lower than untreated control	
		Color (L, a, b)	Nd[a]	
		Lycopene content	Nd[a]	
		Particle size	Smaller	
		°Brix	Nd[a]	
		pH	Nd[a]	
		Viscosity	Nd[a]	
		Sensory evaluation	Preferred to thermally processed juice (92°C for 90 s)	
Cranberry juice	20–40 kV/cm, 50–150 μs, square waveform pulses, 2 μs width	Anthocyanin content	Nd[a]	Jin and Zhang (1999)
		Color (L, a, b)	Nd[a]	
		Aroma compounds retention	Nd[a]	
Apple juice	22–34 kV/cm, 166 μs, bipolar square waveform pulses, 4 μs width	Color (L, a, b)	Nd[a]	Evrendilek *et al.* (2000)
		Vitamin C retention	Nd[a]	
		Sensory evaluation	Slight preference for untreated juice	
	50–66 kV/cm, 2–16 pulses	Soluble solids	Nd[a]	Zárate-Rodríguez *et al.* (2000)
		pH	Nd[a]	
		Acidity	Nd[a]	
		Color	Lightening	
Fruit beverage	28 kV/cm, 100–600 μs, square waveform pulses, 2 μs width	Vitamin C retention	96–87%	Sharma *et al.* (1998)
		Color (L, a, b)	Nd[a]	
		Protein denaturation	6–7%	
		Viscosity	Nd[a]	
Horchata	20–35 kV/cm, 100–475 μs $T^a < 35°C$	pH	Nd[a]	Cortés *et al.* (2005)
		Total fat	Nd[a]	
		Peroxide index	Nd[a]	
		TBARS (thiobarbituric acid-reactive substances index)	Nd[a]	
		Formol index	Nd[a]	
IgG-enriched soymilk	Up to 41 kV/cm, 54 μs, bipolar square wave pulses	IgG activity	Nd[a]	Li *et al.* (2003)
		Color (L, a, b)	Nd[a]	
		Electric conductivity	Nd[a]	
		°Brix	Nd[a]	
		Viscosity	Nd[a]	
Liquid whole egg	25 kV/cm, 250 μs, bipolar square wave pulses, 2.12 μs width + mild heat (55°C, 3.5 min)	Viscosity	Nd[a]	Hermawan *et al.* (2004)
		Electrical conductivity	Nd[a]	
		Color (L, a, b)	Nd[a]	
		pH	Nd[a]	
		°Brix	Nd[a]	
	35 kV/cm, 20 μs, 2 μs width + 0.15% w/v citric acid	Sensory evaluation	No difference with commercial brand (scrambled eggs); preferred to commercial brand (overall appearance)	Qin *et al.* (1995)

Nd[a]: No difference with untreated control.

emulsions treated by PEF and fat oxidation degree. Barsotti *et al.* (2002) studied the effect of PEF treatments (200 μs, exponential decay pulses, at 29–32 kV/cm) on the fat globules size distribution of various oil-in-water emulsions, determined by Malvern laser granulometry. Despite the fact that the stability of protein-stabilized emulsions is partly charge-dependent, no marked modifications, nor in the size droplet distribution nor in the stability indexes could be detected. The only noticeable effect caused by PEF treatments was the rupture of fat globules aggregates into smaller droplets, in a similar way as anionic detergents such as SDS do. However, the extent of this droplet dispersion effect was very small. These conclusions were obtained in model oil-in-water emulsions stabilized by β-lactoglobulin, and further confirmed in food emulsions, specifically pasteurized half skimmed milk, pasteurized whole milk, and dairy cream (35% fat).

Also, the degree of lipid oxidation seems not to be affected by PEF. Cortés *et al.* (2005) have reported that TBARS index (thiobarbituric acid-reactive substances index) of horchata, which is a fat-rich vegetable beverage typical from Spain (more than 2% fat), did not vary with treatments of 100–475 μs at 20–35 kV/cm. The authors concluded that these treatments did not cause oxidation of fatty matter.

3.3. Vitamins

It is generally acknowledged that PEF treatments do not affect vitamin content and therefore PEF-treated foods should be nutritionally more complete than heat-treated foods. However, these assumptions have been made upon a limited number of results. From the scarce studies published up to date, it can be concluded that most vitamins are not affected by PEF treatments of such an intensity enough to attain a reasonable degree of microbial inactivation. The only vitamin that seems relatively more sensitive is ascorbic acid.

Early studies by Grahl and Märkl (1996) reported that high-energy input PEF treatments (>300 kJ/L) did not affect vitamin A content. However, they reported a 90% destruction of ascorbic acid (vitamin C). The experimental conditions applied were not specified. In any case, this is probably the only study reporting a high degree of loss of ascorbic acid. Bendicho *et al.* (2002a) investigated the retention of various water-soluble (thiamine, riboflavin, and ascorbic acid) and fat-soluble vitamins (cholecalciferol and tocopherol) in milk and simulated milk ultrafiltrate (SMUF) after PEF treatments of up to 400 μs at field strengths from 18.3 to 27.1 kV/cm. Also, the effect of the processing temperature was evaluated (20–25°C vs. 50–55°C). Thermal treatments at 63 and 75°C were also applied for comparison purposes. The content in fat-soluble vitamins and thiamine and riboflavin did not change with neither PEF treatments nor thermal treatments tested. According to results reported by Grahl and Märkl (1996), ascorbic acid content decreased after PEF treatment. However, the percentage of retention of this water-soluble vitamin was much higher, both in SMUF and in milk, than the 10% reported by Grahl and Märkl (1996). For instance, retention of ascorbic acid in milk was 93% after 400 μs at 22.6 kV/cm. This percentage of retention was higher than that determined by the same authors for both low (63°C, 30 min, 49.7% retained) or high (75°C, 15 s, 86.7% retained) heat pasteurization treatments. Under the more severe treatment conditions used in this study (27 kV/cm for 400 μs), ascorbic acid loss was approximately 20%. Similar experimental conditions have been proven to be effective to attain 2 log cycles inactivation of *Listeria monocytogenes* and 4 log cycles inactivation of *Salmonella dublin* in milk.

Loss of ascorbic acid increased exponentially with treatment time at any electric field strength tested, following first-order kinetics. However, there was not a relationship between loss of ascorbic acid and electric field strength. In other words, PEF treatments carried out at 18 kV/cm were more deleterious for this vitamin than treatments performed at 22 kV/cm. In any case, maximum loss of ascorbic acid reported was 30%.

An interesting observation is that ascorbic acid retention seemed to be higher in milk than in SMUF, indicating that protein content of milk may exert a protective effect on this vitamin.

The retention of ascorbic acid has also been studied in orange, apple, cranberry juice, and also a fruit-based drink. Most investigations have also reported either slight reductions or no reductions in the content of vitamin C (Evrendilek *et al.*, 2000; Yeom *et al.*, 2000a; Hodgins *et al.*, 2002; Min *et al.*, 2003a,b). Evrendilek *et al.* (2000) treated apple juice at 34 kV/cm for 166 μs (bipolar pulses of 4 μs length). Under these experimental conditions, they achieved a 4.5-log cycles reduction in the population of *E. coli* O157:H7, and no decrease in the ascorbic acid content. Also, Min *et al.* (2003b) analyzed ascorbic acid content of tomato juice processed at 40 kV/cm for 57 μs, and they concluded that there was no decrease due to PEF treatment.

3.4. Pigments

Color is one of the most important parameters determining food acceptability. It is well known that foods with altered color are rejected by the consumer. Perception of food color by the human eye depends on several factors, which include water content and distribution, occurrence of browning reactions, or light scattering due to fat globules, among others. One of the most relevant parameters in many foods of vegetable and animal origin determining color is the content in natural pigments. Type and nature of natural pigments is quite variable, and so is their stability to preservation procedures. Most investigations about color retention in PEF-treated foods use the Hunterlab measurement system, and scarce data are available on the stability of single pigments. However, data available on both water-soluble and fat-soluble pigments suggest that they are not significantly degraded during PEF treatments.

The concentration of anthocyanins in PEF-treated cranberry juice did not change after treatments of 50–150 μs at 20 and 40 kV/cm (Jin and Zhang, 1999). Min *et al.* (2003b) studied the effect of a PEF treatment (40 kV/cm, 57 μs) in tomato juice on lycopene concentration. Lycopene is a carotenoid pigment responsible for the red color in tomato. PEF treatment did not cause any decrease in the lycopene concentration of tomato samples. Also, the color (Hunter lab color parameters) of a nutraceutical beverage, containing annatto and turmeric as sole colorings, was not modified by a treatment of 28 kV/cm for 200 μs (Sharma *et al.*, 1998).

The influence of PEF treatments in color of various food products is described more extensively below in this chapter.

4. GENERATION OF NEW COMPOUNDS

The safety of PEF processing, specifically the possibility of the electric current generating harmful compounds, has raised some concern. Obviously, before new food preservation procedures get approval from the corresponding regulatory bodies, clear evidence of the safety of the processed foods has to be obtained.

Since the initial development of PEF technology, it has been questioned whether the lethal effect observed on microorganisms was due to the electric field itself or to compounds or ions generated by electrochemical reactions. It is known that many chemically active species can be produced by an electric discharge in the food and by electrode processes, which may decompose the chemical structure of liquids close to the electrode surfaces (electrolysis), eventually producing toxic chemical species, such as oxygen peroxide, hydroxyl radical, or chloride ions. If this was so, toxic species could increase the total lethality of the PEF process on microbes, which is desirable effect, but they

could also cause undesirable chemical reactions affecting quality and, more important, cause harmful effects on the consumers.

Some authors have investigated this topic. Hülsheger and Niemann (1980) suggested that hypochloric acid (HClO), generated from chloride in buffers under the action of an electric field, contributed to the lethality of PEF treatment, but Wouters *et al.* (1999) later demonstrated that the inactivation degree of *Listeria monocytogenes* in buffer systems containing chloride ions was not significantly higher than in buffers without chloride. They argued that the length of the pulse is a critical parameter controlling undesirable electrochemical reactions. In other words, if pulses are short enough, electrochemical reactions have little chance to take place (Morren *et al.*, 2003).

Studies available have shown that undesirable chemical species appear extensively when electric fields are applied in the form of high-voltage arc discharges and needle-shaped electrodes. This is not the case for PEF treatments in most laboratories and pilot-plant scale equipments worldwide. Stainless steel plate–plate geometry electrodes, short duration pulses, and bipolar pulses (Morren *et al.*, 2003) seem to be effective means in controlling the electrolysis reactions.

In summary, available data regarding the possible generation of toxic compounds by PEF treatments is inconclusive, and research in this direction is needed, although it appears that under adequate processing conditions the electrolysis phenomenon is minimum.

5. QUALITY OF PEF-PROCESSED FOODS

This section tries to summarize the information available about various quality parameters of PEF-processed foods, such as color, flavor retention, protein functionality, among others. Potential applications include mainly liquid or semisolid foods which can continuously flow between two electrodes. The use of PEF in solid foods has generally a different objective, which is not the production of a safe and stable food, but the extraction of components or acceleration of processes, and is dealt with in detail in other chapters of this book. Thus, this section is focused on the effects of PEF on constituents and quality parameters of liquid foods.

5.1. Milk

Milk was the first product proposed to be processed by PEF. Many studies have been focused on the inactivation of several pathogenic and spoilage microorganisms, as well as various enzymes of interest. Results regarding microbial inactivation clearly show that PEF could be an adequate alternative to heat pasteurization treatments, since it attains between 3 and 6 log cycles of destruction of most vegetative pathogenic species studied. Concerning enzyme deactivation, results are contradictory, as it has been described in Section 2 of this chapter. Nevertheless, information available suggests that enzyme inactivation in milk is lower than in buffer systems, and that insufficient inactivation of lipases and proteases could be expected. However, enzymatic activity of lipases and proteases, while is the most important factor determining shelf life of sterilized milks, play a minor role in pasteurized milk stability.

Although it is assumed that the small amount of heat generated during PEF should not cause detrimental changes in milk components and properties, little research effort has been done to prove it. There is a lack of documentation about the effect of PEF treatments under different experimental conditions on milk constituents, and conclusions exposed in this section have been taken from scattered investigations.

Qin *et al.* (1995) suggested that milk could be processed at 40 kV/cm for 80 μs, and a maximum temperature of 50°C, rendering a product with a shelf life of 2 weeks in refrigeration. According to

these authors, no apparent changes in the physical and chemical properties of milk were induced. Grahl and Märkl described in 1996 that several vegetative cells could be effectively inactivated by PEF treatments while some components such as vitamin A and whey protein did not undergo changes. Sensory evaluations showed no deterioration of milk. However, experimental conditions were not detailed. The lack of effect of PEF on milk properties was confirmed by Michalac *et al.* (2003). These authors used a PEF treatment consisting of 188 μs (bipolar pulses of 3 μs length) at 35 kV/cm in a continuous bench scale system. Maximum temperature attained by processed milk was 52°C. Color, particle size, total solids content, protein content, pH, electrical conductivity, viscosity, and density analysis showed no differences between PEF-treated and heat-pasteurized (73°C/30 s) milk. However, reduction of the natural microflora of milk was greater in heat-pasteurization (2.7 log vs. 1 log).

Also, distribution of fat globules in milk and cream does not change with PEF treatment, as it has been discussed previously (Barsotti *et al.*, 2002). Vitamins are also preserved after PEF treatments, except ascorbic acid, whose content decreases slightly (Bendicho *et al.*, 2002b). Finally, structural studies on β-lactoglobulin (Barsotti *et al.*, 2002) also support the view that no major modifications are caused by PEF treatments in proteins of milk.

5.2. Juices

Fruit juices are perhaps the most suitable products to be processed by PEF. Currently, juices have to be heat-treated to be commercialized either at refrigeration or at room temperature. Fruit juices can undergo changes in their quality due to microbiological growth, enzymatic activities, and also chemical reactions. Stability is generally assured by the combination of the heat treatment, the acidity of the product, and sometimes, refrigeration temperature. On the other hand, compounds responsible for flavor in juices are heat-sensitive, and thus heat-treated juices are perceived as significantly different from raw juices. For these reasons, PEF has been proposed as a possible advantageous alternative to current heat-processes. Unfortunately, due to the scarce efficacy of PEF against mold ascospores (Raso *et al.*, 1998) and some enzymes, PEF processing is viewed as an alternative to heat treatment for pasteurized, but not for sterilized juices. In other words, the obtained product would normally have to be kept under refrigeration.

Orange, cranberry, apple, and tomato juices have been processed by PEF in bench and/or pilot plant equipments (Qiu *et al.*, 1998; Jia *et al.*, 1999; Jin and Zhang, 1999; Evrendilek *et al.*, 2000, 2001; Yeom *et al.*, 2000a,b; Ayhan *et al.*, 2001, 2002; Hodgins *et al.*, 2002; Min and Zhang, 2003; Min *et al.*, 2003a,b).

Several authors have focused their investigations on orange juice because it is the most consumed one. Characteristics such as °Brix and soluble solids, acid content, flavor, color, nutritional content, or cloudiness are among those that determine citrus juices quality, together with microbiological quality. Very few systematic studies are available in which all the quality parameters are studied. Nevertheless, from studies published to date it can be concluded that any of the quality parameters of orange juice is negatively affected by PEF processes of intensity levels high enough to produce a microbiologically acceptable product. This means 5–6 decimal reductions in the natural flora and a shelf life longer than 3 months under refrigeration. Some particular parameters such as flavor or color seem even to be improved.

Yeom *et al.* (2000a) and Ayhan *et al.* (2001) performed a series of studies about various aspects of orange juice quality comparing untreated samples, PEF-treated samples (35 kV/cm for 59 μs, temperature under 60°C) and heat-treated samples (95°C for 30 s). They also analyzed the maintenance of the quality along the storage under several conditions of temperature and packaging materials. Parameters studied were pectinmethylesterase (PME) activity, vitamin C content, flavor

compounds content, browning index, color (L, a, and b values), particle size (Malvern granulometry), °Brix, and pH. Microbial load decreased from 10^3 cells/mL, mainly yeast, to less than 10 cells/mL. The processed juice kept these microbial counts for at least 110 days, either at 4°C or at 22°C. Simultaneously, PME activity was decreased by 88%. Other authors have reported similar inactivation percentages of PME in orange juice after 40–60 μs at 80 kV/cm (Hodgins *et al.*, 2002). Yeom *et al.* (2002) also reported that the inactivation level of PME treated by PEF in orange juice could not be solely attributed to the heat generated in the sample during processing, but mainly to the electric field itself. Whether a 90% PME average inactivation is enough for a long shelf life product under refrigeration is something that has yet to be determined, as it has been discussed above.

The content in ascorbic acid is almost not affected by PEF treatment. Initial losses, measured immediately after PEF treatment at various electric field strengths and total treatment times, ranged from 0 to 5% (Qiu *et al.*, 1998; Yeom *et al.*, 2000a; Hodgins *et al.*, 2002; Min *et al.*, 2003a). Decreases in the content of this vitamin are much more marked during storage than due to the treatment itself, especially if treated samples are stored in oxygen-permeable containers (Ayhan *et al.*, 2001).

PEF-treated orange juice maintains color attributes similar to those of untreated juices. Browning index (absorbance at 420 nm) and color parameters of PEF-treated juice are not significantly different from the untreated one (Ayahn *et al.*, 2001), and consistently better than those corresponding to heat-treated juices. In most cases L, a, and b parameters of PEF-treated orange juice have been found to be identical to untreated controls, and only occasionally, they have shown brighter and more yellowish color (Ayhan *et al.*, 2002), possibly due to the smaller particle size of PEF-treated juice.

Also, physicochemical parameters such as pH or °Brix were not modified by PEF processing as compared to untreated controls. Changes in pH and °Brix are mainly associated to microbial activity during storage.

Further attention has been dedicated to flavor compounds modification after PEF treatments. This is due to the great importance of flavor in orange juice consumers' acceptability. Orange juice flavor consists of more than 200 compounds of different chemical nature. The headspace content of some hydrophobic compounds involved in flavor has been found to remain similar or even increase after PEF processing (Ayhan *et al.*, 2002). This is the case for limonene, myrecene, valencene, and α-pinene, whose content increased by 18–32% after 59 μs at 35 kV/cm. The authors suggested that this increase was due to a release of these components from their hydrophobic environment by PEF. Polar compounds such as octanal, decanal, linalool, and ethyl butyrate remained unchanged. Also, little losses in flavor compounds, ranging from 0 to 11%, have been reported by several other authors (Qiu *et al.*, 1998; Jia *et al.*, 1999; Hodgins *et al.*, 2002; Min *et al.*, 2003). These losses could probably be minimized through a better control of treatment temperature, that occasionally reaches values close to 60°C in continuous-flow pilot plant equipments.

Cranberry juice is characterized by its special flavor and attractive color. Cranberry juice treated at 40 kV/cm for 150 μs had a microbial load similar to a heat-treated sample (90°C/90 s) (Jin and Zhang, 1999). However, retention of volatile compounds examined by headspace solid-phase microextraction gas chromatography has showed that PEF-treated samples could not be distinguished from untreated controls, indicating that PEF treatment does not alter flavor or aroma profile of cranberry juice. On the contrary, heat-treated samples had a significant altered flavor profile. Also, color of cranberry juice, which is mainly attributable to its anthocyanin content, was not modified by PEF. Anthocyanin pigments are particularly sensitive to heat treatments and oxidations. Whereas thermal treatment significantly reduced the anthocyanin content and modified juice color, PEF treatment did not cause any noticeable change (Jin and Zhang, 1999; Evrendilek *et al.*, 2001). In addition, the

concentration of the anthocyanin pigments decreased in a similar manner in untreated controls and in PEF-treated samples during storage at 4°C for 14 days.

A limited number of studies by Zhang and colleagues have focused on *tomato juice* PEF processing. Tomato juice acceptability is highly determined by flavor and color. With regards to flavor, in tomato juice there are more than 400 volatile compounds derived from fatty acids, amino acids, and carotenoids. Similarly to what it has been described for orange juice, PEF treatment either does not change or even increases the content of some flavor-related compounds (Min and Zhang, 2003). A PEF treatment of 40 kV/cm for 57 μs (maximum temperature 53.5°C) caused an increase in *trans*-2-hexenal to a 110% and in 2-isobutylthiazole to a 108%, with respect to the untreated control. The authors attributed this effect to a decreased particle size of tomato juice during PEF processing and a subsequent increased release of flavor compounds. Also in this case, nor decrease of ascorbic acid has been reported for PEF-treated tomato (Min *et al.*, 2003b), nor changes in °Brix, pH, and viscosity. The absence in viscosity changes has been attributed by the authors to the inactivation of the pectic enzymes during the hot break procedure employed before PEF treatment.

Tomato juice color is one of its most important quality attributes, and depends on factors such as the content in lycopene and the development of browning reactions, among others. PEF-treated tomato juice did not show differences in lycopene content and Hunter *a/b* ratio with respect to untreated juice (Min *et al.*, 2003b). In addition, brown color development was slower than in untreated juice. These data suggest that color of tomato juice is well preserved by PEF processing.

Finally, a similar trend has been observed for *apple juice*. Little, if any, changes in soluble solids, pH, acid content (Zárate-Rodríguez *et al.*, 2000), color, and ascorbic acid content (Evrendilek *et al.*, 2000) have been reported for PEF-treated apple juice.

Some research papers report sensory evaluation of PEF-treated juices, orange and tomato (Min and Zhang, 2003; Min *et al.* 2003a,b). Results from these sensory evaluations show that PEF-treated juices are preferred in terms of color, appearance, texture, flavor, and overall acceptability to heat-treated ones. However, these results, as well as other comparisons that have been mentioned along this chapter, have to be taken with caution since thermal treatments applied for comparison are often quite intense (90–92°C for 90 s). These heat treatments are close to those required for the production of stable products, and in fact, higher microbial stability and enzyme deactivation is reported, as compared to PEF treatments. More research is needed to determine the sensory acceptability of PEF-treated juices.

In summary, despite some aspects still need further research, fruit juices can be processed by PEF with substantial reduction of the natural microflora and endogenous enzymatic activity, without major changes in essential quality parameters such as color, flavor, cloudiness, and ascorbic acid content. This makes fruit juices one of the most attractive and suitable products to be processed by this technology.

5.3. Egg Products

Egg products, liquid whole egg, egg white, or egg yolk, are normally heat-treated and distributed frozen, dehydrated, or refrigerated. Heat pasteurization is required to guarantee the inactivation of pathogenic microorganisms such as *Salmonella*, which are common contaminants of these foods. The effects of heat on egg constituents are especially harmful because egg products are used as ingredients in the manufacturing of many other foods, due to their foaming, emulsifying, and gelling properties, among others. Therefore, the maintenance of their functional quality, besides their microbiological quality, is essential. Most of the functional properties of egg and egg products rely on their proteins, which are especially thermosensitive. Thus, nonthermal methods that guarantee the microbiological

safety and stability of egg and derivatives have been sought for years. PEF is one of the procedures that have been proposed. The first difficulty encountered for the application of PEF treatments on egg and egg derivatives is the high conductivity of these foods. Most PEF equipments currently available require samples of low conductivity to obtain electric field strengths of high intensity, therefore some of the research works published have been performed with egg products with reduced salt content, through dialysis or ultrafiltration.

As described before in this chapter, most authors have reported no protein coagulation after different PEF treatments in liquid whole egg or egg white (Jeantet *et al.*, 1999; Fernández-Díaz *et al.*, 2000; Ma *et al.*, 2001). With regards to other quality parameters of egg and egg products, very little is known. Qin *et al.* (1995) reported that liquid whole egg with 0.15% citric acid processed at 35 kV/cm for 20 μs and at a maximum temperature of 45°C was preferred over a commercial brand in an acceptance test. Also, scrambled eggs prepared with PEF-treated were not distinguished from a control in a triangle test. Hermawan *et al.* (2004) did not detect significant changes in the viscosity, °Brix, and color parameters (L, a, and b), between untreated liquid whole egg controls and samples treated by PEF (25 kV/cm, 250 μs) plus a following heat treatment at 55°C for 3.5 min. These treatment conditions were chosen to obtain a product with a long shelf life in refrigeration temperatures (more than 60 days) since PEF alone resulted insufficient. It is noteworthy that the combination of PEF processing with moderate heat treatments and/or antimicrobial substances has been suggested by various researchers as the most suitable alternative (Calderón-Miranda *et al.*, 1999; Hermawan *et al.*, 2004; Jeantet *et al.*, 2004) to completely assure the safety of the product with regards to pathogenic microorganisms such as *Salmonella* or *Listeria*.

From these data, it can be concluded that PEF processing is unlike to cause any detrimental effect either on egg protein functionality or color, viscosity, flavor characteristics, proven treatments are carried out at low temperatures. However, information available is scarce and more research effort is needed to fully characterize possible changes in egg product treated by PEF, especially in combination with moderate heat treatments.

5.4. Other Foods

Some other liquid foods and solid foods have been tested for PEF processing.

The maintenance of the immunoactivity of bovine IgG in soymilk subjected to PEF and thermal treatments was studied by Li *et al.* (2003). PEF treatments (41 kV/cm, 54 μs) resulted in no significant loss of antigen-binding activity, whereas thermal treatment yielded 86% decrease. It is noteworthy that both procedures (PEF and thermal treatment) were equivalent in terms of natural flora destruction (5.1–5.3 logs). No changes in color parameters (L, a, and b), electric conductivity, °Brix, or viscosity were detected in PEF-treated milk at various electric field strengths from 26.0 to 38.4 kV/cm, with respect to the untreated controls. This opens the possibility of producing functional foods processed by PEF with better characteristics than those of heat-pasteurized ones.

Also, the possible application of PEF to preserve horchata has been explored (Cortés *et al.*, 2005). Horchata is a beverage made up of tiger nut, which is characterized by a short shelf life of only 48 h. It is rich in starch; consequently it cannot be heated above 72°C to prevent gelation. Shelf life of horchata could be extended with either no fat oxidation or free amino acids release, and lower peroxidase activity than the untreated controls.

The effect of combined mild heat and PEF treatments (60°C/30 s plus 30 kV/cm/32 μs) on physical and sensory characteristics of high-viscosity yogurt-based products has been studied by Yeom *et al.* (2004). Color parameters, °Brix, and pH values of treated samples were identical to those of untreated controls. Sensory evaluation detected no changes in appearance, color, texture, flavor, and overall acceptability between untreated and treated samples.

6. CONCLUDING REMARKS

As it has been exposed along the chapter, there are some inconsistencies regarding the effect of PEF on food constituents, mainly on enzymatic inactivation, probably related to different experimental conditions among laboratories. Further research effort is required to clarify the degree and mechanisms of inactivation of enzymes by PEF before this technology can be implemented for food preservation purposes.

With regards to the effect of PEF treatments on quality parameters of liquid foods, and despite a wider range of experimental conditions and proper comparison with heat-treated and untreated foods would be desirable, it can be concluded that PEF, applied at treatment intensities to obtain pasteurized-like products, exerts a small impact, if any, on protein stability, fat globules distribution, vitamin content, color, flavor, general appearance, and most of the quality parameters of milk, juices, egg products, and some other foods. Juices and other particularly sensitive liquid food seem most adequate for PEF processing.

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CHAPTER 6

EXTRACTION OF INTERCELLULAR COMPONENTS BY PULSED ELECTRIC FIELDS

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1. INTRODUCTION

During the last decades there was observed a growing interest in electric field application for treatment of food and agricultural raw materials. In early studies of Russian researchers the electrical treatment was applied for intensification of the process of juice extraction from fruits and vegetables (Flaumenbaum, 1949), for sugar diffusion from beet (Zagorulko, 1958), and as a promising method of processing vegetable raw materials, meat, and fish (Kogan, 1968; Matov and Reshetko, 1968; Rogov and Gorbatov, 1974). The evident advantages of electrical treatment applications in the food industry are as follows: the method is simple and does not require any complex and expensive equipment; this is an express process and it can be finished within a short period of time; application of the AC electrical fields with industrial parameters is quite possible; the method allows material processing without any food quality deterioration, in particular, as compared with traditional thermal processing methods; the method can be easily applied in a combined mode, as supplementary to any pressing, thermal or microwave treatment, etc.

Recently, the interest in application of pulsed electric fields (PEF) for food processing has revived. The PEF treatment was shown to be very effective for inactivation of microorganisms, increasing the pressing efficiency and enhancing the juice extraction from food plants, and for intensification of the food dehydration and drying (Gulyi *et al.*, 1994; Barbosa-Cánovas *et al.*, 1998; Barsotti and Cheftel, 1998, 1999; Estiaghi and Knorr, 1999; Vorobiev *et al.*, 2000, 2004; Bajgai and Hashinaga, 2001; Bazhal *et al.*, 2001; Taiwo *et al.*, 2002). A strong electric field causes electroporation of cells, an increase in their permeability, and even disruption of their structural integrity, which is possible in certain cases (Zimmermann, 1975, 1986). The nonthermal permeabilization and damage of cellular membranes in plant tissues can be achieved at moderate electric fields of 500–1000 V/cm and short treatment times of 10^{-4}–10^{-2} s (Fincan and Dejmek, 2002; Lebovka *et al.*, 2002). Nowadays, the available high-voltage pulsers make promising the industrial implementation of the PEF treatment of plant foods. This method of processing can be a real alternative to traditional thermal, osmotic, or microwave processing techniques, because it offers products of fresh-like quality and highest purity.

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However, there exist some restrictive factors, which hinder wide practical implementation of PEF-processing technologies in food industry. The effectiveness of PEF treatment depends on type of a raw material, its quality, and pretreatment procedure (e.g., mild heat or osmotic pretreatment). The degree of the PEF-induced damage in real foods is controlled by the PEF treatment protocol (pulse intensity E and duration t_i, number of pulses n, pulse shape) and process parameters (temperature T, pH and conductivity σ of media, textural properties and porosity of tissues, geometry and size of sliced particles, etc.). Finding of the optimal parameters of PEF treatment allowing to ensure high quality of the treated products at minimum power consumptions still remains a complicated technical problem.

This chapter presents the short review of recent works on the problem of PEF application for the treatment of cellular tissues.

2. THEORETICAL ASPECTS OF PEF INFLUENCE ON THE BIOLOGICAL MATERIALS

2.1. Disintegration Index and Characteristic Damage Time

The main aim of the PEF treatment of biological material lies in destruction of cells, their plasmolysis, and increase of the intracellular liquid release from the cells.

For a cellular tissue or a food material, the PEF application results in increase of the electric conductivity, permeability of the whole sample, and solute local diffusivity. The PEF treatment removes the cellular turgor component of the texture and exerts an estimable effect on the viscoelastic properties of plant tissue (Fincan and Dejmek, 2003; Lebovka *et al.*, 2003). The extensive studies showed (Knorr *et al.*, 1994, 2001; Jeyamkondan *et al.*, 1999; Vorobiev *et al.*, 2004) that the material damage degree is controlled mainly by the electric field strength E and the treatment time t_{PEF} (which is a product of pulse duration t_i and number of pulses n).

The typical time dependence of a material property of a tissue (electrical conductivity, elastic module, diffusion coefficient, etc.) is presented schematically in Fig. 6.1. It is useful to introduce,

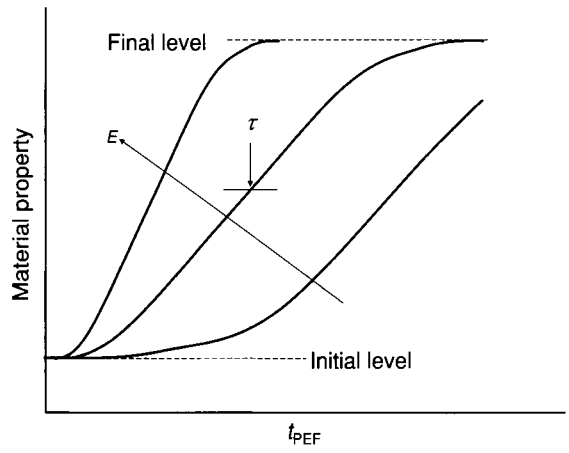

Figure 6.1. Schematic dependence of tissue property (e.g., electrical conductivity, diffusion coefficient, etc.) versus time of the PEF treatment. The final level of material property corresponds to high time of PEF treatment and maximal tissue damage. Here, τ is the characteristic damage time defined in the text, and the arrow shows direction of increase of the electric field strength E.

for purposes of PEF treatment time estimation, the characteristic damage time τ, which is defined as a time needed for attaining a half of the biological material damage ($Z/2$) (Bazhal *et al.*, 2003).

The basic intricacy in development of the optimal PEF treatment procedure is related with the absence of any simple relation between the degree of material damage and PEF-processing protocol, temperature, and biological material characteristics (Vorobiev *et al.*, 2004).

2.2. Selective Concentration of the Electric Fields on Membranes in Biological Materials

Electric field selectively influences the structure of the biological membranes inside the solid water-saturated biological materials. Because of very low electrical conductivity of a membrane material as compared with a surrounding liquid inside the biological tissue, the electric field appears to be concentrated mainly on the membranes.

As for an idealized biological cell of a spherical geometry (Fig. 6.2), the transmembrane potential depends on the angle θ between the external field E direction and the radius–vector on the membrane surface (Schwan, 1957):

$$u_m = 0.75 f d_c E \cos\theta. \tag{6.1}$$

Here, d_c is the cell diameter, and f is a parameter depending on electrophysical and dimensional properties of the membrane, cell, and surrounding media. If we assume a very small relative membrane width $d_m \ll d_c$, the f parameter can be estimated as (Kotnik *et al.*, 1998; Kotnik and Miklavcic, 2000):

$$f \approx (1 + \tfrac{\lambda}{4}(2 + \sigma_c/\sigma_e))^{-1},$$

where $\lambda = \sigma_m d_c/\sigma_c d_m$, and σ_m, σ_c, σ_e are electrical conductivities of the membrane, intracellular, and intercellular media, respectively.

In dilute suspensions of cells and at typical values of parameters $\sigma_m \approx 5.0 \times 10^{-7}\Omega^{-1}m^{-1}$, $\sigma_c \approx \sigma_e \approx 2.0 \times 10^{-1}\Omega^{-1}m^{-1}$, $d_m \approx 5 \times 10^{-9}$ m, $d_c \approx 2 \times 10^{-4}$ m, $\lambda \approx 0.1$ (Kotnik *et al.*, 1998), the value of f is close to 1 ($f \approx 0.93$).

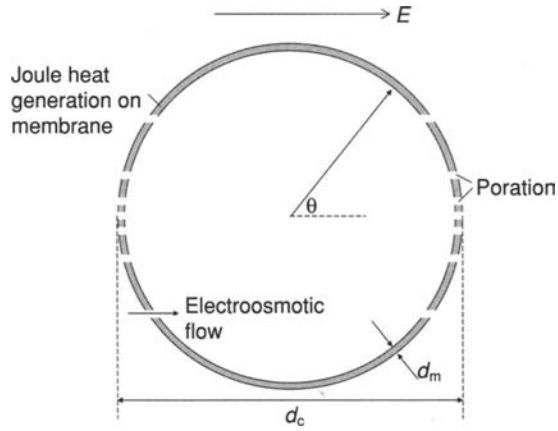

Figure 6.2. A biological cell in the external electric field. Some mechanisms influencing the structure of the cell are depicted here. See the text for details.

Intensity of the electric field generated inside the membrane is $E_m = u_m/d_m$ and the electric field enhancement factor k_m may be defined as

$$k_m = E_m/E \sim d_c/d_m \sim 10^5.$$

Because of selective concentration of the electric fields on membranes, the membrane structure alteration and its damage may occur. Many experimental works evidence that the critical transmembrane potential of the membrane damage is of order $u_m \sim 1$ V (Weaver and Chizmadzhev, 1996), which corresponds to the critical electric field intensity generated inside the membrane of order $E_m \sim 10^8$ V/m. The PEF treatment with short pulses may cause damage of biological cells without noticeable heating of the media. So, such mode of treatment may be nonthermal, with small power consumption.

2.3. Main Mechanisms of Membrane and Cell Damage in External Electric Fields

Causes of the membrane structure alterations and damages of cells in the external electric fields may be different.

For explanation of the selective damage of membranes, the theories of electroporation, electromechanical, electrohydrodynamical, viscous-elastic, electrothermal, and electroosmotic instabilities were proposed (For a review see, Ho and Mittal, 1996; Weaver and Chizmadzhev, 1996). A cell may be damaged owing to the direct rupture of membrane resulting from formation of large pores, or through Joule overheating of the membrane surface. The second possibility of cell lysis may be related to chemical imbalances caused by the enhanced transmembrane transport throughout the membrane pores (Fig. 6.2). Here, some mechanisms of the cellular structure damage under the PEF treatment should be considered.

2.3.1. Electroporation of Membrane

The popular theory of membrane electroporation (Weaver and Chizmadzhev, 1996) supposes that an external electric field stimulates creation and growth of pores in a membrane. The energy of pore formation is

$$W(r) = -\pi\gamma^* r^2 + 2\pi\omega r,$$

where $\gamma^* = \gamma(1 + (u_m/u_0)^2)$, γ and ω are surface and line tensions of a membrane, respectively, $u_0 = \sqrt{2\gamma/(C_m(\varepsilon_w/\varepsilon_m - 1))}$ is a voltage parameter (the dimension of u_0 is voltage), C_m is the specific capacitance of a membrane, ε_w, ε_m are the relative dielectric permittivities of the aqueous phase and of the membrane, respectively.

The typical values of lipid membrane parameters are $\gamma \approx 2 \times 10^{-3}$ N/m, $\omega \approx 1.69 \times 10^{-11}$ N, $\varepsilon_w \approx 80$, $\varepsilon_m \approx 2$, $C_m \approx 3.5 \times 10^{-3}$ F/m^2 at temperature $T = 298$ K (Lebedeva, 1987) and $u_0 \approx 0.17$ V.

The function $W(r)$ is a parabolic barrier and the complete damage of a membrane occurs when the pore radius r exceeds certain critical value r_c. As it follows from the condition of maximum pore formation energy ($(\partial W/\partial r)_{r=r_c} = 0$), the critical pore radius is $r_c = \omega/\gamma^*$ and activation energy of such pore formation is $W_a = W(r_c) = \pi\omega^2/\gamma^*$. Both r_c and W_a increase as the field intensity increases. The equilibrium density of pores in a membrane can be estimated as $C_p \approx \exp(-W_a/kT)$, where $k = 1.381 \times 10^{-23}$ J/K is the Boltzmann constant, T is the absolute temperature.

The PEF parameters (field strength E, pulse duration t_i, and number of pulses n) can influence both the degree of membrane destruction or its structural alteration, and the density of pores in a membrane (Rols and Teissie, 1998; Gabriel and Teissie, 1999).

The lifetime, or the characteristic damage time τ of a membrane can be estimated on the basis of the transient aqueous pore model (Weaver and Chizmadzhev, 1996):

$$\tau(u_m) = \tau_\infty \exp \frac{W/kT}{1 + (u_m/u_0)^2}, \tag{6.2}$$

where $W = \pi\omega^2/\gamma$, τ_∞ is the parameter ($\tau \to \tau_\infty$ in the limit of very high electric fields). For the lipid membranes, $W/kT \approx 109.04$, $u_0 \approx 0.17$ V, and $\tau_\infty \approx 3.7 \times 10^{-7}$ s at $T = 298$ K (Lebedeva, 1987).

The following values can be obtained from above estimations: $r_c \approx 10$ nm, $C_p \approx 4.6 \times 10^{-48}$, $\tau \approx 10^{41}$ s (the membrane is practically stable) at $u_m = 0$ V and $r_c \approx 0.28$ nm, $C_p \approx 4.6 \times 10^{-2}$, $\tau \approx 7.9 \times 10^{-6}$ s at voltage $u_m \approx 1$ V. In most cases $u_m \approx 1$ V is high enough to cause an irreversible damage of a membrane.

2.3.2. Joule Overheating of the Membrane Surface

The electric current flow in a conductive media generates the Joule heat and warms up the medium. The medium temperature increase is proportional to the time t and the current density through the media j:

$$\Delta T = j^2 t/\sigma\rho C,$$

where $j = \sigma E$, and σ, ρ, C are the mean specific electrical conductivity, density, and specific heat of the medium, respectively.

If we take for estimation purposes the values typical for a biological tissue, namely, $C \approx 3$ kJ/kg K, $\rho \approx 10^3$ kg/m^3, and $\sigma \approx 0.1$ $(\Omega m)^{-1}$ as the specific electrical conductivity of a tissue (Lebovka *et al.*, 2000b) the temperature rise ΔT at the electric field strength $E = 100$ V/cm will be 3.3 K/s.

In hypothetical approximation of the absence of any thermal diffusion, the ratio of the local temperature rise in a membrane ΔT_m and in surrounding media ΔT_c is

$$\Delta T_m/\Delta T_c = \sigma_c\rho_c C_c/\sigma_m\rho_m C_m \sim \sigma_c/\sigma_m \sim 10^5,$$

where subscripts m and c correspond to the membrane and to surrounding cellular media, respectively.

So, it can be concluded that membranes are the main Joule heating elements in the cellular systems and the local temperature rise on a membrane is very high

$$\Delta T_m = (\sigma E)^2 t/\sigma_m\rho_m C_m, \tag{6.3}$$

and the external electric field may cause the thermal damage of a membrane (Zagorulko, 1958; Rogov and Gorbatov, 1974).

But heat diffusion effectively ablates the Joule overheating of the membrane surface. The local temperature rise on a membrane in the course of heating ΔT_m averages over the distance $d_T \approx \sqrt{\chi t}$, where $\chi \approx 10^{-7}$ m^2/s is the thermal diffusivity. So, actual temperature rise on a membrane is

$$\overline{\Delta T_m} \approx d_m \Delta T_m/d_T \tag{6.4}$$

and it is much smaller than ΔT_m (Lebovka *et al.*, 2000b).

The temperature rise on a membrane surface after the PEF treatment can be calculated from Eqs. (6.3) and (6.4). For example, if we take electric field strength $E = 500$ V/cm and treatment duration $t = 0.01$ s, and putting for estimation purposes $\sigma \approx 0.1(\Omega m)^{-1}$ in a tissue, and $C_m \approx 2.5$ kJ/kg K, $\rho_m \approx 10^3$ kg/m^3, $\sigma_m \approx 5.0 \times 10^{-7}(\Omega m)^{-1}$, $d_m \approx 5 \times 10^{-9}$ m in a membrane, we get $\Delta T_m \approx 2 \times 10^5$ K (Eq. (6.3)), but actual temperature rise on a membrane is $\overline{\Delta T_m} \approx 26$ K (Eq. (6.4)). So, during PEF treatment the membrane overheating arises, but it is not very high. At the same

time the temperature rise in a tissue media is $\Delta T_c \approx 0.8$ K. The more detailed calculations of the temperature spatial distributions near the membrane in the course of PEF treatment may be found elsewhere (Lebovka *et al.*, 2000b).

2.3.3. Electroosmotic Transport Through the Membrane

Electroosmosis is a phenomenon of the directed electrolyte flow near the charged surface in a pore under the external electric field effect (Hunter, 1981). The linear velocity of electroosmosis is directly proportional to the electric field intensity E

$$V = \mu E,$$

where $\mu = \varepsilon\varepsilon_0\zeta/\eta$ is the electrophoretic mobility, ζ is zeta potential of the pore surface, $\varepsilon\varepsilon_0$ is the permittivity, and η is the electrolyte viscosity inside the pore.

Putting $\varepsilon\varepsilon_0 \approx 80\varepsilon_0 \approx 7.1 \times 10^{-10}$ F/m and $\eta \sim 0.001$ Pa (data for the water at 20°C) and $\zeta \approx 10$ mV (for the surface with low charge), we get $\mu \approx 10^{-8}$ m^2/Vs. The electro-osmotic studies of porous media are usually carried out in low electric fields of order of 1 V/cm, when the electro-osmotic transfer velocity is rather small and makes $V \approx 10^{-6}$ m/s (Alekseev and Ovcharenko, 1992; Tikhomolova, 1993), so this process is rather long. The electroosmosis phenomenon can be used in practical applications for moisture transport in the water-saturated biological materials, their dehydration, and dewatering (Orsat *et al.*, 1996; Banerjee and Law, 1998). At PEF treatment of the biological materials, the critical (sufficient for electroporation) electric field intensity generated inside the membrane is rather high $E_m \approx 10^8$ V/m and the velocity of the electroosmotic flow through the membrane pore is very high also and is of order $V \approx 1$ m/s. So, electroosmosis can be the dominant mechanism, which controls the fast transmembrane exchange (Dimitrov and Sowers, 1990), causes chemical imbalances and enhances lysis of the cells.

2.3.4. Distinctness of the Cell Damage

Effectiveness of the previously discussed membrane damage mechanisms may depend on temperature and PEF treatment protocol. The biological membranes are complex aggregates formed by lipid species, protein channels, and pumps and they display temperature phase transitions within the temperature range of 20–55°C (Exerova and Nikolova, 1992; Mouritsen and Jørgensen, 1997). In the vicinity of phase transition, the fluctuations of the thermodynamical properties are high, and it facilitates origin of pores in the membrane structure.

Because of the temperature dependence of linear and surface tensions, heat capacity, compressibility, and elasticity of membranes (Heimburg, 1998) the electroporation and PEF-induced damage can be very sensible to the structural changes in membranes. It is known also that in a single membrane the breakdown voltage appreciably decreases in the temperature range of 20–50°C (Zimmermann, 1986). So, Joule overheating of the membrane surface, or tissue treatment at temperatures higher than the ambient temperature can enhance electroporation (primary damage processes) or electroosmotic transfer through the pores (secondary damage processes). But physical mechanisms of the thermal effects influencing electroporation are not well understood yet (Weaver and Chizmadzhev, 1996).

The cell's damage mechanisms are even more complex than those of membranes. Equation (6.1) predicts the transmembrane potential u_m distribution over the surface of a biological cell. The highest drop of the potential occurs at the cell poles, it decreases to zero at $\theta = \pm\pi/2$ and the damage probability is maximal at the cell poles. Therefore, there exists heterogeneity of the cell surface damage degree.

The mean characteristic damage time $\tau_c(E)$ of a single cell effected by an external electric field E may be estimated as (Lebovka *et al.*, 2002):

$$\tau_c^{-1}(E) = \frac{1}{2}\int_0^{\pi} d\theta \sin\theta/\tau_m(u_m(E,\theta)), \tag{6.5}$$

where $\tau_m(u_m(E,\theta))$ is determined from Eqs. (6.1) and (6.2).

A more detailed theoretical analysis of $\tau_c(E)$ behavior can be found in (Lebovka *et al.*, 2002).

Moreover, the biological cells always show some distribution of the cell sizes and geometries. The value of u_m is proportional to the cell diameter d_c (Eq. (6.1)), and larger cells get damaged before smaller ones. The value of f in Eq. (6.1) can also deviate noticeably from 1 for biological tissue cells, and it depends on the degree of tissue damage (Lebovka *et al.*, 2000a). All these factors can influence mechanisms and kinetics of the damage of cells induced by PEF treatment (Lebovka *et al.*, 2002; Lebovka and Vorobiev, 2004).

2.4. PEF-Induced Structural Changes in a Cellular Tissue

The biological tissues have a complex structure and their material properties are spatially dependent and highly inhomogeneous. The density, porosity, electrical conductivity, and thermal properties of the fresh tissues can vary substantially. For example, the apple tissue bulk porosity is high enough and falls within the interval of 14–25%, but it is small for many other fresh plant materials and makes 4% for carrot and 2% for potato (Karathanos *et al.*, 1996). Usually, the outer parenchyma porosity is higher than that of the inner parenchyma (Mavroudis *et al.*, 1998) and this may cause a gradient of the electrical conductivity inside the sample.

The cell damage effectiveness is determined by the local electric field $E = E_{loc}$ near the cell. Distribution of the local electric fields inside the inhomogeneous material is a complex function of electrical properties of material constituents, porosity, and structure (Sahimi, 1994) and it changes in the course of PEF treatment.

2.4.1. Electrical Conductivity of PEF-Treated Tissues

The popular methods of experimental estimation of the material damage degree or disintegration index Z are based on electrical conductivity measurements. The conductivity disintegration index Z_σ can be defined as (Rogov and Gorbatov, 1974)

$$Z_\sigma(t) = \frac{\sigma(t) - \sigma_i}{\sigma_d - \sigma_i}, \tag{6.6}$$

where $\sigma(t)$ is the measured electrical conductivity at low frequency (~1 kHz) and the subscripts "i" and "d" refer to the conductivities of intact (initial level) and totally destroyed (final level) materials, respectively.

Application of Eq. (6.6) gives $Z_\sigma = 0$ for an intact tissue and $Z_\sigma = 1$ for a disintegrated material. We need the value $\sigma_f = \sigma(t \to \infty)$ in order to use this definition. Sometimes, the value of σ_f may be approximated by the electrical conductivity of the maximally destroyed material measured for samples after their freeze-thawing treatment (Lebovka *et al.*, 2002; Bazhal *et al.*, 2003).

Another definition of the conductivity disintegration index Z_σ (Angersbach *et al.*, 1999):

$$Z_\sigma(t) = \frac{K\sigma(t) - \sigma_i}{\sigma_{i,\infty} - \sigma_i},$$

where $K = \sigma_{i,\infty}/\sigma(t)_\infty$, includes the conductivities of the treated ($\sigma(t)_\infty$) and intact ($\sigma_{i,\infty}$) tissues measured at high frequency of order 50 MHz.

It is possible to introduce the diffusivity disintegration index Z_D, based on diffusion coefficient measurements in PEF-treated biological materials (Jemai, 1997; Jemai and Vorobiev, 2001)

$$Z_D(t) = \frac{D(t) - D_i}{D_d - D_i},$$

where $D(t)$ is the measured apparent diffusion coefficient and the subscripts "i" and "d" refer to the values for intact and totally destroyed material, respectively.

Unfortunately, the diffusion technique is indirect, it is invasive for biological objects, and it may impact the structure of the sample (Vorobiev *et al.*, 2004).

The experimentally observed curves of $Z_\sigma(t)$ are qualitatively similar to those depicted in Fig. 6.1, and empirical time dependencies of $Z_\sigma(t)$ can be approximated satisfactory by the simple transition function (Bazhal *et al.*, 2003)

$$Z_\sigma(t) \approx \frac{1}{1 + (t/\tau)^k},$$

where k is an empirical parameter, and $Z_\sigma = 1/2$ at $t = \tau$.

The main weakness of the experimental methods used for damage degree or disintegration index Z estimation is the fact that the relation between Z and Z_σ or Z_D is not linear and in fact is unknown. The direct method for estimation of the biological tissue damage degree Z is not developed as yet. It was hypothesized (Lebovka *et al.*, 2002) that Z and Z_σ may be related through Archie's equation

$$Z_\sigma \approx Z^m, \tag{6.7}$$

where m is an empirical parameter (Archie, 1942), which may depend on the ratio of conductivities σ_d/σ_i, the porous space configuration and the connectivity of phases inside the cellular material (Glover *et al.*, 2000).

Archie's exponent is roughly estimated as $m = 3/2$ for randomly spaced spherical cells, $m = 1$ for needle-shape spheroids oriented along the electric field and $m = 2$ for needle-shape spheroids oriented perpendicularly to the electric field (Peters *et al.*, 2001).

The relation between Z and Z_σ can be also estimated on the basis of an effective media model for electric conductivity σ calculation. The structure of a PEF-treated tissue is simulated by a two-phase composite material, where the low conductivity phase with the electrical conductivity σ_i corresponds to the intact tissue and the high conductivity phase with the electrical conductivity σ_d corresponds to the damaged tissue. In this case, the volume fraction of the high conductivity phase (or regions with damaged tissue) corresponds to the tissue damage degree Z. The existing models of the PEF-treated plant tissues account for electrophysical properties of the intact and ruptured plant cells, including plasma and vacuole membranes, and intra- and extracellular compartments (Angersbach *et al.*, 1999; Lebovka *et al.*, 2001).

The generalized effective media (GEM) equation based on the percolation theory (Stauffer and Aharony, 1992) can be successfully used for prediction of the effective electric conductivity of a two-phase composite media σ (McLachlan, 1989)

$$\frac{(1 - Z)(\sigma_i^{1/\theta} - \sigma^{1/\theta})}{\sigma_i^{1/\theta} + [(1 - Z_p)/Z_p]\sigma^{1/\theta}} + \frac{Z(\sigma_d^{1/\theta} - \sigma^{1/\theta})}{\sigma_d^{1/\theta} + [(1 - Z_p)/Z_p]\sigma^{1/\theta}} = 0, \tag{6.8}$$

where θ is the critical exponent of electrical conductivity, and Z_p is the percolation threshold.

It was demonstrated that GEM equation nicely fit a large amount of existing experimental data for different types of objects (McLachlan, 1989). For three-dimensional random systems the

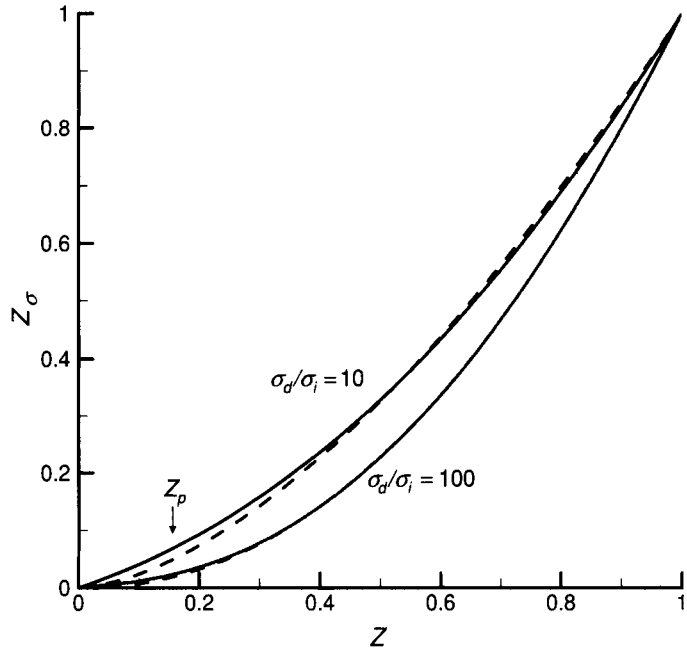

Figure 6.3. Conductivity disintegration index Z_σ versus tissue damage degree Z estimated from GEM equation (6.8) at different σ_d/σ_i ratios (solid lines). Dashed lines correspond to the least square fitting of the calculated relations $Z_\sigma(Z)$ to Archie's equation (6.7).

percolation volume fraction Z_p is approximately 16% and theoretical value of θ is 2 in a three-dimensional case (Stauffer and Aharony, 1992; Kovacik, 1998). These values were put into Eq. (6.8) and the relation of Z_σ versus Z was estimated using Z definition from Eq. (6.6) as shown in Fig. 6.3 (solid lines). Dashed lines correspond to least square fitting of the calculated data to the Archie's equation (Eq. (6.7)). The calculations give $m = 1.62$ at $\sigma_d/\sigma_i = 10$ and $m = 2.13$ at $\sigma_d/\sigma_i = 100$. Note that ratio of conductivity of a PEF-damage tissue to the conductivity of an intact tissue σ_d/σ_i is of order 10–20 for apple, carrot, and potato tissues (Angersbach *et al.*, 1999; Lebovka *et al.*, 2000a). The experimentally estimated index m falls within the range of 1.8–2.5 for apple, carrot, and potato tissues (Lebovka *et al.*, 2002), so we see that the theory predicts the values of m quite satisfactory.

2.4.2. Characteristic Damage Time and Optimization Criteria

There is no simple method for theoretical estimation of the characteristic damage time of cells in the biological tissues, either for a membrane (Eq. (6.2)), or for a single spherical cell (Eq. (6.5)). The observed experimental dependencies of the characteristic damage time τ versus electric field strength E for a potato tissue, which are presented in Fig. 6.4, are typical for different fruit and vegetable tissues (Lebovka *et al.*, 2002; Bazhal *et al.*, 2003).

The characteristic electrical damage time τ decreases with increase of both temperature T and electric field strength E. Lebovka *et al.* (2005) proposed the following empirical equation for description of the experimental data of characteristic electrical damage time $\tau(E,T)$ in a cellular tissue:

$$\tau = \tau_\infty \exp \frac{W/kT}{1 + (E/E_0)^2}. \tag{6.9}$$

Here, τ_∞, W, and E_0 are adjustable parameters.

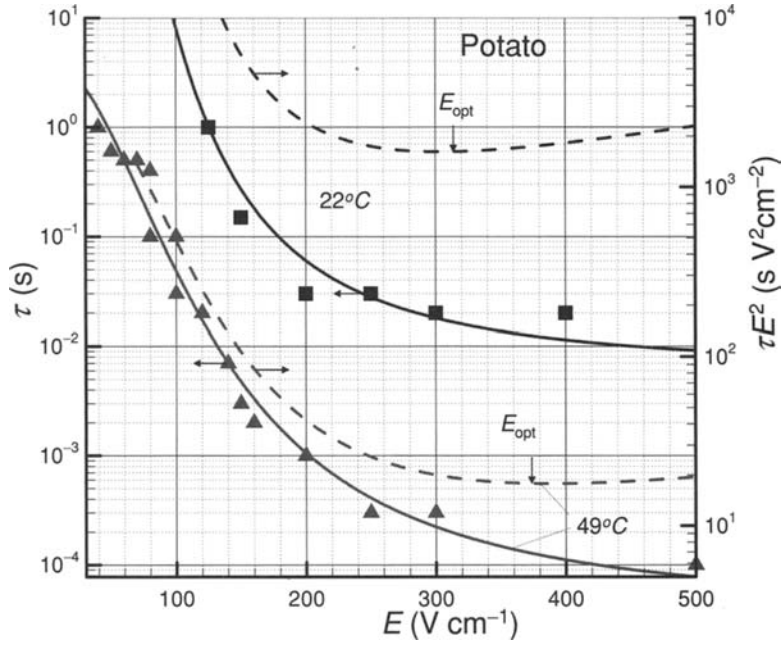

Figure 6.4. Characteristic damage time τ (solid lines and points) and optimization product τE^2 (dashed lines) versus electric field strength E at different temperatures. Points are the experimental data for a potato tissue, dashed lines in Fig. 6.3 correspond to the least square fitting of these data using Eq. (6.3) (Lebovka *et al.*, 2005).

Though this equation has no theoretical justification, it has a simple form similar to that of Eq. (6.2) and it works well for fitting of experimental data.

The characteristic electrical damage time decreases considerably at elevated temperatures as compared with the room temperature, which possibly reflects the temperature structural transitions inside the membrane structure and the more pronounced electroporation efficiency at high temperatures (Zimmermann, 1986). Note, that in a case of potatoes, the direct thermal damage is practically absent at temperatures of order $T = 50°C$ (Lebovka *et al.*, 2004a) and the observed effects are related to the PEF-induced damage and electroporation. At small electric field strengths ($E < 100$ V/cm), the noticeable electroporation effects occur in potato tissue at the room temperature for treatment duration of the order of 10–1000 s, but the effective electrical damage of the tissue takes place at the treatment times of the order of 10^{-2}–1 s at $T = 49°C$.

The energy input during the treatment time $t = \tau(E)$ can be characterized by an optimization product of $\tau(E)E^2$ (Lebovka *et al.*, 2002). The treatment time decreases significantly with E increase, approaching the τ_∞ value in the limit of high fields ($E \approx 1000$ V/cm) and the optimization product of $\tau(E)E^2$ goes through a minimum (dashed lines in Fig. 6.4). So, the PEF treatment at high electric field strength E may cause the high energy consumption without any noticeable increase of the damage degree Z.

The most preferable PEF treatment requires the minimum value of the optimization product at corresponding electric field strength $E \approx E_{opt}$. It was found for different plant tissues (apple, carrot, potato) (Lebovka *et al.*, 2002) that experimental curves $\tau(E)E^2$ versus E pass through minimum at $E = 200$–400 V/cm and this minimum corresponds to the minimum power consumption. Treatment at the elevated temperatures results in considerable decrease of the optimization product, but optimal electric field strength E_{opt} increases slightly. Therefore, it would be quite reasonable to predict that

combination of PEF and thermal treatments at moderate temperatures ($E \approx 50°C$) can give a unique opportunity to reach high tissue disintegration degree at moderate electric field strengths and without noticeable product quality losses.

2.4.3. PEF-Induced Secondary Effects in a Cellular Tissue

The PEF treatment initiates certain secondary effects in a cellular tissue. An important effect is related with possible resealing of the cells after the PEF-induced damage. Thus, for a single membrane, if the time of treatment exceeds considerably the characteristic time, $t \gg \tau$, the rupture covers total of the membrane surface and is irreversible. In cell membranes the rupture of a portion of the membrane, which is bounded by nonmembrane structure (e.g., cytoskeleton) is possible (Weaver and Chizmadzhev, 1996) and electroporation may be partially reversible (Gabriel and Teissie, 1995, 1999). The membrane damages may vanish and cells may reseal in a while $t > \tau_r$ after their PEF treatment. The time constant of the resealing processes τ_r may be rather high, its values are of order 1–100 s (DeBruin and Krassowska, 1999; Neumann *et al.*, 1999; Knorr, 1999). The ability of cell membranes to reseal depend on the electric field intensity E and the treatment time t_{PEF}. Resealing may be complete or partially complete at moderate treatment conditions (small E or t_{PEF}), and also it may be short- or long-lived. The PEF treatment may stimulate hidden damage processes that reveal a day and even later after the PEF treatment (Teissie *et al.*, 1999).

Another secondary effect is related to the additional moisture release from the damaged cells resulting from the PEF-induced structural changes in a tissue. The diffusion migration, osmotic flow, long-lasting moisture, and air redistribution processes develop inside the tissues (Labuza and Hyman, 1998; Lebovka *et al.*, 2001). The time constant of the moisture transfer may be estimated as (Gulyi *et al.*, 1994)

$$\tau_d \approx \frac{d_c^2}{6DZ}. \tag{6.10}$$

Here, D is the moisture diffusion coefficient, and Z is the membrane damage degree.

Assuming the total damage of the cell membranes, i.e., $Z = 1$, $d_c = 10^{-4}$ m, and $D \approx 2 \times 10^{-9}$ m^2/s (water diffusion coefficient at 25°C) (Mank and Lebovka, 1988), we estimate from Eq. (6.10) that $\tau_d \approx 1$ s, i.e., the moisture redistribution processes are rather long-lasting.

The PEF-induced secondary effects cause dependence of the damage kinetics from repetition time Δt in the PEF protocol. The evolution of tissue properties after the PEF treatment may be considered as a correlated percolation phenomenon governed by the resealing of cells and moisture transfer processes inside the cellular structure (Lebovka *et al.*, 2001). Figure 6.5 demonstrates that evolution of the damage degree is sensitive to the repetition time Δt of the PEF treatment. When the repetition time exceeds the time of the moisture transfer, $\Delta t > \tau_d$, the damaged regions have larger spatial dimensions and the total degree of tissue damage Z is higher than in case when $\Delta t < \tau_d$.

The PEF treatment can also initiate the so-called hidden damage. At moderate PEF treatment (small values of E or t_{PEF}) the actual damage degree is hidden and does not affect the electrical conductivity of a sample during some time after the PEF treatment (Lebovka *et al.*, 2002, 2003). Figure 6.6 displays the excess value of the relative mass losses ΔY of potato slices during their pressing at $P = 5$ bars. Here ΔY is a difference between the relative mass losses with (Y_{PEF}) and without (Y_0) PEF treatment. PEF treatment induces the additional liquid releases inside the tissue, but the process starts and PEF enhances expression considerably only after some latent period of time $t > t_H$. At $E = 200$ V/cm and $t_{PEF} = 0.01$ the latent period t_H is rather long and is about 10^3 s, but it decreases with t_{PEF} or E increase (Lebovka *et al.*, 2003).

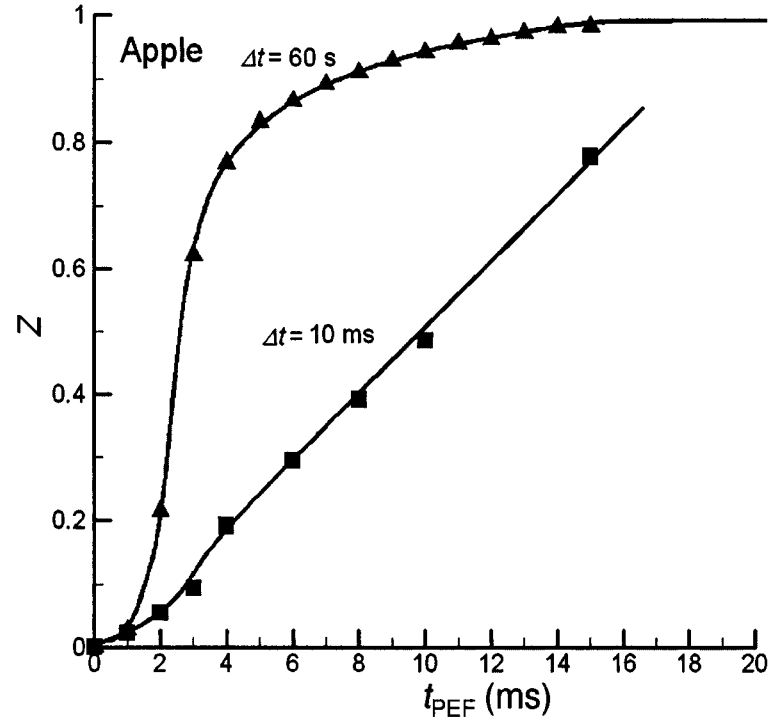

Figure 6.5. Damage degree Z versus time of the PEF treatment t_{PEF} of apple at different pulse repetition time Δt. Symbols are the experimental data obtained for the apple tissue at the electric field strength $E = 500$ V/cm and single pulse duration $t_i = 1$ ms (Lebovka *et al.*, 2001).

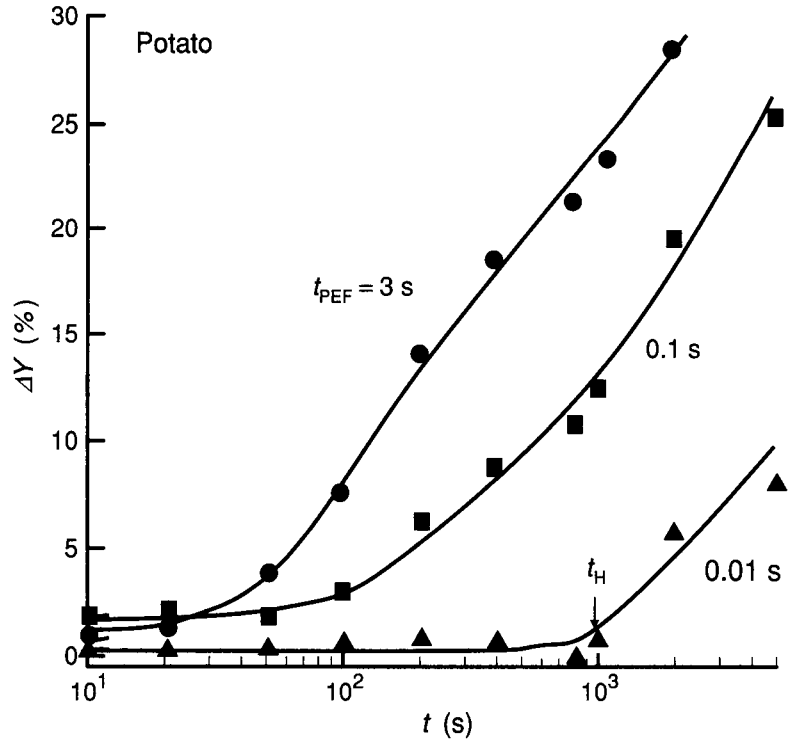

Figure 6.6. Excess value $\Delta Y = Y_{PEF} - Y_0$ versus expression time after the PEF treatment t ($E = 200$ V/cm, $t_i = 100$ μs, $\Delta t = 10$ ms) at different treatment time t_{PEF} (Lebovka *et al.*, 2003).

3. APPLICATION OF PEF FOR SOLID/LIQUID EXPRESSION

Expression (pressing) is the act of liquid expelling from a solid/liquid mixture by mechanical compression, which can be produced by movable elements, for example, piston, screw, rolls, etc. Different equipment like screw presses, belt presses, hydraulic presses, or filter presses is employed for expression of juices from the raw food materials. Pressing is a mechanical process and it is less energy consuming comparing to hot water extraction. Furthermore, it offers fresh-like juices without thermal degradation of a plant tissue. The juice is initially contained in closed cells, which have to be ruptured to facilitate rapid juice expulsion. Therefore, the pressing is usually associated with the material fragmentation. However, the very fine grinding of juicy food materials like roots and fruits producing the mash is not always appropriated.

Intensive mechanical destruction of cells leads to the passage of cell components like pectins and cellulose into the extracted juice thus increasing its cloudiness. If the limpid juice should be produced, the multi-stage clarification is then needed (Albagnac *et al.*, 2002). In some food branches like sugar production, the fine root grinding into the mash is not industrially implemented because of the difficulties of extracted juice purification (Van der Poel *et al.*, 1998). To overcome the difficulties with juice clarification or purification, the coarse fragmentation of the raw material (e.g., by slicing) is needed. However, the juice yield obtained by pressing of coarse particles is insufficient. Therefore, the particles are frequently treated thermally to destroy the cells remaining intact after their coarse fragmentation (Nagy *et al.*, 1993). The PEF application as a pretreatment operation before pressing and combination of PEF with pressing allows to increase significantly the juice yield from coarse particles and to obtain products of higher quality.

3.1. Example of a Laboratory Device for the Combined Pressing and PEF Treatment

The laboratory device developed in the University of Technology of Compiegne (UTC) for the combined pressing and PEF treatment is presented in Figs. 6.7 and 6.8. The compression chamber

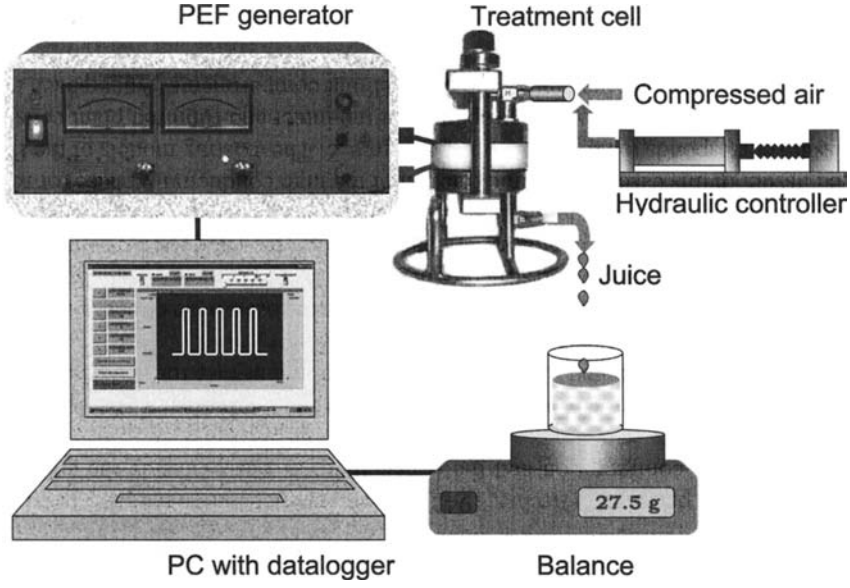

Figure 6.7. Laboratory device for the combined pressing and PEF treatment.

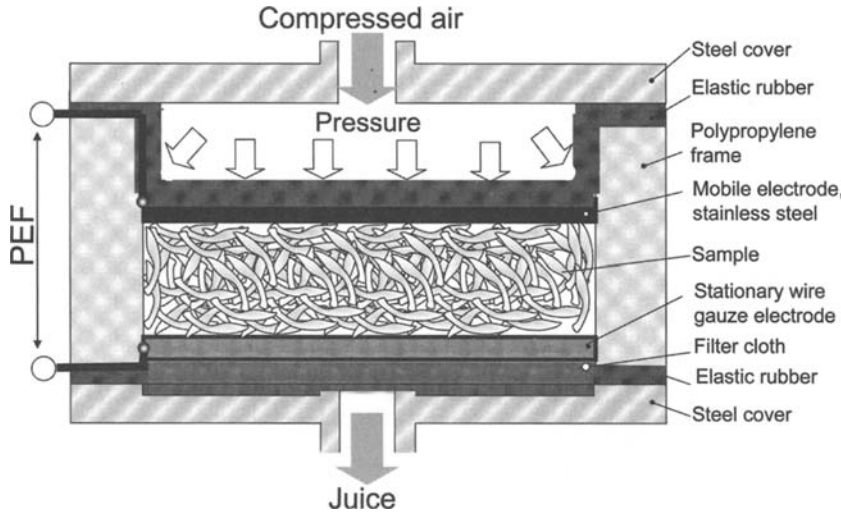

Figure 6.8. Compression chamber.

of the treatment cell has a polypropylene frame with a cylindrical cavity compartment (20 mm thick, 56 mm in diameter) (Fig. 6.8). The cavity compartment of the frame should be initially filled with tissue slices and then tightly closed from both sides by steel covers. The mobile electrode is attached to the elastic rubber diaphragm. A stationary wire gauze electrode is installed between the filter cloth and the layer of tissue slices.

The electrodes of the compression chamber are connected to the PEF generator (Fig. 6.7), which can provide the monopolar or bipolar pulses of near-rectangular shape. The pulse duration t_i can be varied within the interval of 10–1000 μs, and the pulse repetition time Δt can be varied within the interval of 1–100 ms. Pressure is applied to the layer of slices through the mobile electrode and elastic diaphragm. The compression chamber can be connected with a hydraulic pressure controller or with air compressor. Pulse protocols (E, t_i, Δt, number of pulses n) and all the output data (current, voltage, electrical resistance, and actual mass of extracted juice) are collected using a data logger and special software.

3.2. Mechanism of Solid/Liquid Expression from Biological Tissue Treated by PEF

The mechanism of solid/liquid expression from biological materials is complicated by different simultaneous phenomena influencing the process kinetics: air expulsion, tissue collapse, cell wall breakage and separation, internal liquid flows between intracellular, extracellular, and extraparticle spaces, biological transformation of cellular tissue, etc. Usually, simulation of the solid/liquid expression of plant materials is based on the theory of consolidation adopted for the liquid-containing particles (Schwartzberg, 1997). The complicated mechanism of expression from the cellular materials containing viscoelastic and plastic components leads to the multi-staged consolidation behavior (Lanoisellé *et al.*, 1996).

Terzaghi's rheological model (dashpot cylinder with inserted Hookean spring simulating consequently the porous liquid and the solid structure) is often used for simplified simulation of expression. For liquid expression from the biological materials, the inserted spring can be replaced or supplemented by one or several Kelvin–Voight elements modeling the viscoelastic properties of the cellular tissue structure (Lanoisellé *et al.*, 1996). Figure 6.9a shows the typical rheological model representing

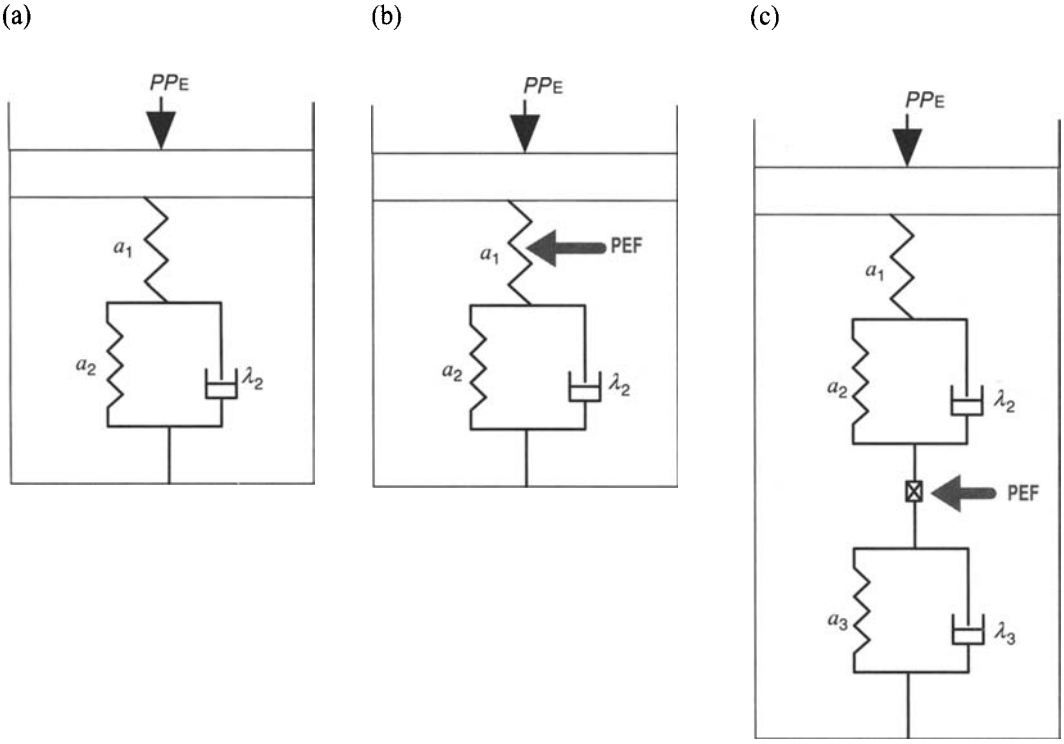

Figure 6.9. Rheological model representing the liquid expression from a biological material. Terzaghi's cylinder with spring is supplemented by one Kelvin–Voight element modeling the viscoelastic properties of a cellular tissue structure (a). The PEF application (b), (c) may influence properties of the tissue structure.

expression of liquid from a biological material, which is simulated by one Kelvin–Voight element. When a time-dependent effective stress $P_s = P - P_l$ (where P is the applied pressure and P_l is the liquid pressure) effects a layer of the fragmented biological material, the spring a_1 compresses instantaneously, but compression of the compound Kelvin–Voight element a_2–λ_2 is retarded because of the presence of the dashpot λ_2. The stress–strain relationship can be therefore presented in the following form (Shirato *et al.*, 1986):

$$e = a_1 P_S(t) + \int_0^{\tau} P_S(t) e^{-(\lambda_2(t-\tau))/a_2} d\tau. \tag{6.11}$$

Equation (6.11) shows that during the initial period of pressing, when the total compression is dominated by the spring compression a_1, the layer strain (e) is proportional to the effective stress P_s. This initial period of expression is often called the hydrodynamic period and the consolidation that occurs is the primary consolidation (Shirato *et al.*, 1986). The later period of compression, governed by compression of the compound element a_2–λ_2 and represented by the integral term in Eq. (6.11), corresponds to the secondary consolidation. The number of consolidation periods (and the number of the compound Kelvin–Voight elements in a rheological model) depends on the type of the biological material (Lanoisellé *et al.*, 1996).

The degree of material fragmentation and the type of pretreatment essentially effect the viscoelasticity of material and the mechanism of compression. Consequently, pretreatment of the material may influence the properties and even the number of elements in the rheological model presented in Fig. 6.9. The electropermeabilization of the cell membranes by PEF modify the viscoelastic

properties of the cellular tissue and facilitates the liquid expulsion during pressing. As far as the PEF treatment can be applied either before, or during the solid/liquid expression, the rheological properties of material may be influenced in a different manner. The PEF application before starting the pressing may influence essentially the initial hydrodynamic period of consolidation. The PEF application during the pressing may prolong the primary consolidation (Fig. 6.9b) or even induce the additional consolidation periods. The fracture element (Fig. 6.9c) conserves its rigidity (which corresponds to rigidity of the press-cake structure) before the PEF application. However, it loses its rigidity because of the cell rupture after the PEF treatment (Bouzrara and Vorobiev, 2003). Several studies were devoted to investigation of the different modes of PEF application.

3.3. Effect of PEF on the Behavior of Constant Pressure Expression from Food Plants

3.3.1. Consolidation Behavior

The constant pressure (CP) solid/liquid expression is usually performed on the industrial pressing equipment like filter presses or tube presses. This regime of pressing can also be realized using hydraulic piston presses. Application of the rheological model of solid/liquid expression (Fig. 6.9) under constant pressure P leads to the multi-exponential equation presenting several Kelvin–Voight elements corresponding to different consolidation periods (Bouzrara and Vorobiev, 2003):

$$U = \sum_{i=1}^{m} a_i(1 - \exp(-\nu_i t)) \Big/ \sum_{i=1}^{m} a_i, \tag{6.12}$$

where U is the consolidation ratio, a_i represent the Hookean springs, $\nu_1 = b\pi^2/4h^2$ (b is the primary consolidation coefficient, h is the layer thickness), and ν_i ($i = 2, \ldots m$) represent the dashpots of the compound Kelvin–Voight elements.

Equation (6.12) can be rearranged to express the juice yield (Lebovka *et al.*, 2003):

$$Y = Y_{\max}(1 - p_1 \exp(-t/\tau_1) - p_2 \exp(-t/\tau_2) - p_3 \exp(-t/\tau_3) - \cdots), \tag{6.13}$$

where $Y_{\max}$ is the maximal mass loss, τ_1, τ_2, τ_3 are the characteristic times of different consolidation periods, and p_1, p_2, p_3, ... are the fraction coefficients, respectively.

Figure 6.10 presents the relative mass losses (or juice yield) $Y = \Delta m/m$ (open squares) versus time t at the constant pressure $P = 5$ bars for the fresh nontreated potato slices. Dashed line in Fig. 6.10 represents the least square fit of the three exponential version of Eq. (6.13) with a correlation coefficient $r^2 = 0.998$. The characteristic time of the initial expression period is $\tau_1 = 10 \pm 2$ s, but during this short period about of 20% of juice was extracted ($p_1 = 0.20 \pm 0.05$). This initial consolidation period may be attributed to the external juice and air removal. It is very short for fresh potato slices and somewhat longer for fresh sugar beet slices (about 50 s) (Bouzrara and Vorobiev, 2003).

On finer material fragmentation and involvement of other methods of the cell damage, the quantity of the external juice increases. Then the first consolidation period may be much longer, as it was demonstrated for thermal pretreatment in (Schwartzberg, 1997). It follows from Fig. 6.10 that the characteristic time of the second expression period is $\tau_2 \approx 500 \pm 150$ s and $p_2 = 0.20 \pm 0.05$. The end of this period can be estimated as $t_2 \approx -1000$ s (Fig. 6.10). The third consolidation period was most prolonged $\tau_3 \approx 40,000 \pm 10,000$ s. The biggest part of liquid was expressed during this period ($p_3 = 0.60 \pm 0.05$). After a certain transition time, kinetics of this longest consolidation period may be approximated by the Buisman's logarithmic law (linear approximation of Y versus $\ln t$ dependency, Fig. 6.10).

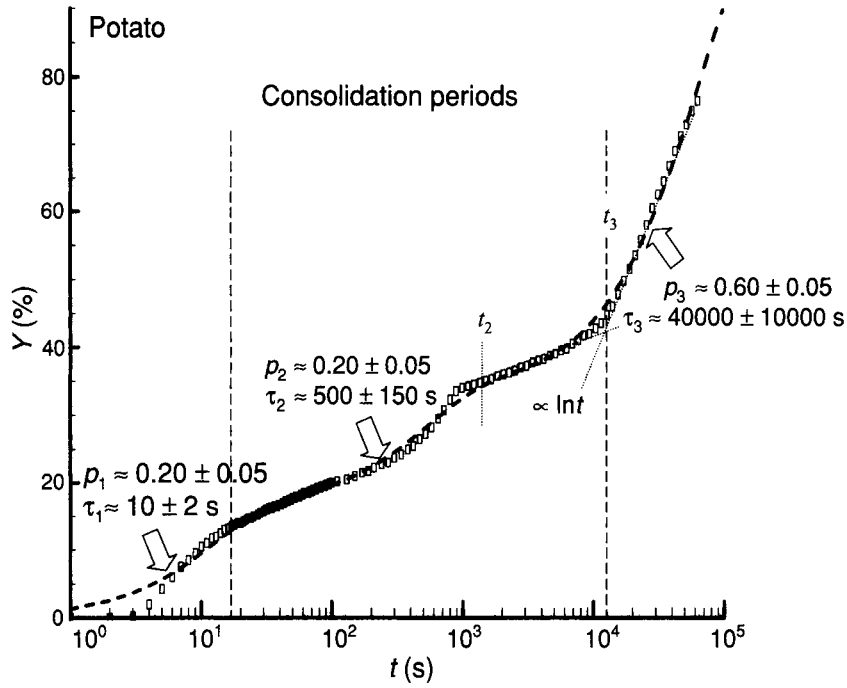

Figure 6.10. Relative mass losses Y versus expression time t at pressure $P = 5$ bars for potato slices (Lebovka *et al.*, 2003).

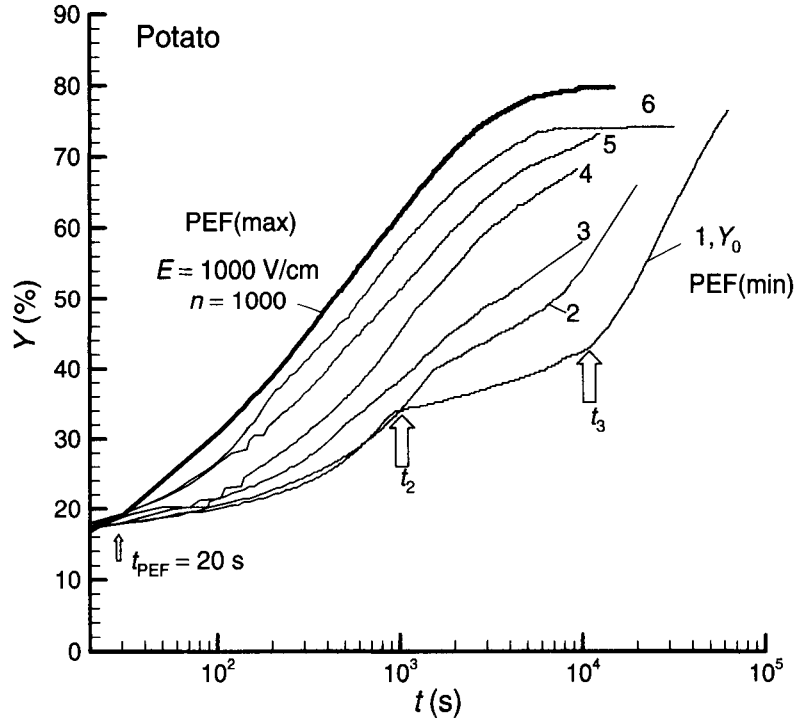

Figure 6.11. Consolidation curves $Y(t)$ for potato slices at the constant pressure $P = 5$ bars at variable total time of PEF treatment t_{PEF}(s) = 0(1), 0.005(2), 0.01(3), 0.1(4), 2(5), 3(6) ($E = 200$ V/cm, $t_i = 100$ μs, $\Delta t = 10$ ms) (Lebovka *et al.*, 2003).

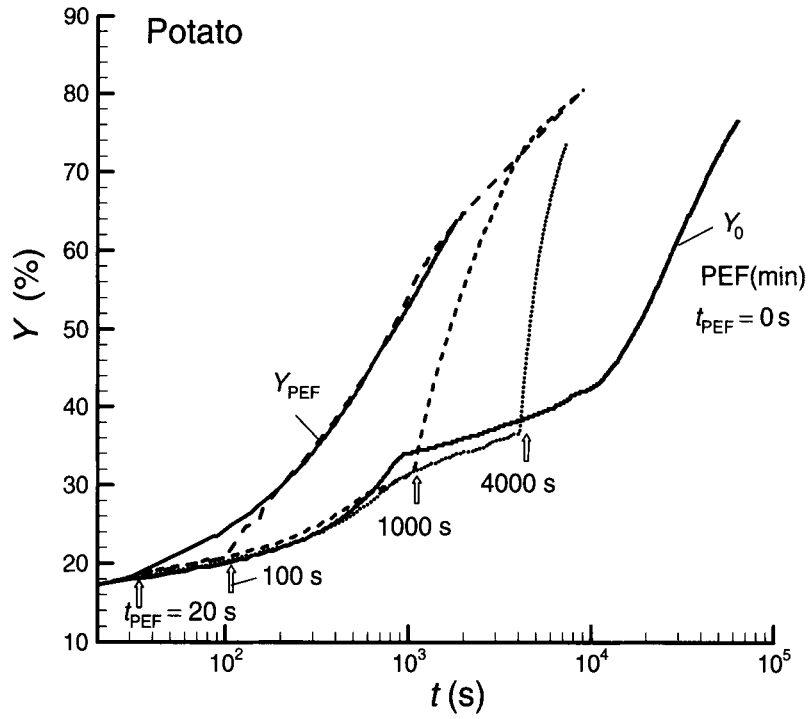

Figure 6.12. Consolidation curves $Y(t)$ for potato slices, treated by PEF ($E = 300$ V/cm, $t_i = 100$ μs, $\Delta t = 10$ ms, and $n = 1000$) after different periods of consolidation. Arrows show the time of PEF application (Lebovka *et al.*, 2003).

Figure 6.11 shows the typical consolidation curves $Y(t)$ obtained for potato slices at the constant expression pressure $P = 5$ bars, electric field intensity $E = 200$ V/cm, pulse duration $t_i = 100$ μs, pulse repetition time $\Delta t = 10$ ms and variable total time of PEF treatment $t_{PEF} = nt_i$. In these experiments, PEF treatment was applied just after the termination of the first consolidation stage when about 20% of juice was extracted. After the PEF application, the additional juice releases from electropermeabilized cells and the consolidation process begins to develop more intensively. Increase of the PEF intensity and duration leads to acceleration of expression kinetics. The difference between consolidation periods, which is noticeable at $E = 0$ V/cm, disappears even after the PEF of short duration $t_{PEF} > 0.01$ s (Fig. 6.11).

Figure 6.12 shows typical juice yield curves $Y(t)$ obtained at $P = 5$ bars for potato slices treated by PEF after different periods of consolidation. All the curves of juice yield join after the PEF application (Fig. 6.12). However, too late application of PEF ($t_{PEF} > 1000$ s) retards the consolidation process and increases a process duration.

The characteristic consolidation time after the PEF treatment τ_{PEF} as estimated by fitting of experimental data to the equation

$$\Delta Y = \Delta Y_{max}(1 - \exp(-t/\tau_{PEF}))$$

is equal to $\tau_{PEF} = 1400 \pm 20$ s. Note that this characteristic consolidation time is larger than the corresponding consolidation time without PEF treatment $\tau_2 \approx 500 \pm 150$ s.

From an industrial point of view, it is important to reduce the pressing time as much as possible. The time at which the PEF treatment starts t_{PEF} exerts no significant effect on the PEF-enhanced juice yield for the case when $t_{PEF} < t_2 \approx 1000$ s. The PEF protocol (pulse duration and pulse repetition time) can be freely constructed with the only restriction that all the pulses should be applied during

the time $t < t_2$. So, power input into the system may be distributed over the time period $t < t_2$ and it is important, because it allows application of a less expensive industrial PEF generator and avoidance of undesirable ohmic overheating.

3.3.2. Solid/Liquid Expression with Intermediate PEF Treatment

3.3.2.1. Sugar beets

Intermediate PEF treatment improves the juice expression from sugar beet slices (Bouzrara and Vorobiev, 2000, 2001; Bouzrara, 2001). The slices were obtained by grating a beet root on a 6-mm grater. After the first pressing period at 5 bars, the juice yield was about 19.1%. Application of the PEF (500 pulses, pulse duration of 100 μs, pulse frequency 100 Hz) markedly enhanced the juice yield, which rises to 43, 68, and 79%, respectively for voltage gradients of 215, 300, and 427 V/cm. Such results confirm the significant effect of moderate PEF treatment combined with pressurization on the juice yield.

Besides, Bouzrara and Vorobiev (2001) demonstrated the effect of change in the number of pulses on the efficiency of pressing. Three pulse numbers 250, 300, and 500, were tested with PEF intensity of 427 V/cm and pulse duration of 100 μs. The juice yield was increased approximately to 60, 73, and 79%. Taking into account the juice yield evolution as a function of both intensity of PEF and pulse number, Bouzrara and Vorobiev (2001) demonstrated dependence of the additional juice yield on energy input generated by PEF. A correlation between the energy input by PEF and juice yield was demonstrated. Indeed, until 6×10^{-4} kWh/kg the juice yield was rising drastically with energy input increase. After a certain energy threshold, it becomes more difficult to enhance the extraction, an improvement of about 6% of juice yield requires the double of the applied energy. It was concluded that the energy threshold suggests the destruction of quasi-totality of cells and inefficacy of further energy input by PEF.

In addition to PEF parameters, other factors, such as compressive pressure and size of sliced particles, have been reported to have significant effects on efficiency of the combined extraction process (Bouzrara and Vorobiev, 2000, 2001; Bouzrara, 2001). Increase of the applied pressure from 0.5 to 10 bars enhanced the juice yield extracted from the sliced particles from 1.6 to 34%, which suggests that a large quantity of cells was not destroyed even at pressure of 10 bars. The PEF pretreatment (500 V, pulse duration of 100 μs and 1000 pulses) followed by pressing, enhanced the juice yield up to 62.3% at 10 bars. The combined process with an intermediate PEF application was most effective and enhanced the juice yield up to 82.4% at 10 bars. Even at rather low pressure of 5 bars the effect of intermediate PEF was significant (78.5%). The size of the sliced particles and the type of the mechanical slicing operation significantly influenced the extraction performance.

Bouzrara (2001) studied the effects of an intermediate PEF application on the juice yield for both small and coarse particles prepared from sugar beets. The widths of sliced particles were 1.5, 3, 4, 5, 6, and 7 mm, their length and thickness were fixed (5 and 1.5 mm, respectively). Pressure was fixed at 5 bars, and PEF treatment parameters were 500 V/cm, 100 μs, and 1000 pulses. As expected, the quantity of the first juice expressed before the PEF application increased for the smallest particles (up to 40% for particles with 1.5 mm of width as compared to 15% for particles with 7 mm of width). Nevertheless, the total quantity of the first and second juices (after the PEF application) was nearly the same for particles with sizes in the range of 1.5–6 mm (76–78%). The yield was lower for larger particles of 7 mm (73%).

The attractive feature of pressing with an intermediate PEF treatment is a better purity and less color of the sugar juice. Bouzrara and Vorobiev (2001) compared the physicochemical characteristics of the two juices: after the first pressing (before PEF application) and after the second pressing. The second pressing juice after the PEF application was less colored than the first pressing juice.

Moreover, the juice had higher sugar concentration and did not contain any pectin substances, which is advantageous for subsequent filtration and purification in a sugar refinery.

3.3.2.2. Apples

Bazhal and Vorobiev (2000) have demonstrated improvement of the juice extraction from Golden Delicious apple slices by solid/liquid expression with an intermediate PEF treatment. The slices were obtained by grating an apple on a 6-mm grater. The laboratory filter-press cell was similar to that presented on Fig. 6.7. When pressure was varied from 1 to 30 bars, the juice yield after the first pressing increased from 28 to 61%. An intermediate PEF treatment leads to an increase of the total juice yield. The quantity of additional juice extracted during the second pressing depends on the PEF parameters and on the compressive pressure. The additional juice yield was more appreciable for the lowest pressure value (31% at 1 bar against 11% at 30 bars). The total juice yield was approximately the same in the range of pressures from 3 to 30 bars. So, it was suggested to make expression at lower pressures of 3–5 bars. Increase of the voltage from 200 to 1000 V/cm, the number of pulses from 3 to 1000 and pulse duration from 20 to 100 μs, resulted in the juice yield increase. The correlation between the energy input by PEF and apple juice yield was established (Bazhal and Vorobiev, 2000; Bazhal, 2001). The maximum juice yield was attained at minimum energy input of 3 kJ/kg of apples.

Bazhal and Vorobiev (2000) presented the absorption spectral curves of juices after the first and second pressing at 3 bars. The juice after the first pressing was turbid and required filtration. After the second pressing the juice transmittance was significantly higher and filtration did not increase the juice lightness. The values of dry matter were higher for juices obtained by PEF treatment.

3.3.2.3. Carrots and spinach

Bouzrara (2001) studied the solid/liquid expression of carrot slices with an intermediate PEF treatment at voltage gradients of 180, 225, 270, and 360 V/cm, pulse duration of 100 μs and frequency of 100 Hz. The treatment time t_{PEF} was 5 s. The carrot slices were grated on a 6-mm grater and pressed at 5 bars. The juice yield after the first pressing was 25.6%. An intermediate PEF treatment permitted to increase the juice yield to 38.3% at 180 V/cm and to 72.4% at 360 V/cm. However, the additional juice yield was not significant if voltage gradients were increased from 270 to 360 V/cm. Some threshold of juice yield was noted for energy input about of 1.5 kWh/kg of carrots. The second pressing juice was lighter and more concentrated with soluble matter than the first pressing juice.

Bouzrara (2001) demonstrated the effect of intermediate PEF treatment at 875 V/cm, 100 μs, and 100 Hz on juice extraction from spinach. Juice yield after the first pressing attained 26.1, 30, and 37.7% at pressures 5, 10, and 15 bars, respectively. An intermediate PEF treatment applied during 5 s followed by the second pressing resulted in juice yield of 60.6% (at 10 bars).

3.4. Effect of PEF on the Behavior of Constant Rate Expression from Food Plants

The constant pressure regime of expression provides a sudden pressure increase up to some given value, which causes a "shock" compression of material. For biological materials, gentler regime of a constant rate (CR) expression with gradually increasing pressure is often preferable. Such regime of pressing may be implemented using hydraulic piston presses and some approximation in screw presses.

Figure 6.13 shows the juice yield curves obtained during CR solid/liquid expression of sugar beet slices (grated with 6-mm grater) under different modes of PEF application (Praporscic *et al.*, 2004). In all experiments presented in Fig. 6.13, the velocity of cake deformation (pressure loading) was 0.65 mm/min, the voltage was fixed at $U = 500$ V, the duration of each pulse was $t_i = 100$ μs

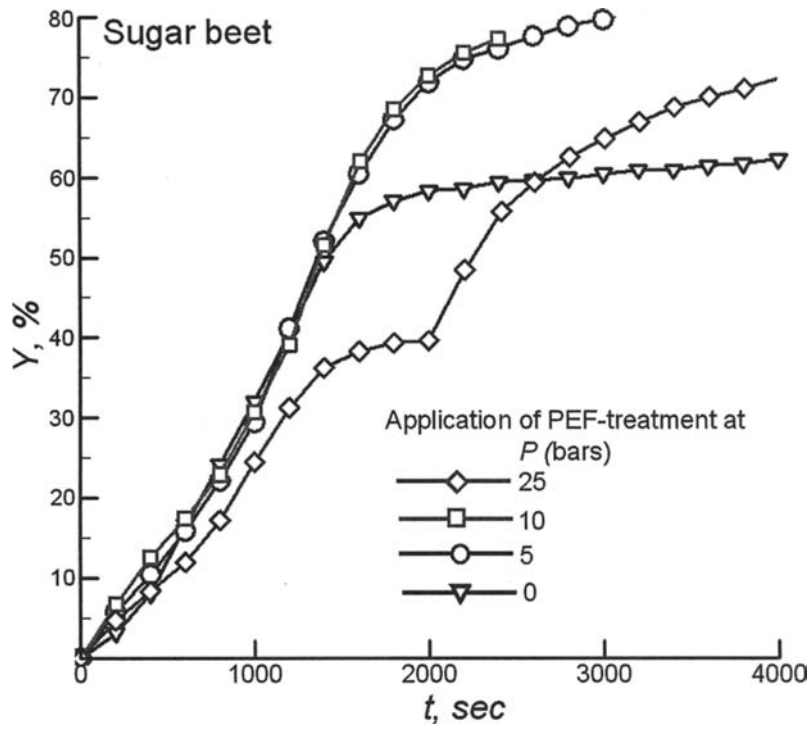

Figure 6.13. Juice yield Y as a function of the expression time t for different modes of PEF application at different pressures P (Praporscic *et al.*, 2004).

and the number of pulses was $n = 1000$. So, the total time of PEF application was $t_{PEF} = nt_i = 1000 \times 10^{-4} = 0.1$ s. As it can be seen from Fig. 6.13, the best results were obtained with an intermediate PEF treatment of press-cakes pressurized at 5 and 10 bars (83% juice yield attained after 1 h of pressing). With PEF application before pressurization, the 65% juice yield was attained just after 1 h of pressing. The later PEF application to the press-cake pressurized at 25 bars gives an intermediate result: the juice yield of about 75% was attained after the 1 h of pressing. However, the juice yield curves obtained after the PEF treatment combined with pressurization at different pressures (5, 10, and 25 bars) approached asymptotically the same final "equilibrium" value with continuation of pressing. This corroborates well with results, obtained for solid/liquid expression at the constant pressure.

The juice yield, obtained under different intensities of the PEF treatment and fixed number of pulses ($n = 300$), is shown in Fig. 6.14. The juice yield, obtained under different number of pulses and fixed number of PEF intensity ($E = 670$ V/cm), is shown in Fig. 6.15. In these experiments, the solid/liquid expression was effected with a constant rate of 0.65 mm/min (up to $P = 25$ bars) and following the constant pressure of 25 bars. The PEF was applied after the cake pressurization at 5 bars. As it can be seen from the Fig. 6.14, increase of E from 0 to 250 V/cm did not enhance the juice yield. It can be assumed that the electrical damage of cell membranes was not attained at this intensity of the PEF treatment. The critical value of PEF intensity permitting to attain a noticeable juice yield increase was 300 V/cm. The following increase of the PEF intensity resulted in the noticeable improvement of efficiency of the solid/liquid expression up to 500 V/cm. And afterward the juice yield was enhanced just slightly (Fig. 6.14).

Figure 6.15 shows that increase of the number of pulses n up to 10 has exerted a limited effect at $E = 670$ V/cm and pulse duration $t_i = 100$ μs. On the contrary, enhancing of the juice yield was

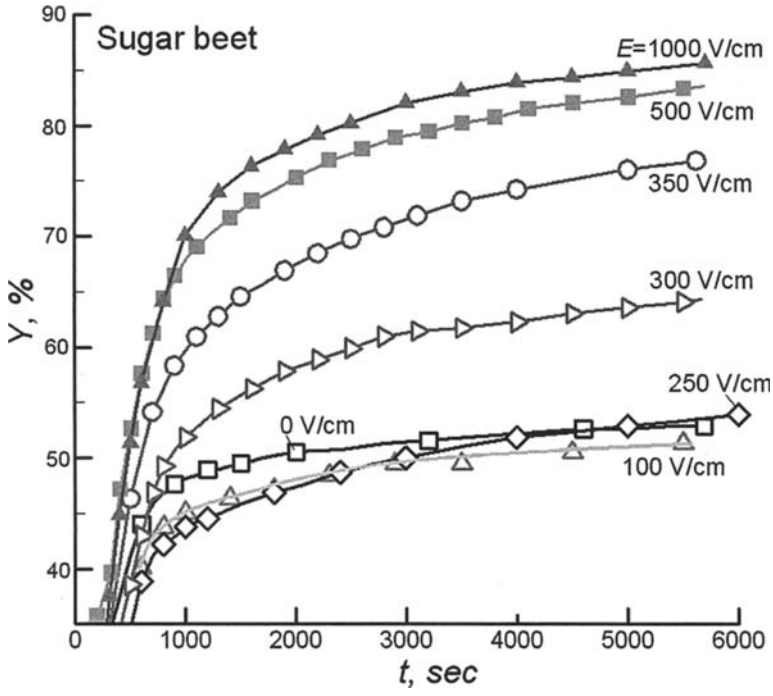

Figure 6.14. Juice yield Y obtained under different intensities of the PEF treatment $E(t_i = 100\ \mu\text{sec}, \Delta t = 10\ \text{msec}, n = 300)$ and pressure $P = 5$ bars (Praporscic *et al.*, 2004).

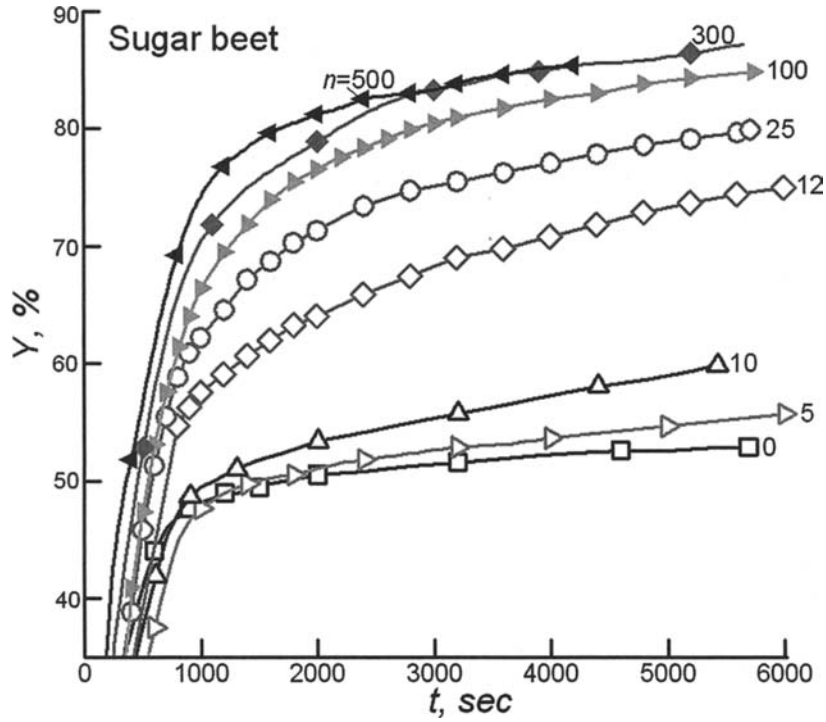

Figure 6.15. Juice yield Y obtained under different number of pulses $n(E = 670\ \text{V/cm}, t_i = 100\ \mu\text{sec}, \Delta t = 10\ \text{msec})$ and pressure $P = 5$ bars (Praporscic *et al.*, 2004).

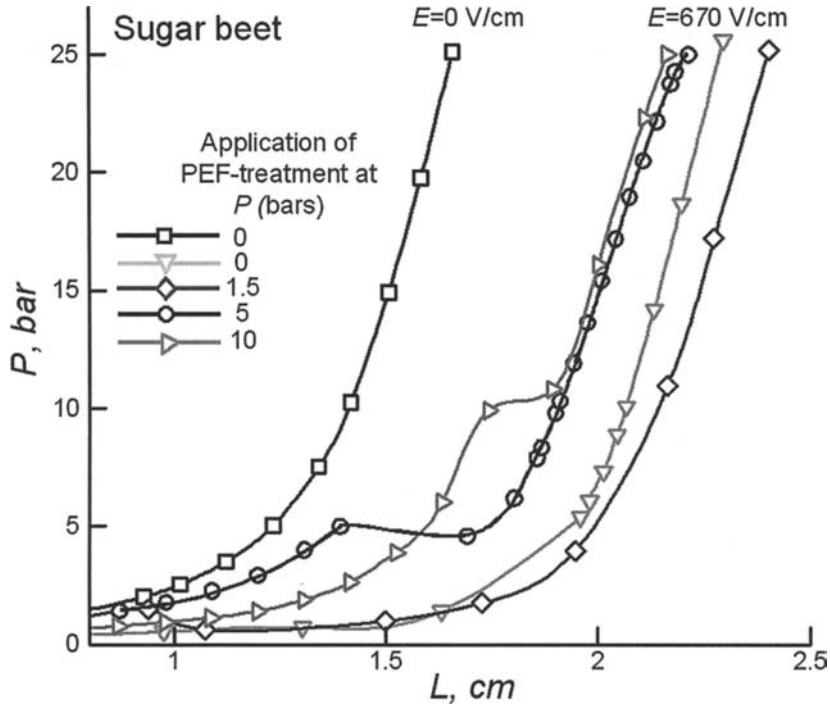

Figure 6.16. Loading pressure dependence of cake displacement at different degrees of sugar beet slices pressurization before the PEF treatment ($t_i = 100$ μs, $\Delta t = 10$ ms, $n = 500$) at different pressures P (Praporscic *et al.*, 2004).

significant with 12 and more pulses. It can be supposed that the indicated critical number of pulses, which corresponds to the PEF treatment time $t_{PEF} = n \cdot t_i = 12 \times 10^{-4} = 0.0012$ s, was sufficient to attain a noticeable electrical damage of membranes. An increase of the number of pulses was efficient just to $n = 300 - 500$. The following increase of n just slightly improved the juice expression.

Figure 6.16 presents the curves of loading pressure versus the cake displacement, obtained at the rates of cake deformation 0.65 mm/min and different degrees of sugar beet pressurization before the PEF treatment (Praporscic *et al.*, 2004). The presented curves clearly demonstrate existence of two different parts: linear part, which corresponds to the elastic deformation of a cake layer and nonlinear part, which corresponds to the viscoelastic deformation of a cake layer. The linear part of a curve corresponds to the primary consolidation (the first term in Eq. (6.13)). The elasticity modulus can be defined for linear part of a stress–strain curve as $G_E = P/e$ (e is the dimensionless strain of deformation, $e = \Delta h/h_0$, Δh is the linear part of the cake deformation and h_0 is the initial thickness of the cake). If no PEF treatment is applied, then the linear part of a stress–strain curve is rather short and $G_E = 3.14$ bars. If PEF treatment is applied before pressurization, then the linear part of a stress–strain curve (primary consolidation) is prolonged and $G_E = 1.55$ bars. It can also be seen from Fig. 6.16 that application of an intermediate PEF treatment during cake pressurization causes the tissue failure. As a result, the cake deformation arising after the PEF treatment is accompanied by certain pressure fall (when PEF was applied at $P = 1.5$ and 5 bars) or slowed pressure increase (when PEF was applied at $P = 10$ bar, Fig. 6.16). The observable fall of the load pressure P was provoked by the effective stress decrease on the solid structure P_S.

As it can be seen from Figs. 6.13 and 6.16, conditions of the cake pressurization previous to PEF application influence considerably kinetics of the solid/liquid expression. The best method for juice yield enhance under a CR expression was an intermediate PEF application after the end of the primary consolidation period. For sugar beet slices, the optimal cake pressurization was attained at 1.5–5 bars (Praporscic *et al.*, 2004). From the other hand, the cake pressurization considerably decreases the energy consumption W. For example, specific energy consumption due to PEF application (670 V/cm and 500 pulses) was (4–4.5) $\cdot 10^{-3}$ kWh/kg for nonpressurized cake of the sugar beet slices, (3.3–3.4)$\cdot 10^{-3}$ kWh/kg for cake pressurized at 1.5 bars, and (1.2–1.8)$\cdot 10^{-3}$ kWh/kg for cake pressurized at 10 bars. As excessive cake pressurization before the PEF treatment retard the juice yield, therefore, some compromise should be found for the pressurization pressure.

3.5. Combination of PEF with a Moderate Heating

Fincan and Dejmek (2002) showed that despite the electropermeabilization of the cellular membranes, PEF seems to retain intact the cell wall architecture in plant tissues. The rigidity of cell walls may hinder the consolidation behavior during the solid/liquid expression. Lebovka *et al.* (2004a,b) demonstrated that mild heating at 45–50°C leaded to softening of a plant tissue and influenced textural properties of different foods (apples, carrots, potatoes).

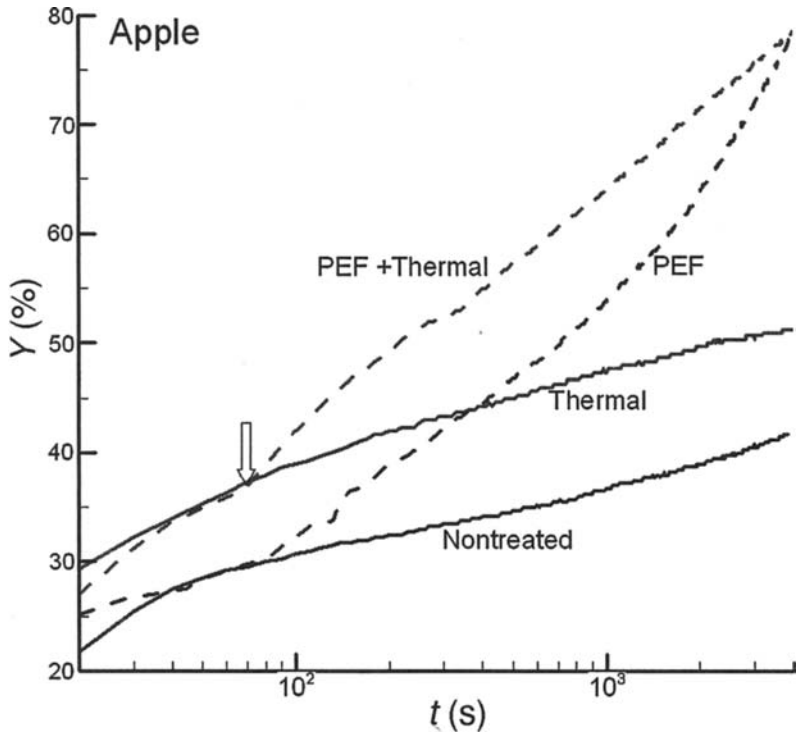

Figure 6.17. Juice yield Y versus expression time t obtained by compressing of apple slices at the constant pressure P = 5 bars combined with different types of treatment. Arrow shows an instant of the PEF application (E = 500 V/cm, t_{PEF} = 0.01 s) (Lebovka *et al.*, 2004a).

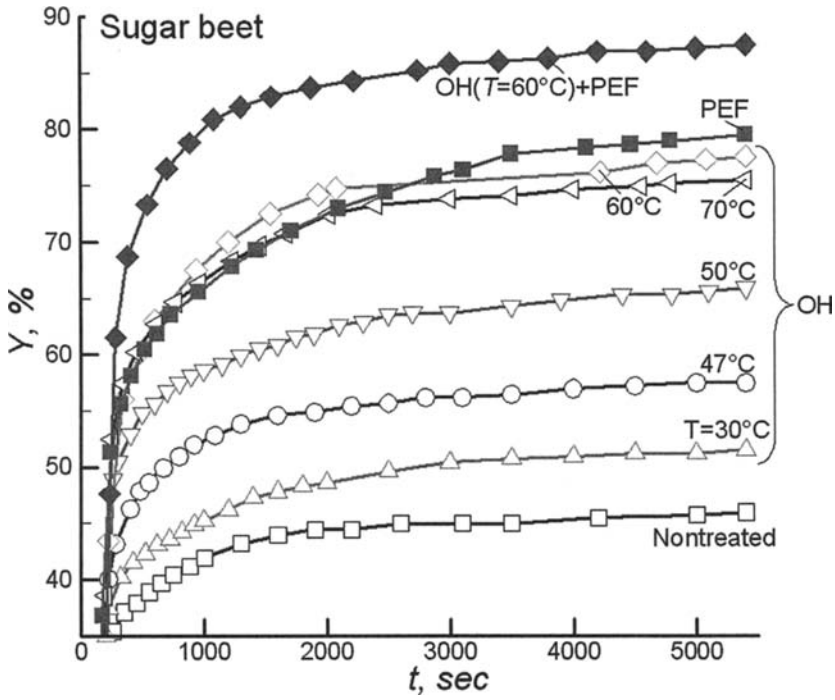

Figure 6.18. Juice yield Y versus expression time t at different temperatures T of ohmic heating (OH) and PEF treatment ($E = 600$ V/cm, $n = 400$ pulses, $t_i = 100$ μsec, $t_{PEF} = 0.04$ sec, $\Delta t = 10$ msec) (Praporscic *et al.*, 2005a).

Figure 6.17 presents the CP expression kinetics ($P = 5$ bars) for the layer of fine-cut apple slices (1.5 mm width, 40–50 mm length) under different types of treatment. Thermal pretreatment was applied at $T \approx 50$°C during 10 min, after which the slices were cooled to the ambient temperature and either treated or not by the PEF. As it can be seen from Fig. 6.17, the PEF treatment increases the juice yield for both nontreated and thermally pretreated samples. But the juice yield enhancing effect of PEF is significantly higher for the thermally pretreated samples.

Ohmic heating (OH) additionally to thermal effect is believed to induce an electropermeabilization of cell membranes (Wang and Sastry, 2002). Praporscic *et al.* (2005,2006) have compared the effects of moderate OH and PEF treatments on kinetics of the juice extraction from sugar beet cuts and to explore the combined action of the said two treatments. Figure 6.18 presents kinetics of the juice yield from sugar beet cuts (1.5 mm thickness, 6 mm width, and 35 mm length) both for nontreated and differently treated samples. The OH duration at fixed temperatures (30–70°C) was 10 min. After that the cuts were cooled to the ambient temperature and treated by PEF (600 V/cm, 400 pulses of 100 μs, $t_{PEF} = 0.04$ s).

As it is shown in Fig. 6.18, the OH accelerates the extraction kinetics even at mild temperatures (30–50°C). However, only the higher temperature (60°C) ohmic heating permits to attain a juice yield comparable to that one obtained from a PEF-treated tissue (Fig. 6.18). Increase of the temperature of sugar beet cuts from 60 to 70°C is not effective and even leads to some reduction of the rate of extraction kinetics, probably, due to deterioration of tissue drainage properties. An interesting feature of the combining OH (60°C) and PEF treatment is the fact of existence of a certain synergetic effect causing a noticeable enhancement of the juice yield (up to 87.5%, Fig. 6.18).

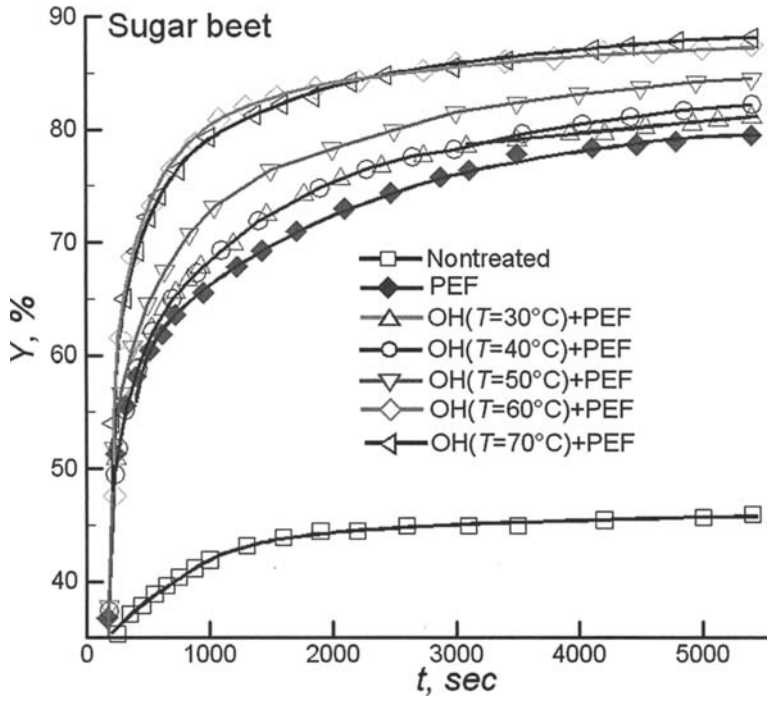

Figure 6.19. Juice yield Y versus time of expression t at PEF treatment ($E = 600$ V/cm, $n = 400$ pulses, $t_i = 100$ μsec, $t_{PEF} = 0.04$ sec, $\Delta t = 10$ msec) combined with ohmic heating (OH) at different temperatures T (Praporscic *et al.*, 2005a).

Figure 6.19 shows the extraction kinetics after the combined OH (at different temperatures) and PEF treatment (600 V/cm, 400 pulses of 100 μs, $t_{PEF} = 0.04$ s). The OH temperature elevation up to 60°C leads to a more profound structure modification and improves efficiency of the combined treatments. One should consider different factors to choose the appropriate temperature of OH preceding the PEF treatment. Mild temperatures should be preferable to preserve the product quality and to limit the energy consumption. The energy consumed by PEF remains low (1–5 kWh/ton of sugar beet cuts) (Praporscic *et al.*, 2005). The OH applied at 40°C during 10 min consumes nearly 40 kWh/ton of sugar beet cuts. The OH temperature elevation to 60°C leads to the energy consumption increase up to 80 kWh/ton of sugar beet cuts (Praporscic *et al.*, 2005).

Figure 6.20 shows the effect of the size of particles on the final juice yield reached after different treatments, the tissue structures of coarse and middle cuts without any treatment remain less destroyed and less hydraulically permeable compared to fine cuts. Therefore, the juice yield is substantially higher from pressed fine cuts. This tendency is maintained after PEF treatment. However, after combined OH and PEF treatment, the difference in juice yield obtained from fine, middle, and coarse cuts almost disappears (Fig. 6.20). It means that the additional softening effect of OH can be especially useful for extraction from coarse particles and pieces, generally used in industrial applications like sugar beet technology.

Figure 6.21 shows the juice yield obtained during the CP solid/liquid expression ($P = 5$ bars) from potato cuts (1.5 mm in width, 1 mm in thickness, and 30–40 mm in length) and apple cuts

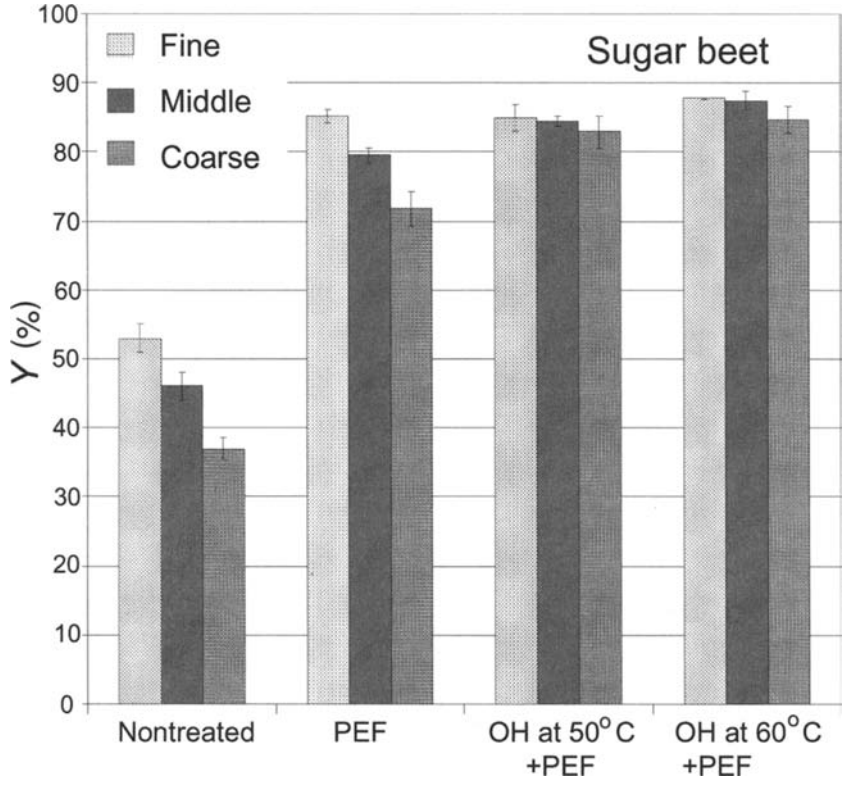

Figure 6.20. Final juice yield Y for the untreated and treated fine (1.5 × 1 × 35 mm), middle (6 × 1.5 × 35 mm) and coarse (7 × 3 × 35 mm) sugar beet cuts (Praporscic *et al.*, 2005).

(6 mm in width, 1.5 mm in thickness, and 30–40 mm in length) treated by OH or PEF (Lebovka *et al.*, 2005). The juice yield enhancing, observed for ohmically heated cuts treated by AC electric field ($E = 50$ V/cm, $t_{OH} = 45$ s) can even be higher (for apple tissue) than the observed one for cuts treated by PEF ($E = 850$ V/cm, $t_{PEF} = 1$ s). This effect can be explained by a synergetic temperature and electric field effect at mild heating conditions, which may be more significant for apples than for potatoes (Lebovka *et al.*, 2004a,b).

Note that ohmic heating needs higher energy consumption than nonthermal PEF treatment. Therefore, the choice between two technologies has to account for electrophysical properties of the treated product, its required quality and the cost of electrical treatment.

3.6. Pilot Studies of Solid/Liquid Expression Combined with PEF

The laboratory scale results of the solid/liquid expression enhanced by PEF were validated in the University of Technology of Compiegne on a larger scale (Bouzrara, 2001) using a pilot filter-press (5–15 bars of pressure; 4.5–15 kg of raw material filling) and a PEF generator (1000 V–100 A) (Fig. 6.22).

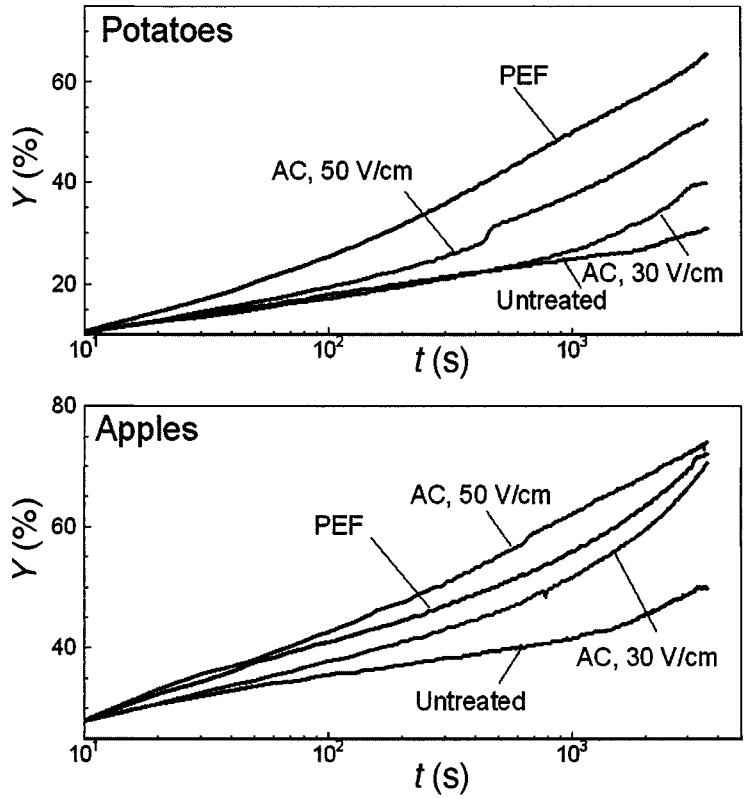

Figure 6.21. Juice yield at pressure $P = 5$ bars for the untreated, PEF-treated or AC-treated potato and apple cuts (Praporscic *et al.*, 2006).

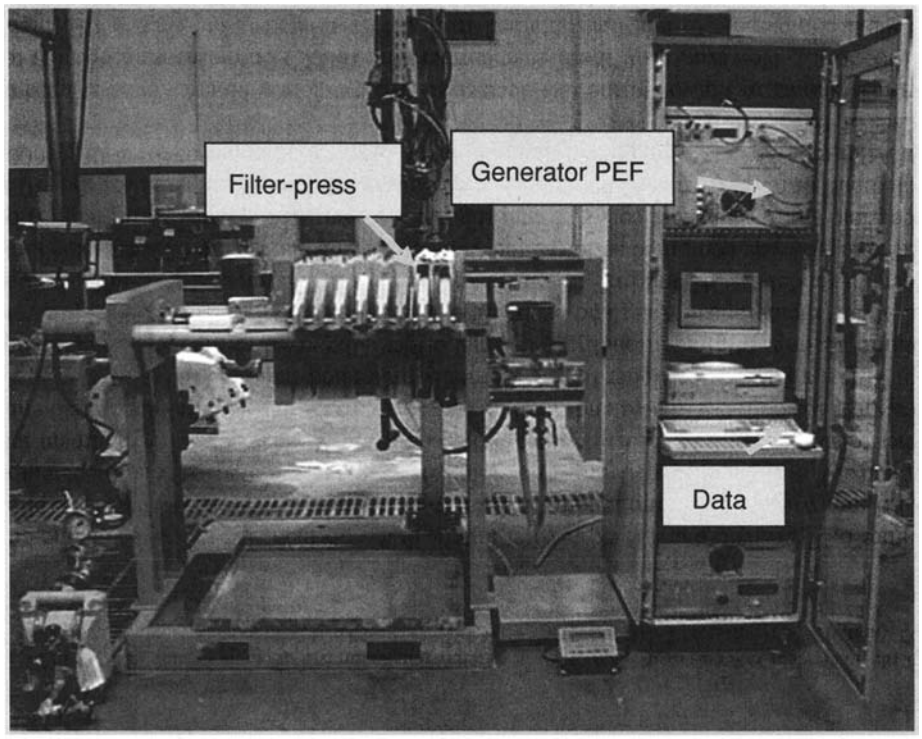

Figure 6.22. Pilot equipment for the solid/liquid expression combined with PEF.

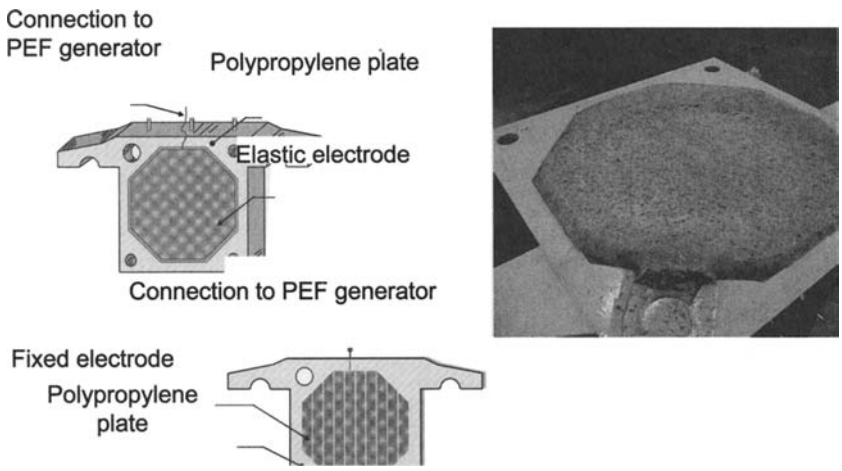

Figure 6.23. Filter-press plates.

Each chamber consists of a plate covered by a filter cloth and a flexible electrode (metallic grid) on the one side and a rigid (fixed) electrode on the other one. The pressure (compressed air) is applied to the membrane of the plate, which distributes the pressure over the layer of cuts placed between the plate and the rigid electrode (Fig. 6.23). Juice is drained through channels leading to the outlet of the liquid phase.

Kinetics of juice accumulation can be monitored by a balance connected to a data acquisition system (Fig. 6.23). In general, a PEF-assisted pressing process includes a first pressing step (FP), an intermediate PEF treatment, a second pressing step (SP), and eventually subsequent washing and pressing of pulps. Experiments were performed with different raw materials (sugar beet, apple, carrots) fragmentized into fine cuts (cossettes).

Table 6.1 summarizes the scale-up performances based on the pilot scale study of pressing of the sugar beet cossettes (Jemai and Vorobiev, 2002b). It has been shown that using 4.5 kg (i.e., 112.5 times laboratory scale) or 15 kg (i.e., 375 times) of initial cossettes, similar juice yields were obtained compared to laboratory scale juice yields from only 40 g of cossettes.

It can be seen that juices following PEF (second juice) have higher purity values (90–93% compared to 95–98%) than that of the first pressing. The color of juices following PEF is systematically three to four times lower than juice before PEF and factory juices; this observation is confirmed by the color difference of sugar crystals obtained from PEF juices compared to factory juice crystals

Table 6.1. Scale-up performance of pilot scale results compared to laboratory scale data.[a]

	Scale-up factor				
	$x1$[a]	x	112.5	x	375
	% Yield	% Yield	Purity	% Yield	Purity
First juice	27.5	29	90–93	27	93–94
Second juice	48.5	47	96–98	46	95–97

[a]Using 40 g of the grating-type cossettes (Bouzrara and Vorobiev, 2000)

Table 6.2. Color of PEF-treated and factory juices and final crystals in ICUMSA units.[a]

	Initial product	Crystals
Thick juice from factory[b]	1150–1740	280–470
PEF-treated juice	350	100

[a]Summarized from Jemai and Vorobiev (2002b).
[b]Béghin-Say factory at Chevriére, France.

(Table 6.2). Therefore, PEF juices are preferably handled separately in order to take advantage of this quality difference.

Another important criteria characterizing performance of the pressing process are characteristics of the final pulps. It is the general tendency that PEF pulps contain three to five times more α-amino nitrogen and two to three times more sodium and potassium compared to sugar industry value.

It should be noted that following said pressing steps the pulps still continue to retain significant amounts of sugar. Consequently, ways to optimize this process were explored. Thus, for example, one or more washing steps were performed in order to extract further amounts of sugar remaining in the pulps (Jemai and Vorobiev, 2002b). Continuous pressing equipment (screw presses, belt presses) should be used instead of a batch filter-press for the large-scale production (Vorobiev *et al.*, 2000).

In view of the results obtained in Jemai and Vorobiev (2002b), the best possible scheme for a PEF-assisted cold pressing of the sugar beet cossettes was proposed (Fig. 6.24). Some interesting aspects of the process described by the Fig. 6.24 flow diagram should be pointed out; for instance, significant quantities of high quality juices were obtained following the PEF application.

Consequently, separate handling of different juices may be advantageous. The final pulps have high dryness (i.e., of the order of 35% DW). This eliminates the need to dry the pulps which commonly occurs in industry.

4. EFFECT OF PEF-ENHANCING ON DIFFUSION OF WATER AND SOLUBLE SUBSTANCES FROM TISSUES

Transfer of solute from biological solids to hot water is a traditional unit operation in different food applications (extraction of sugar, tea, coffee, and many others) (Schwartzberg and Chao, 1998). For example, the thermal treatment of the beet tissue at 70–74°C, used in industrial sugar processing, permits denaturing of the cellular membranes and accelerates sugar extraction from interior of the cells. Unfortunately, cell walls are also subjected to thermal destruction, thus resulting in loss of nondesirable substances (like pectin) getting into the juice, which should be purified (Van der Poel *et al.*, 1998). The purification of juice needs numerous operations.

In recent decades, a drastic increase of the use of electrical treatment for water extraction (diffusion) from biological solids was observed. It has been shown that a low gradient electric field (direct or alternating current), in the range of 100–200 V/cm may enhance diffusion of soluble substances (Katrokha and Kupchik, 1984; Botoshan *et al.*, 1990; Jemai, 1997). However, a DC electric field leads to product electrolysis and loss of its quality (Bazhal and Gulyi, 1983), while an AC electric field results in significant energy consumption and temperature elevations to high points in many of the cases (ohmic heating) (Jemai, 1997). The PEF treatment may be a suitable alternative to continuous DC or AC field applications in order to achieve membrane breakdown with minimal electrolysis and heating effects. Recently, several studies dealing with the PEF effect on

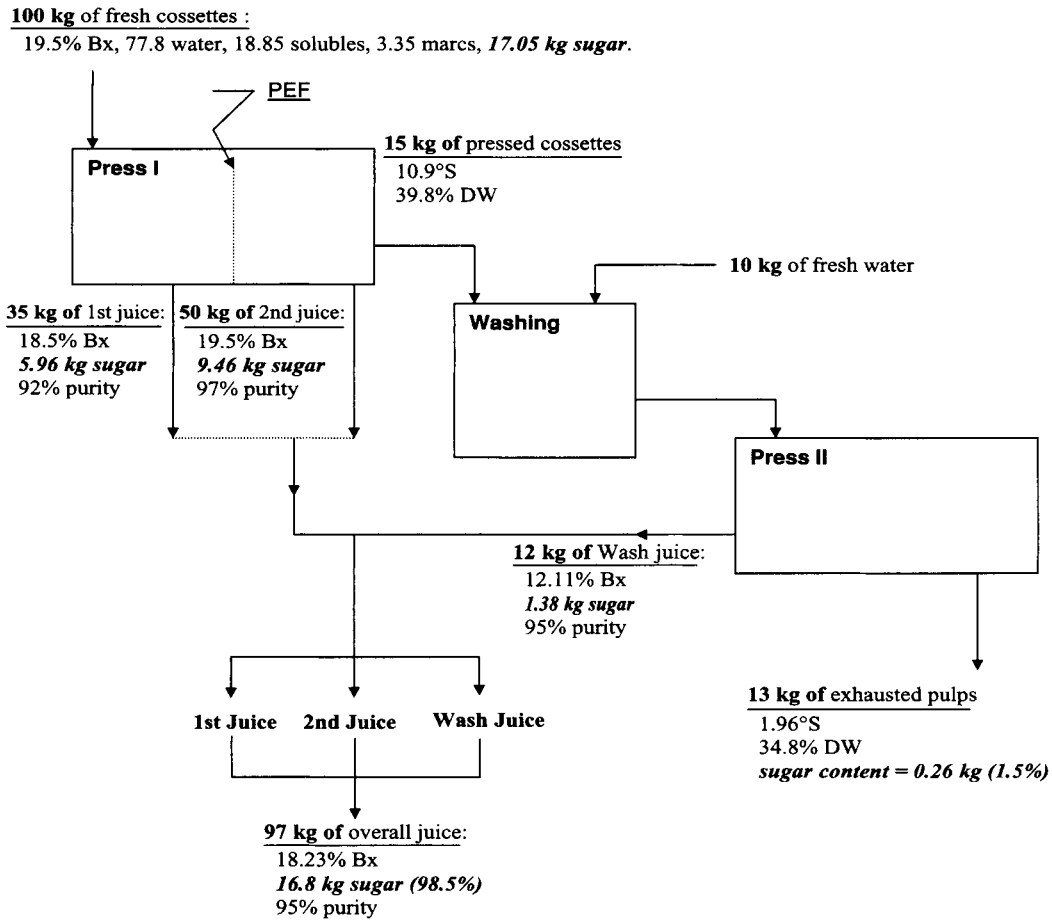

Figure 6.24. Best scheme scenario for a PEF-assisted cold pressing of sugar beet cossettes (Jemai and Vorobiev, 2002b).

solute extraction from food plant tissue were performed (Jemai and Vorobiev, 2002a,b, 2003; Fincan *et al.*, 2004; Chalermchat *et al.*, 2004; El-Belghiti and Vorobiev, 2004, 2005a,b).

4.1. Models for PEF Treatment Enhanced Diffusion

For the infinite slab geometry, the diffusion coefficient can be estimated from Fick's diffusion equation:

$$y = \frac{c_s - c_{s\infty}}{c_0 - c_{s\infty}} = \sum_{n=1}^{\infty} A_n e^{\lambda_n t}, \tag{6.14}$$

where $\lambda_n = -Dq_n^2/l^2$, l is the solid thickness/2, y is the concentration ratio, c_s, c_0, and $c_{s\infty}$ are the actual, initial, and final (at $t = \infty$) solute concentrations, respectively, in the cellular juice of solid, D is the diffusion coefficient. For solute diffusion to a large volume of well-stirred solution, $q_n = (2n-1)\pi$, $A_n = \frac{8}{(2n-1)^2\pi^2}$, and $c_{s\infty} = 0$ (Schwartzberg and Chao, 1982).

Jemai and Vorobiev (2002a) have demonstrated that a moderate pulsed electric field exerts an enhancing effect on the diffusion of soluble substances from the apple tissue. The experimental

technique consisted of measuring kinetics of diffusion from apple discs (Golden delicious) after their different pretreatments: (1) thermal denaturation (75°C, 2 min) followed by diffusion (60 min) at different temperatures (20, 40, 55, 60, and 75°C); (2) electric field pretreatments (1000 pulses of 50, 100, and 200 μs duration at various PEF intensities) before diffusion at 20°; and (3) standard electric field pretreatment (1000 pulses of 100 μs duration and 500 V/cm field intensity) before diffusion (60 min) at 20, 30, 40, 50, and 75°C. Diffusion took place in a temperature-controlled water bath with agitation.

Diffusion in electrically treated samples at 20°C revealed that a detectable enhancement of the diffusion kinetics starts at the field intensities of 100–150 V/cm. The further increase in both of the field intensity and pulse duration leaded to enhancement of the diffusion kinetics. Jemai and Vorobiev (2002a) showed that for thermally treated samples, the temperature variation of the diffusion coefficient D is of Arrhenius type with two diffusion regimes: (1) without thermal pretreatment ($E_a \sim$ 28 kJ/mole) and (2) after thermal denaturation ($E_a \sim 13$ kJ/mole). Only one regime with intermediate activation energy ($E_a \sim 20$ kJ/mole) was observed for electrically treated samples.

El-Belghiti and Vorobiev (2004) have demonstrated that the Fick's diffusion law can be insufficient to explain the overall mass transfer from the PEF-treated plant tissue. During the PEF treatment, some of the cellular juice release through the electropermeabilized membranes to exterior of the cells, thus humidifying the tissue surface. When following contact of the humidified tissue with water occurs, the solute convection to surrounded water is more rapid than the internal diffusion phenomena.

A two-exponential kinetic model was proposed to describe extraction from the plant tissue treated by PEF to a limited volume of solution (El-Belghiti and Vorobiev, 2004). This model involves two simultaneous processes, each having different kinetics: (1) washing, which corresponds to a high rate of transfer from tissue surface to the solvent and (2) diffusion having lower rate of transfer from the plant tissue and becoming significant when the washing stage is completed. These two mechanisms were treated separately and then summed up to obtain the following equation:

$$c^* = c_w^*[1 - \exp(-k_w t)] + c_d^*[1 - \exp(-k_d t)], \tag{6.15}$$

where $c^* = c/c_\infty$, c is the actual solute concentration in solution, c_∞ is the theoretical equilibrium solute concentration in solution $c_\infty \cong c_0/(i+1)$, i is the ratio of liquid to solid; $c_w{}^* = c_w/c_\infty$, c_w is the equilibrium solute concentration in solution due to the washing stage alone; $c_d{}^* = c_d/c_\infty$, c_d is the equilibrium solute concentration in solution due to the diffusion stage alone; k_w is the kinetic coefficient for the washing stage in min^{-1}; k_d is the kinetic coefficient for the stage of diffusion in min^{-1}; and t is the time in min. If the total quantity of solute is available for extraction, the value of $(c_w{}^* + c_d{}^*)$ is 1.

Figure 6.25 shows kinetics of sugar extraction from electrically treated sugar beet slices, obtained on a 6-mm grater (El-Belghiti *et al.*, 2005). Extraction occurred to a limited volume of well-stirred surrounded water ($i = 3$) kept at the ambient temperature of 25°C. As it can be seen from Fig. 6.25, nearly 40% of extracted solute were attained after 2 h of extraction even in the absence of PEF. It indicates that some cells were broken mechanically during slicing of the beet. The yield of solute was significantly increased with PEF treatment: it attained 93% after the PEF treatment at 670 V/cm and 2 h of extraction. Majority of the cellular membranes were probably permeabilized at this value of PEF intensity. Further increase of PEF intensity up to 800 V/cm was not effective (Fig. 6.25).

Data presented in Fig. 6.25 were fitted by two-exponential equation (6.15) with correlation coefficient $r^2 = 0.996$. The model values of coefficients are shown on Fig. 6.26a,b. As it can be

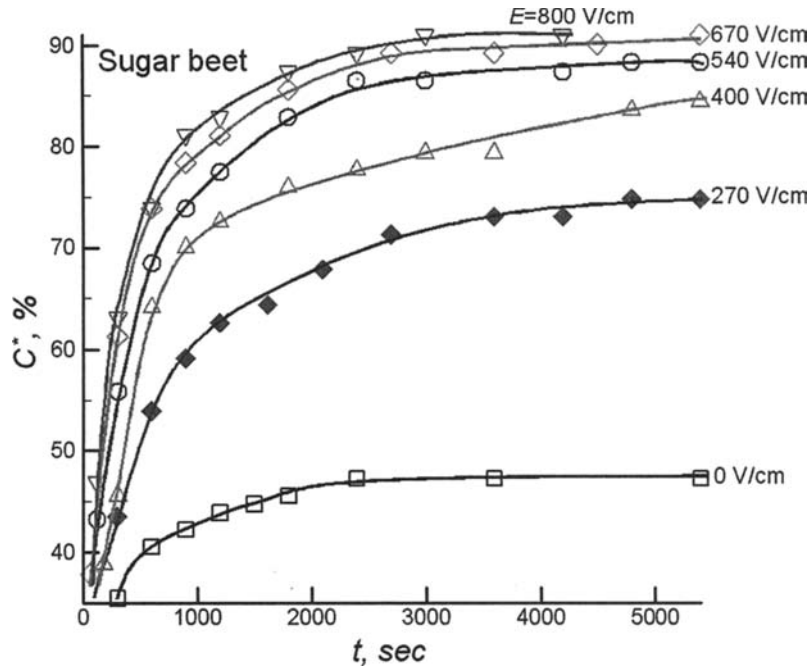

Figure 6.25. Yield of solute C^* during extraction from slices treated at $t_i = 100$ μsec, $\Delta t = 10$ msec, $n = 1000$ pulses and different field intensities E (El-Belghiti *et al.*, 2005a).

seen from Fig. 6.26a, the values of coefficients c_w^* and c_d^* increase up to $E = 670$ V/cm as PEF intensity increases. It means that the quantity of extracted solute increases with increase of the PEF intensity. Comparison of the said coefficients indicates that the higher quantity of solute was extracted during the initial washing stage of extraction ($c_w^* > c_d^*$). Figure 6.26b shows that values of the kinetic coefficients k_w and k_d are almost independent from the PEF intensity. Comparison of these coefficients indicates that the first stage of extraction is significantly more rapid than the second (diffusion) stage.

Experimental data, presented in Fig. 6.25, were fitted to calculate the diffusion coefficient D from Eq. (6.14) (El-Belghiti *et al.*, 2005). For this purpose, the initial period of rapid solute extraction was neglected and only data of the steady-state period of extraction, when $Dt/l^2 > 0.13$ (Schwartzberg and Chao, 1982), were taken into account. Results, presented in Table 6.3, show that D does not depend on PEF intensity and values of $-\lambda_1 = \pi^2 D/l^2$(Eq. (6.14)) correspond well to values of the kinetic coefficient k_d (Fig. 6.26b).

4.2. Influence of PEF Protocol, Temperature, and Fragmentation of Particles on the Extraction Kinetics

El-Belghiti and Vorobiev (2005a) have also investigated influence of PEF duration on the extraction kinetics. On fixed intensity of PEF (670 V/cm) and number of pulses increasing from 50 to 250 ($t_i = 100$ μs, $\Delta t = 10$ ms), the yield of solute was increased from 60 to nearly 93%. However, further increase of the number of pulses up to 1000 did not improve the yield of solute. This result shows inefficiency of long PEF treatment times. For PEF treatment duration

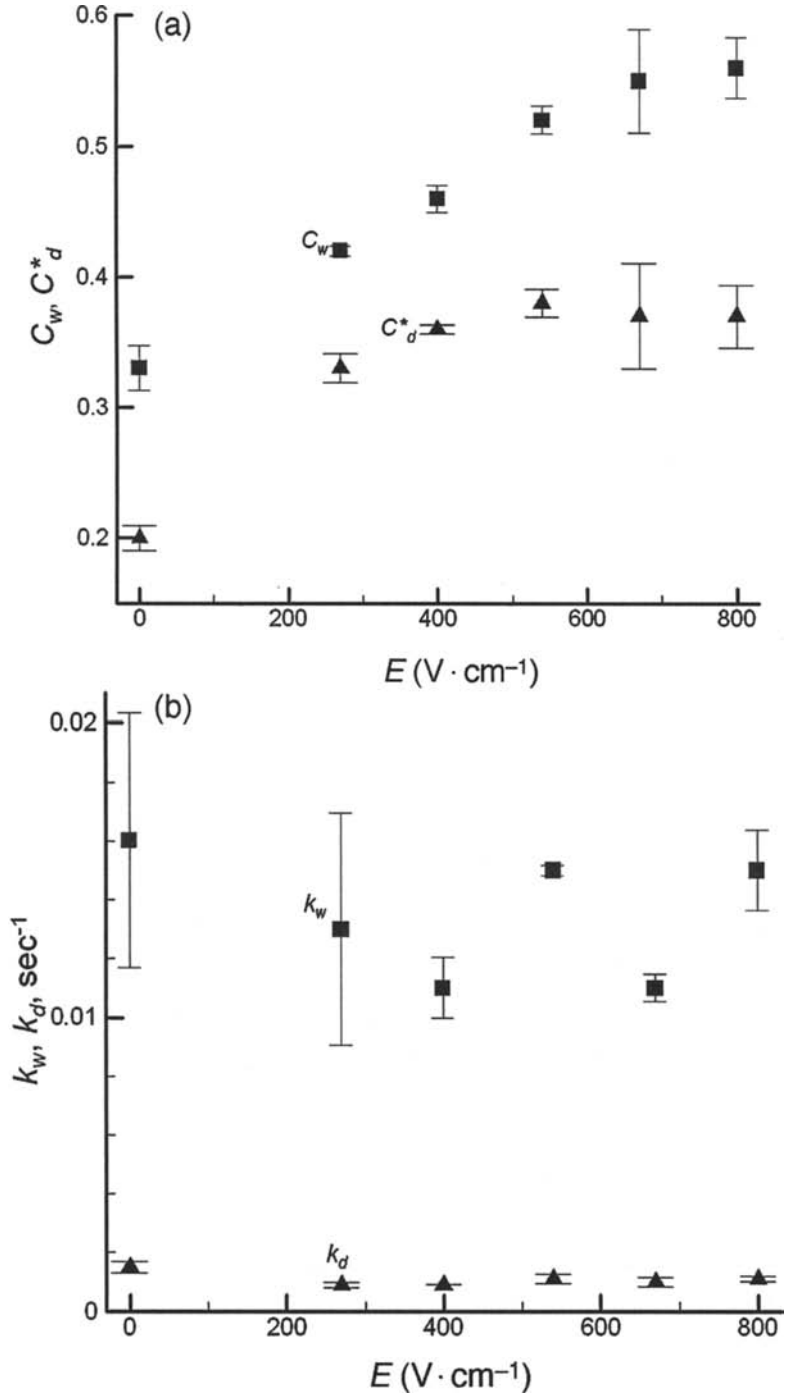

Figure 6.26. Effect of the PEF intensity E on the values of model coefficients: □, c^*_w; ■, c^*_d (a), ◊, k_w ♦, k_d (b). The slices were treated at $t_i = 100$ μs, $\Delta t = 10$ ms, $n = 1000$ pulses (El-Belghiti *et al.*, 2005).

Table 6.3. Diffusion coefficient as a function of PEF intensity at 1000 pulses.

PEF intensity (V/cm)	0	270	400	540	670
D (10^{-10} m^2/s)	1.68 ± 0.40	1.56 ± 0.23	1.3 ± 0.37	1.82 ± 0.12	1.74 ± 0.48
$-\lambda_1(10^{-3}$ s$^{-1})$	0.0013 ± 0.0002	0.001 ± 0.0001	0.0008 ± 0.0001	0.0011 ± 0.0001	0.0011 ± 0.0002
r^2	0.99	0.98	0.95	0.97	0.99

$t_{PEF} = t_i \cdot N = 10^{-4} \cdot 250 = 0.025$ s corresponding to 250 pulses, effective permeabilization of the cellular membranes was attained.

To enhance extraction kinetics after the PEF treatment, El-Belghiti *et al.* (2005) provided extraction at different moderate temperatures (30, 40, and 50°C). For instance, about 70 min was needed to attain the 93% yield of solute at ambient temperature and around 40 min to obtain the same yield at 50°C. Both kinetic coefficients k_w and k_d as well as the diffusion coefficient D increased with temperature increase.

Energy provided by PEF is considered to be united parameter accounting both for PEF intensity and duration (number of pulses). El-Belghiti and Vorobiev (2005b) studied influence of the energy provided by PEF on the kinetics of extraction from carrot slices obtained by grating carrot in a 6-mm grater (1.5 ± 0.05 mm in thickness) (Fig. 6.27).

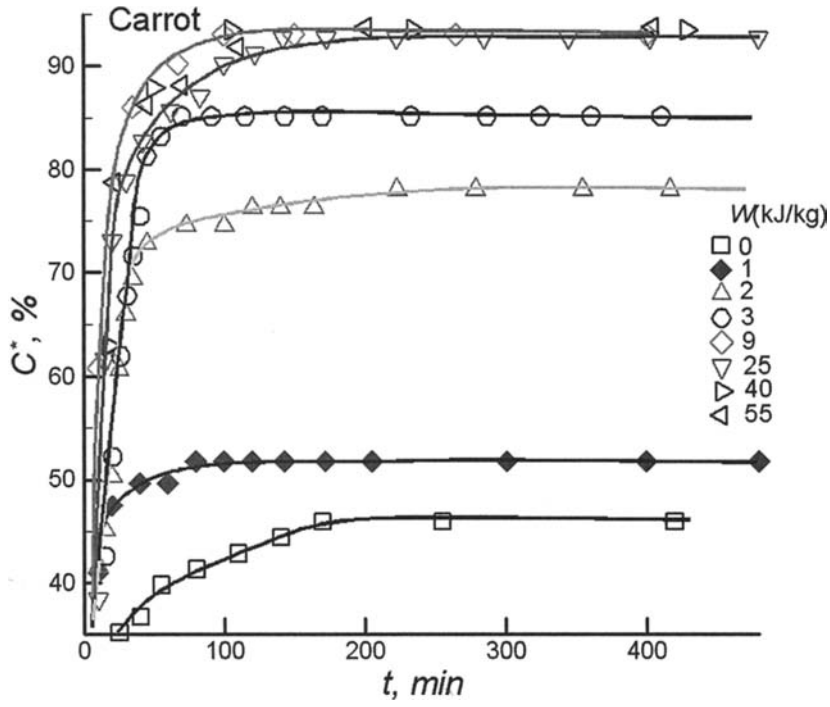

Figure 6.27. Yield of solute C^* from coarse carrot slices treated under stirring speed of 250 min^{-1} with different energy supply W (El-Belghiti and Vorobiev, 2005b).

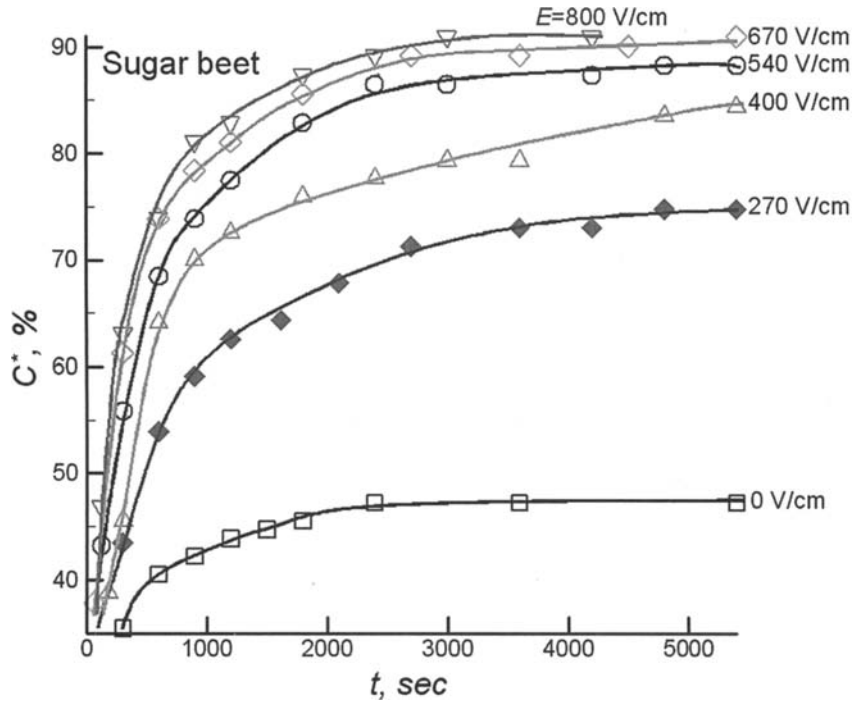

Figure 6.28. Yield of solute during extraction from samples treated by PEF with energy supply 99 (kJ/kg) (El-Belghiti and Vorobiev, 2005b).

It can be seen from Fig. 6.27 that in the absence of PEF pretreatment only 45% of the solute was obtained from coarse slices after 8 h of extraction. On increase of energy provided during the PEF pretreatment, the quantity of the extracted solute increased accordingly, until the threshold value of 9 kJ/kg was attained. No further enhancement of the solute yield was observed above this threshold. It can be assumed that this energy is sufficient for electropermeation of the majority of cells.

In order to compare the effect of sample fragmentation on the extraction kinetics, El-Belghiti and Vorobiev (2005b) conducted experiments with coarse slices, fine slices, and disc with thicknesses of 0.5, 1.5, and 8.5 mm, respectively. The energy provided by PEF was fixed as W of 9 kJ/kg.

Figure 6.28 indicates that fine and coarse slices showed almost the same extraction kinetics. About 2 h were needed to attain nearly the stabilized solute yield for both types of slices. Extraction was considerably slower for the disc sample. The two-exponential model (Eq. (6.15)) was used to estimate the effect of both convection and diffusion stages of extraction on the total solute yield. In the case of slices, the majority of solute was extracted by convection during the washing stage ($c_w^* = 0.70$ and 0.73 for coarse and fine slices, respectively). Nearly all the remaining quantity of solute was extracted by diffusion ($c_d^* = 0.24$ and 0.20 for coarse and fine slices, respectively). On the contrary, for the disc sample only 25% of solute was extracted during the rapid washing stage ($c_w^* = 0.25$), and the remaining solute was extracted slowly by diffusion ($c_d^* = 0.62$). As a result, the quantity of solute available for extraction was lower for the disc sample with ($c_w^* + c_d^*$) equal to 0.87. It can be suggested that some cells of the disc sample were not destroyed by the PEF pretreatment. These intact cells did not contribute to the solute transfer. The difference in the values of coefficient

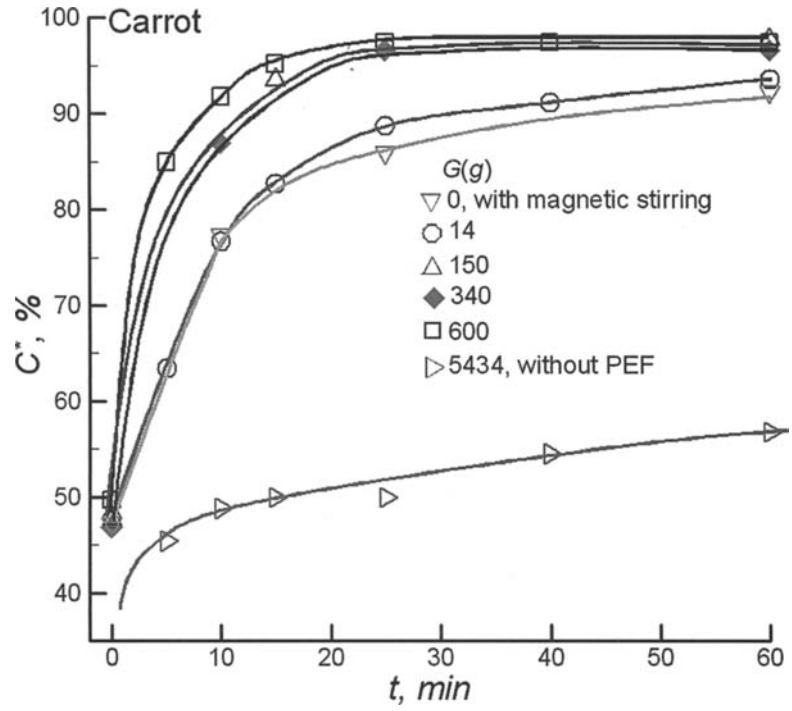

Figure 6.29. Yield of solute during centrifugal extraction from the PEF-treated ($E = 670$ V/cm, $n = 300$, $t_i = 100$ μsec, $t_{PEF} = 0.03$ sec, $\Delta t = 10$ msec) carrot slices under different centrifugal accelerations G (El-Belghiti and Vorobiev, 2005c).

k_w was not significant. More noticeable difference was found for the coefficient k_d, which reflects the slower diffusion of solute from the disc sample compared to slices (El-Belghiti and Vorobiev, 2005b).

Acceleration of extraction from electropermeabilized carrot tissue was attained using centrifugal extraction (El-Belghiti and Vorobiev, 2005c). Figure 6.29 presents solute concentration during aqueous extraction at different centrifugal accelerations from both electrically treated ($E = 670$ V/cm, $t_i = 100$ μs, $\Delta t = 10$ ms, $n = 300$ pulses) and nontreated carrot slices. In the absence of PEF only a 50% yield of solute was obtained after 60 min of extraction under the high centrifugal acceleration of $5434g$ (Fig. 6.29). This amount of solute was extracted from mechanically broken cells. At the same time, the yield of solute in presence of the PEF was significantly higher and reached 97% after 60 min of extraction even at low centrifugal acceleration ($14g$). It can also be noted that there exists an acceleration threshold ($150g$) beyond which no further enhancement of the solute concentration was observed. At this acceleration threshold, the solute concentration of 97% was reached after 25 min of extraction.

The moderate heating permits further enhancement of the extraction kinetics. El-Belghiti and Vorobiev (2005b) carried out the centrifugal extraction at various temperatures (18, 25, and 35°C) and fixed centrifugal acceleration ($150g$). The final yield of solute (97%) was identical for all the temperatures. However, this yield was attained after 15 min at 35°C against 40 min at 18°C. It was concluded that within the studied range of temperature it influenced only the extraction kinetics and did not have any apparent effect on the total amount of extracted solute.

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PART III

APPLICATIONS AND EQUIPMENTS

CHAPTER 7

APPLICATIONS OF PULSED ELECTRIC FIELDS TECHNOLOGY FOR THE FOOD INDUSTRY

Stefan Toepfl, Volker Heinz, and Dietrich Knorr

1. INTRODUCTION

It has been shown that exposing biological cells to an electric field provides a potential to influence permeability of cell membranes and to induce structural changes and a local membrane breakdown. Based on this phenomenon called electroporation a broad variety of applications in food processing and bioprocessing have been studied since the 1960s (Doevenspeck, 1960; Sale and Hamilton, 1967; Flaumenbaum, 1968). In plant or microbial genetics an electroporation is used to induce foreign material such as DNA into cells. The process of reversible pore formation has to be perfectly controlled to maintain viability of the organisms during the application of the PEF. This principle can also be utilized to induce stress reactions and secondary metabolites biosynthesis, which can be desirable food constituents.

Increasing treatment intensity an irreversible membrane breakdown of plant, animal, or microbial cell membranes can be achieved. Disintegration of biological cells and break of permeability barrier often is a crucial pretreatment step to improve mass transfer rates. A PEF treatment can be utilized to substitute conventional disintegration techniques such as grinding, thermal, or enzymatic treatments prior to processes such as drying, extraction, or pressing. Pore induction by PEF can, dependent on treatment intensity, also cause a loss of vitality and be utilized to achieve an inactivation of microorganisms in liquid media.

Several attempts to achieve an industrial exploitation have been made (Toepfl *et al.*, 2005a), in 2006 the first commercial application of the technique has been reported (Clark, 2006). Within this chapter potential applications will be shown along with estimations of costs for operation and investment and a discussion of advantages, challenges, and pitfalls of this emerging membrane permeabilization technique.

2. PERMEABILIZATION OF CELL MEMBRANES

It has been shown that an electroporation of biological membranes can be achieved by exposure to an external electrical field (Sale and Hamilton, 1967; Zimmermann *et al.*, 1974). The underlying

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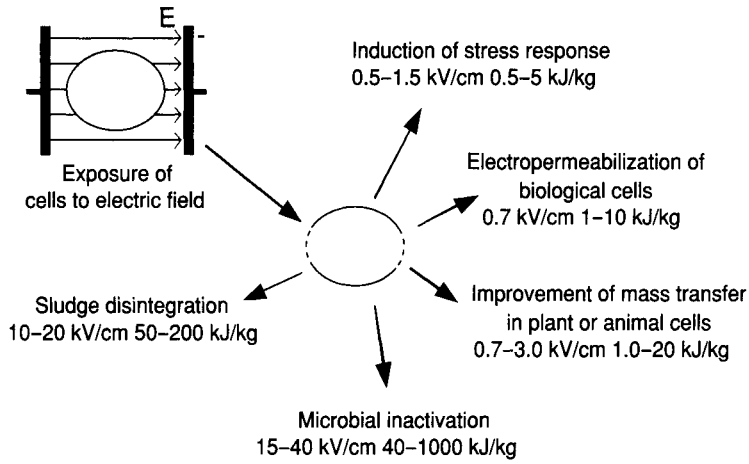

Figure 7.1. Exposure of biological cells to an electric field and applications in food, bio, and wastewater processing with typical electric field strength and energy input requirements.

mechanisms of action have not been fully elucidated up to now, the theory of electromechanical breakdown of the cytoplasmatic membrane is generally accepted but the precise mechanisms of membrane permeabilization and microbial inactivation are not well understood. A review of theories and mechanisms discussed can be found in Chapter 3. The intrinsic properties of the cells or the cellular tissue to be treated determine the processing parameters required for a successful and efficient electroporation. A cell placed in an electric field can be regarded as a capacitor filled with material with a low dielectric constant of $\varepsilon \approx 2$. When exposed to an external electrical field an accumulation of charges, due to movement of ions along the electric field lines, will increase the natural transmembrane potential of the cells. Exceeding a transmembrane potential in a range of 1 V has been shown to cause formation of a pore (Zimmermann *et al.*, 1976). A drastic increase in permeability re-establishes the equilibrium of the electrochemical and electric potential differences of the cell plasma and the extracellular medium. The electric breakdown can be reversible if the pores induced remain small in comparison to the membrane area. An increase of treatment intensity, in terms of electric field strength and pulse width or number will promote formation of larger pores and reversible damage will change into irreversible breakdown. In this case the increase of permeability is impairing on physiological control systems of the cell such as osmoregulation, resulting in a loss of viability.

The occurrence of a critical electrical field strength has been shown, dependent on size and geometry of the cell. For plant or animal cells, which have a diameter in the range of 120 μm an external field of 1–2 kV/cm is required to achieve a cell permeabilization (Angersbach *et al.*, 2000). For electroporation of microbial cells with characteristic diameters from 1 to 10 μm the external field strength necessary is in the range of 10–15 kV/cm (Sale and Hamilton, 1967; Pothakamury, 1995). Treatment parameters and treatment intensity are determined by the type of cell to be permeabilized and the type of PEF application. An overview of possible applications of PEF in food processing and bioprocessing along with typical electric field strength and specific energy requirements is shown in Fig. 7.1. For induction of stress response treatment intensity has to be carefully controlled to maintain cell vitality and to allow formation of secondary metabolites. For improvement of mass transfer and for microbial inactivation treatment intensity has to be increased to achieve an irreversible membrane breakdown and a loss of its barrier function. The electric field strength has been identified as one main processing parameter, a cell-specific threshold has to be exceeded to induce a membrane pore. Further increasing the electric field strength has been found to enhance the efficiency of the treatment (Boyko *et al.*, 1998; Heinz *et al.*, 1999; Heinz and Knorr, 2000; McDonald *et al.*, 2000). Apart from the peak

electric field strength the treatment time, product of pulse width, and pulse number have often been used to evaluate treatment intensity (Hülsheger *et al.*, 1983; Zhang *et al.*, 1995; Barbosa-Cánovas *et al.*, 1999). Increasing the treatment time resulted in more efficient microbial inactivation. The specific energy input has been suggested as an alternative parameter to describe treatment intensity, it can be calculated by the electric energy delivered to the product and its flow rate. As the energy is dissipating into the media the energy input can also be estimated by the temperature increase during the treatment. From a processing point of view the energy input seems to be advantageous as it can also indicate the costs of operation.

3. APPLICATIONS OF PEF TECHNOLOGY FOR THE FOOD INDUSTRY

The application of PEF in food processing gained considerable attention within the last decades, utilizing its impact on cell membranes. Apart from preservation the disintegration of biological tissue is often a key step in food processing prior to winning of intracellular compounds. It is noteworthy that an electropermeabilization can be performed continuously and in a time-scale of seconds, the treatment therefore can easily be implemented into existing processing lines. Within this section the applicability of PEF for different purposes will be shown along with cost estimations and a discussion of technical feasibility.

3.1. Induction of Stress Response

The application of a low intensity treatment, though initiating formation of a conductive channel across the membrane does not necessarily cause irreversible cell rupture. For example, for potato tissue a pore formation was observed when a transmembrane potential of 1.7 V was achieved, but electrically insulating properties have been found to be recovered within seconds (Angersbach *et al.*, 2000). As vitality and metabolic activity are retained a potential to induce stress reactions in plant systems or cell cultures is provided, as also described for high pressure processing (Dörnenburg and Knorr, 1998). It was shown that metabolic activity can be stimulated and extractability was improved. As a low intensity PEF treatment does not damage proteins or enzymes it can be utilized to release valuable intracellular components from the cells. It was shown that a sublethal treatment of maize germs can influence the production of phytosterols (Guderjan *et al.*, 2005), after a treatment of soybeans and olives an increased content of isoflavonoids was found. The application of PEF can provide an eminent potential to activate and stimulate cells as "bioreactors," to produce high quality food or food ingredients. Ye *et al.* (2004) have shown enhanced accumulation of taxuyunnanine C, a bioactive secondary metabolite, by exposing cells of *Taxus chinensis* to PEF. As the electric field as well as the energy input required is moderate, a PEF system for treatment of cell cultures or tissue is comparably easy to establish, but still many questions remain unanswered. Metabolic activity of cells, complex systems, is influenced by the stress induction, and in addition to desired components also the formation of undesired such as allergens might be stimulated. More research work will be required to exclude adverse effects in particular when the treated product shall be consumed instead of being used for extraction of single ingredients.

3.2. Treatment of Plant or Animal Cellular Tissue

3.2.1. Juice Processing

Disintegration of cellular material, a key step prior to juice winning operations such as extraction or pressing is often performed by an enzymatic maceration, a thermal treatment or a mechanical grinding. These techniques may require a significant amount of thermal or mechanical energy as

well as holding times and storage tanks for enzymatic maceration. Side activities of enzymes (added or natural) and thermal degradation during holding time can cause losses of nutritionally and physiologically valuable compounds and lower product quality. Applying PEF to cellular tissue an increase in mass transfer coefficients was observed due to cell membrane permeabilization (Knorr *et al.*, 1994; Bazhal and Vorobiev, 2000; Fincan *et al.*, 2004). Based on this effect a PEF application can replace or substitute conventional techniques in fruit and vegetable juice processing. First, attempts to apply PEF technology for disintegration of cells have been made by Krupp, Germany, in the 1960s, developing the ELCRACK process (Sitzmann and Münch, 1988) based on experiments conducted by Doevenspeck (1960, 1961). Flaumenbaum (1968) reported a 10–12% increase of juice yield when applying electroplasmolysis to apple tissue. The energy input required to achieve a disintegration of plant cells is in the range of 10–20 kJ/kg, causing a temperature increase below 5°C and indicating that product quality and fresh-like character will be retained in contrast to thermal treatments. Bazhal *et al.* (2001) investigated juice expression of apple under simultaneous mechanical pressing and PEF treatment, a significant increase in juice yield was reported after a treatment at 520 V/cm and 100 μs treatment time. For carrot juice an increase of juice yield from 60.1 to 66.4% was found in comparison to an untreated sample; in the same way the dry matter of the sample was increased from 13 to 15% (Knorr *et al.*, 2001). The influence of a PEF pretreatment of apple mash (Royal Gala variety) prior to pressing was investigated by Toepfl *et al.* (2005b), at a field strength of 0.8–5.0 kV/cm, applying 10–40 pulses. After a treatment at 3 kV/cm and 20 pulses a juice yield of 83.0% was obtained after pressing in comparison to 80.1% after enzymatic maceration and 75.4% for the untreated control. A PEF treatment of grapes resulted in a juice yield of 87%, similar to that after an enzymatic maceration, whereas an increased content of soluble solids and pigments was reported (Eshtiaghi and Knorr, 2000). The beneficial impact of a PEF treatment for wine production was also reported by Sigler *et al.* (2004).

A method has been developed to evaluate the damage of cellular tissue after a PEF treatment (Angersbach *et al.*, 1997, 1999) based on determination of the frequency dependent electric conductivity. It has been shown that a permeabilization of potato tissue can be achieved after a transmembrane potential of 1.3 V for potato and 1.0 V for apple tissue, which can be induced by an external electrical field of 250 and 200 V/cm, respectively. The potential to enhance efficiency by further increasing electric field strength was investigated and three areas of external electrical field strength were defined: a subcritical range below 150 V/cm, a transitional range, and a supercritical range. In the latter one aqueous pore formation was so large that the contribution of the cell membrane properties to the total electric conductivity was negligible and a finite maximum conductivity was reached (Angersbach *et al.*, 2000). The membrane breakdown occurred within the first μs of the pulse, and the membrane polarization time was shorter at higher electric field strength. From an engineering point of view the electric field applied should exceed a value of 1 kV/cm for plant cell tissue and a repetition of pulses should be applied to achieve an irreversible electroporation. The impact of an electroporation on mass transport coefficients in red beet root was investigated by Fincan (2004), suggesting bimodal Fickian diffusion model. Viscoelastic properties of the tissue changed due to loss of turgor and release of intracellular compounds. Lebovka *et al.* (2004, 2005) investigated the stress deformation and relaxation of carrots, potatoes, and apples. It was shown that though formation of pores and loss of turgor a PEF treatment alone does not completely eliminate the textural strength of the tissue similar than after a thermal treatment. It was shown that the press ability of juices from soft vegetables such as potato, after a PEF treatment at field strength of 300 V/cm was improved after a PEF treatment, a pretreatment or a treatment during pressing significantly reduced the time required for pressing.

Application of PEF for juice processing provides a potential to replace or enhance conventional cell disintegration techniques, in particular due to its short residence time and ambient temperature

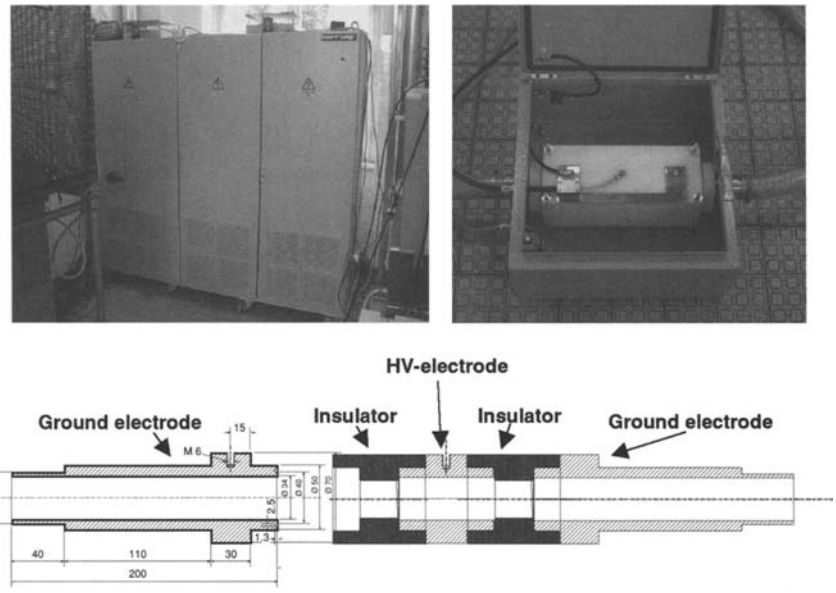

Figure 7.2. Power supply (top left) and treatment chamber (top right) of a pilot scale PEF system for fruit mashes. The treatment chamber is a co-linear configuration of 3 cylindrical electrodes separated by insulators (bottom).

processing. The continuous operability and the high degree of cell disintegration might also allow a continuous liquid–solid separation, e.g., by a decanter centrifuge. In the context of consumer demand for functional food with a composition close to fresh products and a high content of physiologically valuable compounds an increased extractability of intracellular compounds such as pigments or phenolic substances could provide an enormous potential for product development. Fincan *et al.* (2004) investigated extractability of red beetroot pigment after a treatment at 1 kV/cm and an energy input of 7 kJ/kg, a 90% release of color was reported. Jemai and Vorobiev (2002) showed the enhancing effect of a PEF treatment on the diffusion coefficients of soluble substances in apple slices. The results available in literature clearly indicate that PEF can be successfully applied to disintegrate biological tissue and to improve the release of intracellular compounds, though an industrial application has not been achieved up to now. At Berlin University of Technology a system with a peak voltage of 20 kV, an average power of 7 kW and a production capacity of 2 ton/h has been developed for the treatment of fruit mashes. The power supply and the treatment chamber are shown in Fig. 7.2. It is noteworthy that avoiding an enzymatic maceration the pectin fractions will remain in a native, highly esterified structure. This provides a potential to extract high quality pectin from the pomace after juice winning and therefore a step toward a more economic and sustainable processing.

3.2.2. PEF Treatment of Microalgae, Seaweed, and Other Aquatic Species

In addition to winning of fruit juices or extraction of intracellular compounds of interest for food industry, the winning of bioactive substances for cosmetic or biotechnological use gained considerable interest recently. Different varieties of macro- and microalgae are sources of vitamins, pigments, proteins as well as antixodative and bioactive substances (Stolz and Obermayer, 2005). *Chlorella vulgaris*, a protozoic green algae was shown to possess a high content of proteins and minerals and to have a positive influence on collagen synthesis. *Spirulina platensis* contains a high

amount of proteins as well as the Vitamins E, B1, B2, B3, B6, B12, and H as well as carotenoids. Algae extracts are commonly produced by hot water extraction, but a PEF treatment could provide a potential toward a more gentle downstream processing. The extractability of proteins, chlorophyll, and carotenoids as well as protease activity of extracts from *Spirulina* and *Chlorella* after a PEF treatment has been investigated by Koehler *et al.* (2005). An increase of 27, 80, and 52.5% has been found for protein, chlorophyll, and carotenoids content in chlorella extract after a treatment at 15 kV/cm and a specific energy input of 100 kJ/kg, respectively. Antioxidative activity of the extract was increased by almost 100%. In mice cell studies, a higher growth stimulation has been found in comparison to a conventional extract. It is noteworthy, that microalgae disintegration by PEF requires comparable high treatment intensity in contrast to plant or animal cells, as the characteristic diameter of *Chlorella* cells is in the range of a few μm only. A treatment with the parameters given was shown to result in a microbial decontamination of previously inoculated extracts of 7.1, 6.0, and 4.1 log cycles of *Escherichia coli*, *Bacillus subtilis*, and *Candida utilis*, respectively.

In contrast to microalgae multicellular algae such as Kelp (*Laminaria digitata*) with larger cell diameters in a range of 100 μm can be electroporated with processing parameters similar to that of plant cells. The extractability of growth hormone formulations from Kelp after a PEF treatment has been investigated by Heinz and Klonowski (unpublished data), indicating an improved yield after pressing. The expressible moisture of seaweed, a very tough material rich of alginate, was increased from 2 to 6.5% after a PEF treatment (Hafsteinsson *et al.*, 2000). The potential of a PEF treatment on aquatic nuisance species such as zebra mussels, hydrozoans, or barnacles was reported by Schoenbach *et al.* (1996). After a treatment of tidal water at a field strength of 12 kV/cm biofouling was prevented, indicating that a PEF treatment can be utilized to protect lake or river water operated cooling systems from clogging due to biofouling. Abou-Ghazala and Schoenbach (2000) showed that even at treatment intensities as low as 1 kV/cm and an energy input in range of 16 kJ/kg, 90% of barnacles could be inactivated. A 100% protection against fouling was obtained after a treatment at 6 kV/cm and an energy input of 560 kJ/kg. Energy efficiency was found to be increased when reducing pulse width of the rectangular pulses from 10 to 0.5 μs.

3.2.3. Plant Oil Extraction

Sitzmann and Münch (1988) reported an enhanced separation of tankage emulsions when extracting protein and fat from animal tissue. A similar effect can be expected after PEF treatment of oil seeds prior to recovery. Yield and quality of oils from plant origin has been studied by Guderjan *et al.* (2005) and a modified processing scheme for production of maize germ oil with an increased amount of phytosterols and high oil yield was developed. Wet milled corn, steeped for 48 h at 30, 40, and 50°C in water, was treated at a field strength of 3.0 kV/cm, 120 pulses. Subsequently, oil was separated by hexane extraction, pressing, and supercritical CO_2 extraction. At an electrical field strength of 3.0 kV/cm and a steeping water temperature of 50°C the oil yield could be increased by 27.8% for hexane extraction, 25.2% for pressing, and by 14.9% for supercritical CO_2 extraction.

A low intensity treatment at a field strength of 600 V/cm was shown to induce secondary metabolites formation, the phytosterol content was increased from 785 to 929 mg/100 g oil. Different fractions of maize germ hulls have been analyzed regarding their phytosterol content and it was shown that fine hull fractions can be used to improve the physiological value of maize germ oil. Oil recovery from olives was improved by 7.4% after a PEF treatment at 1.3 kV/cm in comparison to the control sample; in soybean oil an increase in isoflavonoid content was reported. Treatment of rapeseed at a field strength of 5 kV/cm and 60 pulses increased oil yield from 34 to 42% after pressing in a lab-scale screw press.

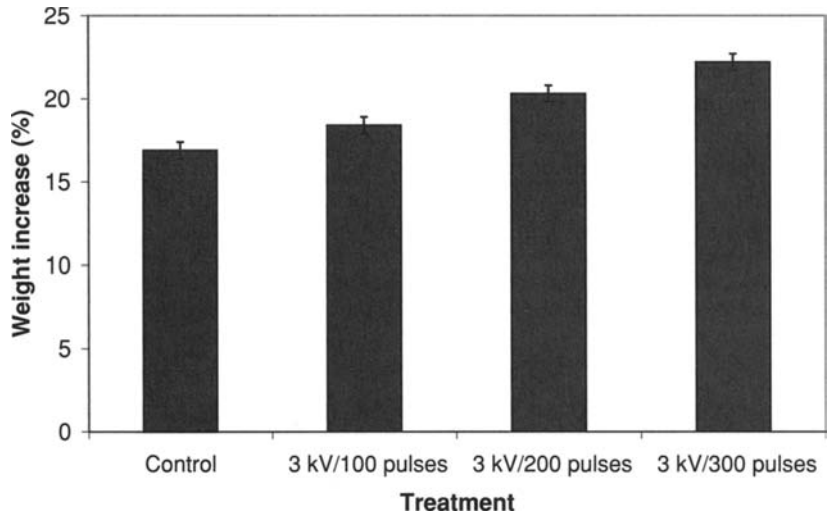

Figure 7.3. Weight increase of brine marinated cod fillets after a PEF treatment in comparison to untreated sample (adapted from Hafsteinsson *et al.*, 2000).

3.2.4. Meat and Fish Treatment

Disintegration of animal cellular tissue might also be utilized to enhance processes where an uptake of substances is required, such as marination or curing of fish or meat products. Salt or a combination of salt and antimicrobial agents such as nitrite is often applied for preservation, spices are added to develop product-specific taste and aroma profiles. In case of raw ham such as Parma- or Serrano-type ham a long-term curing and air drying is applied. During such procedures a PEF treatment can be applied to improve mass transfer processes and to accelerate curing, reducing the time requirements. Not many research work is available in this field of PEF application, but recently the interest in the potential of PEF application for meat and fish products increased. In contast to plant material or liquid food the effect of a PEF treatment on protein-based, solid food structures such as meat and fish tissue or protein gels has been studied in a few reports only (Sitzmann and Münch, 1988; Hafsteinsson *et al.*, 2000; Barsotti *et al.*, 2001; Gudmundsson and Hafsteinsson, 2001). As shown in Fig. 7.3, the weight increase of brine marinated cod fillets was enhanced after a PEF treatment in comparison to the control sample (Hafsteinsson *et al.*, 2000). The membrane permeabilization resulted in an enhanced mass transport of brine and weight increase. The impact of a PEF treatment on chicken muscle as well as salmon meat has been investigated by Gudmundsson and Hafsteinsson (2001). A treatment at 1.36 kV/cm and 40 pulses resulted in a reduction of cell size and gaping between cells. Research work on feasibility of a PEF treatment in meat processing is currently performed at Berlin University of Technology, it was shown that the fermentation of raw sausages (Salami type) can be accelerated by improving the availability of intracellular liquid for fermenting cultures. The time required to lower the pH by lactic acid formation was found to be reduced (Fueller *et al.*, unpublished data) after PEF treatment of the minced meat at a field strength of 2 kV/cm and an energy input of 10 kJ/kg. A treatment of whole pig haunches during production of Serrano type ham revealed, as a preliminary result, an increased uptake of salt subsequent to a PEF treatment after hand salting, as diffusive mass transport along a concentration gradient is enhanced after breaking the cells permeability barrier. During meat processing salt as well as sodium nitrate are applied for preservation, reduction of water activity as well as meat curing. During production of cooked ham such as Prosciutto commonly salt brine is injected to improve water binding capacity.

It has been shown that after a PEF treatment at 2 kV/cm and 100 pulses the brine uptake as well as distribution within the tissue of pork meat can be significantly improved after injection of brine in a commercially available injector (Toepfl *et al.*, 2005c). Because of more homogeneous salt distribution in the tissue its water binding capacity has been improved, resulting in a reduction of weight loss during cooking as well as softer structure of final product. The impact of a PEF treatment on pork muscle is was investigated by electron micrographs, showing the formation of a swollen, sponge-like tissue structure (Fig. 7.5).

3.2.5. Drying Enhancement

Removal of water to preserve food products such as fruits or vegetable accounts for a significant amount of energy costs in food processing. The electropermeabilization of cell membranes, leading to a drastic increase in mass transfer rates, can be utilized to enhance drying rates of cellular tissue. The drying rates of osmotic drying and the diffusion coefficients of carrots were investigated by Rastogi *et al.* (1999). After a PEF treatment at a field strength of 1–2 kV/cm the time required for fluidized bed drying of potato cubes was reduced. For apple slices an increased osmotic drying rate and improved rehydration capacity as well as shorter rehydration times were reported (Taiwo *et al.*, 2002). Ade-Omowaye *et al.* (2001) investigated the relationship between a PEF treatment and drying rates of plant-based foods during osmotic dehydration and air drying. As mass transport across the cell membrane is influenced by a PEF treatment, it can be utilized to significantly increase mass transfer rates by 10–30% when applying 5–20 pulses at a field strength of 1.0 kV/cm. For convective air drying at 60°C and an airflow of 1 m/s a reduction of drying time of 20–30% was reported, while maintaining other drying parameters constant. The energy required for evaporation of water is, dependent on temperature and pressure in the range of 2.5–2.7 MJ/kg, but total energy input required for conventional drying is in the range of 4–6 MJ/kg of removed water dependent on thermal efficiency of the drying system (Singh, 1986). Dependent on dryer type, heat and mass transfer within the product and losses on heating side as well as to surroundings cause drying efficiencies in the range of 40–70%. An increase in mass transfer rates, resulting in faster water transport to the product surface and therefore reduction of drying time after a pretreatment will lead to drastic saving of energy and better utilization of production capacities during convective air drying. Taking into account the low energy input required for a PEF treatment of plant or animal tissue (2–20 kJ/kg), it is evident that there is a potential to reduce the total energy input for product drying.

3.2.6. Sugar Processing

Conventional procedures for production of sugar from beets involve an extraction at elevated temperatures (68–72°C) after carving the fruits into cossettes. For disintegration and destruction of cell membranes a thermal treatment at temperatures in the range from 70 to 78°C is applied. The membrane denaturation results in an acceleration of sugar release into the extraction media, but also cell wall components such as pectin may become soluble and can diminish juice purity and quality. In addition, the thermal denaturation as well as the hot water extraction require a significant amount of energy, as high as 175 kJ/kg of treated beet (Schultheiss *et al.*, 2002). It has been shown that mechanically pressed, raw juice has a higher sugar concentration and contains less nonsugars, but juice yield remains unacceptable (Bliesener *et al.*, 1991). A PEF treatment of sugar beets prior to extraction could increase mass transfer rates and could allow to reduce extraction temperatures or to apply mechanical pressing. The applicability of a PEF pretreatment prior to an extraction at ambient temperature has been investigated by Eshtiaghi and Knorr (2002). It was shown that after a PEF treatment at 2.4 kV/cm and a pulse number of 60, a similar cell disintegration than after a

thermal treatment at 75°C for 15 min was obtained. A three-step pressing at a pressure of 5 MPa and intermediate addition of water was suggested to achieve a high sucrose content juice after a short processing time of 30 min in comparison to up to 90 min for thermal extraction. The energy input required was 12 kJ/kg (Eshtiaghi and Knorr, 1999). Bouzrara and Vorobiev (2000) reported a juice yield of 78% after application of 1000 pulses with a peak voltage of 1.2 kV. The specific energy input required was in the range of 100 kJ/kg. From an engineering point of view this high energy input could be significantly reduced by selection of appropriate processing parameters. Schultheiss *et al.* (2004) reported an increase of juice yield by a factor of 2.1 after a PEF treatment in comparison to an untreated sample. In contrast to a thermal denaturation the energy input required was in the range of 2–3 kJ/kg to achieve similar juice yield. Raw juice quality was maintained or improved even when using low quality beet as raw material. El-Belghiti *et al.* (2005) developed a two-exponential kinetic model to describe diffusion during extraction of sugar. Optimum PEF processing parameters have been identified as 670 V/cm and a pulse number of 250. A comparison of juice quality to juice from thermally treated beet revealed superior juice quality. These results indicate the tremendous potential of PEF application for sugar processing, in particular against the background of changing legislative situation in the European Community. The reform of the sugar sector in Europe (EU IP/04/915) will remove or at least reduce trade barriers as well as export subsidies, thus reduction of energy requirements and production costs will be inevitable to stabilize the sales of beet sugar in competition to cane products, and PEF could provide an alternative to conventional processing. But considering the production scale of today's sugar refineries—a total average production of 104,644 ton/day has been reported for the nine production sites of Südzucker AG (Kraus, 2003)—it is obvious that the PEF equipment available at present is far away from reaching these capacity levels in the range of up to 500 ton/h per refinery. To treat an amount of 500 ton/h of sugar beet suspended in water with a packing density of 500 kg/m^3 an amount of 1000 ton/h has to be treated. Limiting the flow velocity below 1 m/s a cross section of 2700 cm^2 is required, corresponding to treatment chamber diameters in range of 600 mm. It is evident that a peak voltage of above 200 kV will be required to operate at a field strength of 3 kV/cm when including losses in switching system and connections. The average power consumption required to achieve an energy input of 10 kJ/kg is 2.7 MW. It remains questionable if a power supply with these ratings and in particular a pulse modulator with these power ratings can be integrated into an existing refinery. To limit losses the power supply needs to be set as close as possible to the treatment chamber, but at same hand legal and constructional restrictions have to be accounted when operating at 200 kV. Even when separating the total flow to several lines with capacities of 100 ton/h and limiting the diameter to 300 mm (below clogging might become a problem) a load voltage in the range of 100 kV remains. Up to present pulsed power systems with these power ratings have not been successfully used for PEF application, in particular the pulse modulation has proven to be a challenging task. Dependent on the length of the treatment chamber a minimum pulse repetition rate will be required. To keep the load resistance high the electrode area should be as small as possible, resulting in shorter residence time and higher necessary repetition rate. To provide a pulse with a peak voltage in the range of 100 kV a MARX-Generator (Marx, 1923) has to be applied, as only few switches can handle such parameters with any reliability (Kuthi *et al.*, 2003). Prior to large industrial scale application of PEF as required for sugar processing the development of systems with production scale in a range of 10–20 ton/h and their successful exploitation will be required to confirm the technical feasibility.

3.2.7. Energy Requirements for Tissue Disintegration

The tissue softening effect of PEF, based on cell membrane electropermeabilization and loss of turgor (Bazhal *et al.*, 2003; Fincan and Dejmek, 2003; Lebovka *et al.*, 2004) to induce structural

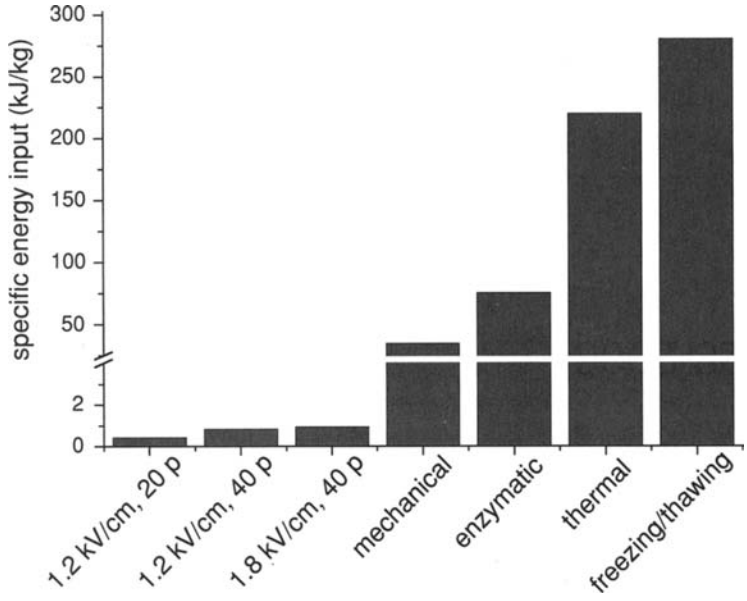

Figure 7.4. Specific Energy Requirements for different pre-treatment techniques.

changes in fruit or vegetable tissue. With a continuous, short time and low energy (~10 kJ/kg) PEF treatment of vegetable tissue, a tissue disintegration and a reduction of tissue toughness similar to that after a thermal or enzymatic treatment can be achieved. For sugar beet a 50 % reduction of cutting force required has been reported (Kraus, 2003). For vegetable tissue an almost total cell disintegration was found after a treatment at 1.8 kV/cm and 40 pulses (Knorr *et al.*, 2005). An estimation of energy requirements for different pretreatments is shown exemplarily in Fig. 7.4. These results indicate that in addition to improvement of extraction and mass transport processes a cell disintegration by PEF can be achieved with lower energy usage and shorter timescale than using other treatments.

3.3. Microbial Decontamination

The applicability of PEF for microbial inactivation of liquid food has been proven by a high number of studies investigating the impact of PEF on vegetative organisms in model as well as real food material. From first experiments, conducted in batch-wise treatment chambers with small treatment volume the technique has been transferred to lab- and pilot-scale equipment with continuous operability and production capacities up to several hundred of liters per hour (Sale and Hamilton, 1967; Zhang *et al.*, 1994; Gaskova *et al.*, 1996; Qin *et al.*, 1997; Jeyamkondan *et al.*, 1999; Ho and Mittal, 2000; Cserhalmi *et al.*, 2002). Even if the underlying mechanism of action have not been fully elucidated up to present, the main processing parameters have been identified, crucial knowledge for design of pulse modulators and treatment chamber geometries. It has been shown that in general yeasts are comparably sensitive against a PEF treatment (Barbosa-Cánovas *et al.*, 1999; Toepfl *et al.*, 2004a), as in addition to cell membrane constitution cell size seems to play an important role. For a broad variety of vegetative microorganisms, spoilage as well as pathogenic strains the applicability of PEF to achieve a significant inactivation has been shown. The products under investigation include different fruit or vegetable juices (Zhang *et al.*, 1994; Raso *et al.*, 1998; Qui *et al.*, 1998; McDonald *et al.*, 2000; Heinz *et al.*, 2003; Molinari *et al.*, 2004) as well as milk

(Reina *et al.*, 1998; Bendicho *et al.*, 2003; Toepfl *et al.*, 2004c; Sampedro *et al.*, 2005; Sepulveda *et al.*, 2005), liquid egg (Martín-Belloso *et al.*, 1997; Fernandez-Diaz *et al.*, 2000; Góngora-Nieto *et al.*, 2003a), model beer (Ulmer *et al.*, 2002) or nutrient media such as broth (Selma *et al.*, 2004) or algae extracts (Koehler *et al.*, 2005). It is noteworthy that, in comparison to disintegration of plant or animal cells a higher electric field intensity is required. The electric field strength required for an efficient microbial decontamination of liquid food has been reported to be in a range of 20–40 kV/cm, an energy input in the range of 50–1000 kJ/kg has been reported. At these treatment parameters plant or animal tissue will be permeabilized, a microbial decontamination will also destroy solid food tissue integrity and structure. Therefore the technique is applicable only for liquid food preservation. One exception has been reported by Gudmundsson and Haffsteinsson (2001), lumpfish roes, which do not possess a cytoplasmatic membrane have been shown to remain their native structure after a PEF treatment and successful microbial inactivation.

Different concepts have been developed to avoid or to minimize the product temperature increase due to electric energy dissipation. The temperature increase of the media can be estimated from the specific energy input and the specific heat capacity of the product under adiabatic conditions. To preserve fresh-like character (and to justify the term nonthermal treatment) a temperature increase over 65°C must be prevented. To raise temperature of fruit juice with a specific heat of 3.8 kJ/kg K from 15 to 65°C, an energy input of 190 kJ/kg is sufficient, indicating that at the energy input range reported significant heating will occur. When operating in batch-mode the pulse repetition rate can be kept low to allow heat removal across the electrodes, but when high flow rates are required during continuous operation other means have to be taken. Application of cooling jackets or series of electrode pairs with intermediate cooling sections have been suggested to maintain temperature below harmful levels (Zhang *et al.*, 1995). It is obvious that for cooling (in addition to the pulsed energy) at least a similar amount of energy will be required to remove the electric energy after or during the treatment, emphasizing the importance to reduce the specific energy input as far as possible. Other processing concepts make use of the temperature increase during treatment. It has been reported that increasing initial treatment temperature can reduce the pulse number and the specific energy input required to obtain a certain level of microbial log reduction (Stanley, 1991; Zhang *et al.*, 1995; Heinz *et al.*, 2003), an effect based on phase transition and structural changes of the cytoplasmatic membrane (Jayaram *et al.*, 1993). Allowing a certain temperature increase during treatment can therefore enhance the efficacy of the PEF application, while still operating at lower temperatures than during conventional, thermal processing. The electric energy, resulting in a temperature increase will have to be removed after treatment to minimize the thermal load, thus a cooling is still required. From an engineering point of view there are not many possibilities to utilize the electric energy dissipated into the product after the preservation as long as there is no need for heat. A processing concept including heat recovery after PEF treatment has been developed by Heinz *et al.* (2003). The energy dissipated during the treatment is used to preheat untreated media, as the PEF application is performed at elevated treatment temperature. Operating at initial treatment temperatures in a range of 35–55°C was shown to drastically reduce the electric energy input required to achieve a certain log-reduction. As thermal energy is required to preheat the media heat recovery is applicable after treatment and can make subsequent cooling obsolete. The energy input required to achieve a 6-log reduction of *Escherichia coli* in apple juice was reduced to 40 kJ/kg when operating at an initial temperature of 55°C. The input of 40 kJ/kg pulse energy will cause a temperature increase of 11°C or up to 66°C. An enthalpy diagram of such a process is shown in Fig. 7.5. A combination of mild heat and PEF might in addition be helpful to achieve sufficient enzyme inactivation to avoid the necessity of chilled distribution and storage. After ambient temperature PEF-treatments contradictory results have been obtained regarding enzyme inactivation (Ho *et al.*, 1997; Ocio *et al.*, 1997; Van Loey *et al.*, 2001; Bendicho *et al.*, 2002; Yang *et al.*, 2004; Bendicho *et al.*, 2005). Giner *et al.* (2005)

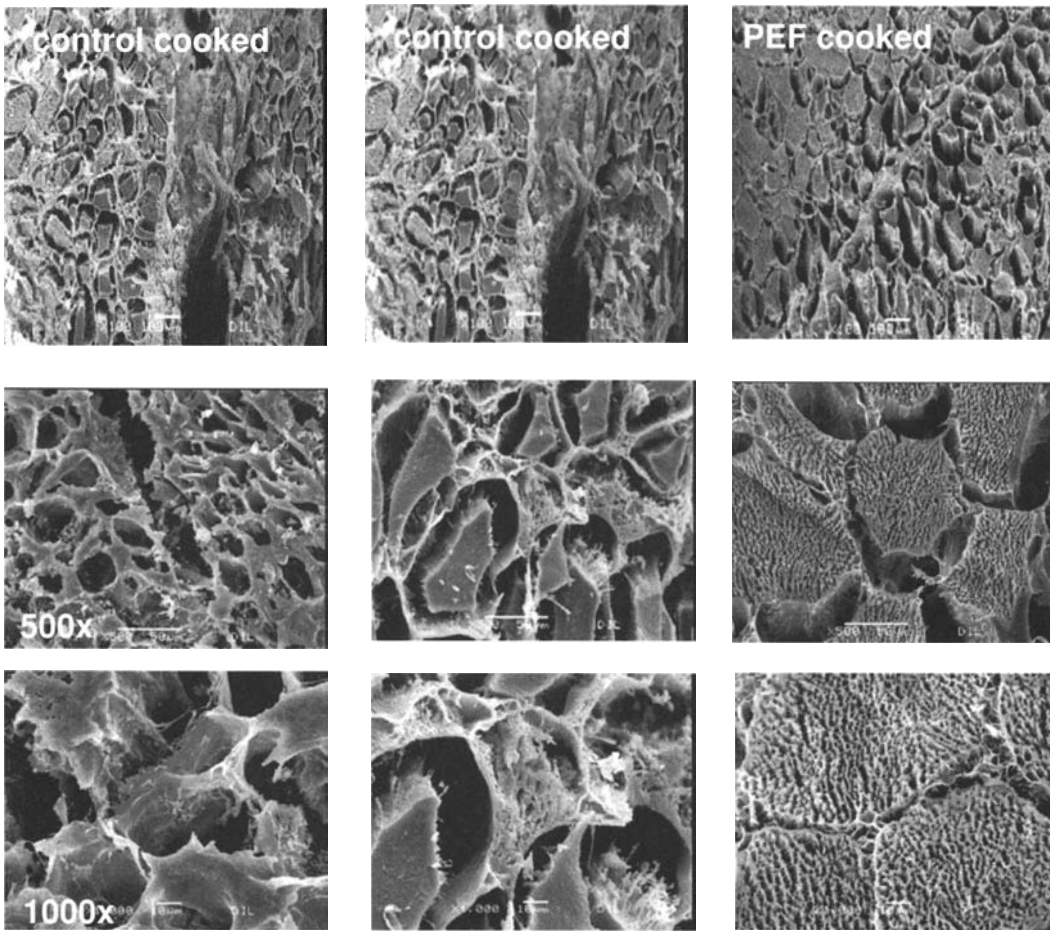

Figure 7.5. Electron micrographs of pork meat untreated (left column), after cooking (center column) and after PEF-treatment and cooking (right column). To all samples an injection of 20 % brine containing 10 % of pickling salt was applied, cooking was performed at 75°C, 90 % humidity until a core temperature of 64°C.

reported an almost total inactivation of pectinesterase in a commercial formulation, but an energy input as high as 8000 kJ/kg was required. To achieve a residual activity of 20%, an energy density of 2000 kJ/kg was reported, far above that used for microbial inactivation. Van Loey *et al.* (2001) related enzyme inactivation after PEF to secondary effects such as local temperature distributions, electrochemical reactions, or formation of free radicals instead of primary effects of the electric field. In general enzyme inactivation is required to obtain sufficient shelf life of the product, in particular for ambient temperature storage of fruit juice (Evrendilek *et al.*, 2000; Schuten *et al.*, 2004). In the case of milk processing, where the naturally occurring lactoperoxidase system with its antimicrobial system is present (Seifu *et al.*, 2005), retaining enzyme activity could be beneficial. It was shown that 90 % of lactoperoxidase activity can be retained after a PEF treatment inactivation up to 4-log of *Pseudomonas flourescens*. Making use of the antimicrobial activity of an activated lactoperoxidase system the shelf life of raw milk could be prolonged (Toepfl *et al.*, 2006). A PEF treatment of milk provides a potential to reduce the maximum treatment temperature, an issue not only important regarding product quality, but also of high importance to increase service life of heat exchangers (Beuf *et al.*, 2004). A combined application of PEF and mild heat could be applied, as biofouling at

the heat exchanger surface, causing a reduction of transfer efficiency and quality problems, could be significantly reduced when operating at lower temperatures.

Estimations of costs of operation and investment will be presented in a later section but at this point it can already be stated that application of PEF as a preservation technique will be limited to products with substantial margins or where other techniques are not applicable. As the application of PEF in food processing is almost unknown at the present state, and no experience on long-term reliability of the impulse generators as well as electrode lifetime is available it reimans challenging to convince potential users to conduct an investment. Costs for investment as well as for operation to achieve a microbial inactivation are higher in comparison to the well-established thermal treatment. Substituting conventional techniques will require significant product quality and consumer benefits to justify the additional costs and to overcome scepticism against novel techniques. The first attempt to commercially apply PEF technology for fruit juice preservation has been reported in the US for treatment of apple, strawberry and other juices (Clark, 2006). Even if the production scale of 200 L/h is comparably small this could be the first step toward an industrial exploitation and hopefully others will follow.

3.4. Wastewater Treatment

During biological wastewater treatment the minimization of excess sludge production due to strengthened ecological and legislative measures became an issue of high importance. A disintegration of excess sludge and destruction of cells, organic matter consisting of a broad variety of different organisms, and subsequent release of intracellular material can be utilized to initiate biodegradation and autolysis of cells (Kopplow *et al.*, 2004). After a treatment at 15 kV/cm, a chemical oxygen demand (COD) release of up to 15% was reported after an energy input of 360 kJ/L. Volatile suspended solids and gas production during anaerobic degradation were found to be improved by 8 and 19% after a PEF energy input of 150 kJ/L in contrast to 445 kJ/L for a thermal treatment. Heat recovery from sludge is difficult; as viscosity is high and particles are present only tubular heat exchangers can be used and will require large exchange surface. Biofouling and deposits on the surface will cause short service times and require intense cleaning. Taking into account a maximum heat recovery in the range of 50% of the energy requirement for thermal treatment could be reduced to approx. 225 kJ/kg. The main advantages of a PEF application in contrast to conventional disintegration techniques such as mechanical rupture, ozone application, thermal, or ultrasound treatment are short processing times and a direct and efficient permeabilization of cell membranes. PEF-treated sludge showed a reduction of biological activity and an increase in organic matter in the water fraction (Loeffler *et al.*, 2001). The chemical oxygen demand of sludge filtrate was increased up to 25% after a treatment at 26 kV/cm and an energy input of 800 kJ/kg, energy requirements were reduced to 250 kJ/kg when a temperature increase above 40°C was allowed. Release of organic material will improve digestion as well as subsequent dewatering.

A total reduction up to 53% of volatile suspended solids and 45% of total suspended solids in the excess sludge was achieved after a PEF treatment at 15 kV/cm, 35°C and an energy input of 100 kJ/kg of sludge (Koners *et al.*, 2004). During these experiments a flow of 5 L/h of sludge (3% dry matter) was subjected to an electropermeabilization and returned to the aerated reactor with a volume of 200 L, corresponding to a stress frequency of 0.47/day. The sludge retention time was 14 days (Fig. 7.6). It has been shown that quality of the treated water was maintained and sludge quality was still acceptable in spite of drastic reduction of excess sludge production. Settling experiments indicated an improved agglomeration and sludge separation (Koners, unpublished data). In comparison to a PEF application for microbial inactivation in food products it has to be considered that the biocenose within the sludge is much more diverse; its impact on the broad variety of organisms has to be further

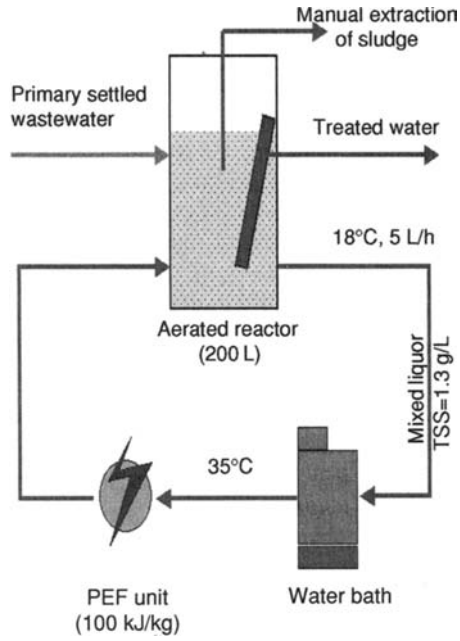

Figure 7.6. Schematic diagram of wastewater treatment pilot system with the application of PEF (adapted from Koners *et al.*, 2004).

studied. Additional research work will be required for optimization of PEF treatment parameters as well as equipment design, but it was indicated that PEF application provides a potential to reduce the amount of excess sludge and can be utilized to improve anaerobic digestion. The average costs for sludge deposition in Germany are in the range of 50 €/ton (Anonymous, 2003), for incineration of mechanically dewatered sludge costs in the range of 300–750 €/ton of original dry matter have to be estimated (Halbach *et al.*, 2003).

As a PEF treatment for excess sludge reduction still needs to be optimized from technical and economic point of view and the results reported are obtained in lab- or pilot-scale the energy requirements can only be estimated at present. To improve treatment efficiency it is suggested to increase the dry matter of the sludge prior to treatment as at low solid concentration the main part of energy is dissipated into the liquid media without benefit. An increase of dry matter from 3 to 5% could lead to a drastic increase of electrical energy utilization. A treatment of sludge with an energy input of 50 kJ/kg of liquid sludge with 5% dry matter, a stress frequency of 0.4, and a sludge retention time of 14 days, will require an energy input of 5.6 MJ/kg or 1.55 MWh/ton of original dry matter. Taking into account a charge of 0.10 €/kWh, this results in energy costs of 155 €/ton of dry matter, indicating that a PEF treatment can be utilized as an effective alternative to reduce the amount of excess sludge from civil or industrial wastewater prior to incineration. Further research work at Berlin University of Technology will focus on determination of process parameter requirements and their optimization with regard to sludge reduction as well as energy efficiency.

4. COST ANALYSIS

Within this section the treatment costs as well as the costs for investment will be estimated exemplarily for disintegration of fruit tissue for juice winning and for pasteurization of liquid food.

These applications have been discussed extensively in literature within the last decades, but still no industrial exploitation has been achieved. In principle the costs for other applications of PEF in food processing can be derived from the examples given, as the processing parameters required are similar for disintegration of other plant or animal cells or for pasteurization of other liquids, respectively.

4.1. Fruit Mash Disintegration for Juice Winning

Considering the processing parameters reported, an electric field strength in the range of 1–2 kV/cm, exponential decay or rectangular pulses with a pulse width in range of μs and a total energy input of 10–20 kJ/kg, the design parameters for an industrial scale system with a production capacity of 10 ton/h of fruit mash can be determined. Based on a treatment chamber diameter of 50 mm, a peak pulse voltage of 20 kV will be sufficient. To limit the impact of product conductivity, use of a colinear treatment chamber is suggested, which provides a high load resistance. Two subsequent treatment zones are formed by a setup of three electrodes, consisting of a high voltage electrode and two grounded counterparts (See Fig. 7.2.). The average residence time within a treatment zone with a diameter of 50 mm and a gap of 50 mm will be 2.8×10^{-2} s. To subject every volume element to an average number of 20 pulses (10 in each zone) a minimum repetition rate of 350 Hz will be required. At a flow rate of 10 ton/h and an energy input of 10 kJ/kg an energy supply with an average output power of 30 kW is required. The price for such a unit can be estimated to be in the range of 150,000 € (see Chapter 2); from an engineering point of view a PEF system for this application appears to be feasible as low peak voltage and pulse repetition rates are required. To achieve larger production capacities a setup of several units in parallel is favorable in contrast to an increase in treatment chamber diameter to avoid the necessity to increase the peak voltage required.

An energy input of 10 kJ/kg corresponds to an electric power consumption of approx. 3 kWh/ton of product. Based on a price of 10 ct/kWh, the pure electric energy costs for the PEF treatment can be estimated as 0.30 €/ton, adding 10% overhead a total power of 0.33 €/ton is assumed. For a conventional enzymatic maceration the treatment costs can be estimated at 7.50 €/ton, where a significant amount is contributed by enzyme costs. A calculation of profitability is shown in Table 7.1, taking into account the costs for investment as well as variable and maintenance costs. It is obvious that the high initial costs for installation of a PEF system can amortize within a very short period of time, as the treatment costs per ton are quite low. Even if under economic pressure the enzyme charges would decrease it is evident that there is a large span in range of 7 €/t of variable costs between both techniques. This estimation is based on the assumption that the same juice yield is obtained after PEF or enzymatic pretreatment, which is confirmed by literature data available. Additional consumer benefit due to less detrimental impact on product quality is not included into this balance, same as the drastic reduction of processing time and a possible potential to extract native structure pectin from the pomace. From this point of view an application of PEF in fruit juice processing provides a tremendous potential to reduce processing times and costs, as soon as a robust and reliable PEF system is available on the market. This comparison indicates the potential of the technique as well as the necessity to develop a turnkey-system for small or medium sized enterprises. For the application of PEF for disintegration of other cellular tissue or to improve drying or extraction, similar processing parameters (1–3 kV/cm, 10 kJ/kg) have been reported, the total costs for the PEF treatment in the range of 2.69 €/ton of product therefore can be utilized to characterize the expenses to expect for other applications with similar production capacity. At present a technical system with a capacity of 1 ton/h is realized at Berlin University of Technology to confirm these estimations in a cooperative project with participation of the association of German fruit juice processing companies.

Table 7.1. Estimation of total costs of a PEF cell disintegration in comparison to an enzymatic maceration for a production capacity of 10 ton/h and an operation time of 1875 h/a.

Cost type	Unit	Enzymatic maceration	PEF
Production p.a.	ton	18,750	18,750
Investment	€	37,500	150,000
Residual value	€	-	-
Replacement value	€	45,000	175,000
Expenditure	€	45,000	175,000
Depreciation range	years	7	7
Interest	%	7	7
Depreciation	€/a	6,000	22,000
Interest	€/a	3,150	12,250
Maintenance	€/a	9,150	10,000
Fixed costs p.a.	€/a	18,300	44,250
Variable costs p.a.	€/a	140,625	6,188
Total costs p.a.	€/a	158,925	50,438
Variable costs per ton	€/ton	7.5	0.33
Total costs per ton	€/ton	8.48	2.69
Δ Total costs p.a.	€/a	108,487	
Δ Total costs per ton	€/ton	5.79	
Reflux time	years	1.38	
Profitability	%	119	

Load voltage, 20 kV; average power, 30 kW; estimated investment cost, 150,000 €.
a., annum; p.a., per annum.

4.2. Cost Estimation for Beverage Pasteurization

For an efficient pasteurization of liquid food a required peak electric field in the range of 30–40 kV/cm has been reported. Dependent on type of product, experimental setup, treatment chamber geometry, and processing parameters such as pulse wave shape and processing temperature the specific energy input requirements are varying in a broad range from 50 up to several hundreds of kJ/kg in particular when enzyme inactivation was taken into consideration, up to 8000 kJ/kg of treated product have been reported (Zhang *et al.*, 1994, 1995; Heinz *et al.*, 2003; Góngora-Nieto *et al.*, 2003a; Aronsson *et al.*, 2005; Evrendilek and Zhang, 2005; Giner *et al.*, 2005; Toepfl *et al.*, 2005b). Treatment intensity required, in comparison to disintegration of plant or animal tissue is much higher in terms of electric field strength as well as energy input. PEF systems for preservation in industrial scale are not on the market, but pilot-scale equipment is available at Ohio State University (USA), Stork Food and Dairy Systems (The Netherlands), SIK (Sweden), and Berlin University of Technology (Germany), pulse modulators are commercially available from Diversified Technologies, USA, exemplarily. Some limitations have to be kept in mind when considering preservation by PEF in comparison to a conventional thermal processing:

- the treatment is affecting vegetative microbes only, spores or viruses are not susceptible to electric fields at the parameters applied
- an aseptic filling subsequent to treatment will be required (in contrast to a high hydrostatic pressure or hot filling process)
- efficiency regarding enzyme inactivation remains unclear, in most cases chilled storage might be necessary.

Table 7.2. Estimation of investment costs for power supply and pulse modulator based on processing parameters from literature (Heinz *et al.*, 2003; Evrendilek and Zhang, 2005) and equipment cost assumptions from Chapter 2.

		Heinz *et al.*	Evrendilek *et al.*
Specific energy input	kJ/kg	50.00	700.00
Electric power consumption	kWh/ton	13.89	194.44
Power price	€/kWh	0.10	0.10
Power costs	€/ton	1.39	19.44
Flow rate at industrial scale	ton/h	10.00	10.00
Average power required	kW	138.89	1,944.44
Power supply cost estimation	M€	0.14	1.94
Overhead for other components		2.00	2.00
Investment cost estimation	M€	0.42	5.83

A thermal preservation of fruit juice is commonly performed at a temperature level of 85°C and a holding time of 30 s (Pandur, 1988), the temperature increase from 20 to 85°C will require an energy input of approx. 250 kJ/kg for apple juice. Taking into account heat recovery with a recovery rate of 90–95% the average energy input required will be in the range of 20 kJ/kg for thermal processing. It is obvious from the energy requirements reported, that a PEF treatment, even when making use of heat recovery to reduce the electric energy input required to 40 kJ/kg as shown by Heinz *et al.* (2003) will require a higher specific energy input, dependent on parameters applied up to 20-fold in comparison to thermal treatment. It has to be considered that use of electric power is commonly connected to higher costs than energy derived by fuel or gas. If a PEF treatment shall be performed at ambient temperature, the electric energy input required will have to be removed by an active cooling system, causing additional costs of operation and investment. The costs for a treatment of apple juice at an energy input of 50 (data from Heinz *et al.*, 2003) applying heat recovery and with an energy input of 705 kJ/kg and active cooling (data from Evrendilek and Zhang, 2005) will be estimated. An overview of these estimations can be found in Table 7.2.

An energy input of 50 kJ/kg can be translated into an electric power consumption of 13 kWh/ton of product or, at a current price of 10 ct/kWh to 1.3 €/ton of treated product. For treatment of a flow of 10 ton/h at an energy input of 50 kJ/kg, an average power of 150 kW is required. Operating at an energy input of 700 kJ/kg will result in a power consumption of 194 kWh/ton, electric power costs of 19.4 €/ton and require an average power of 2 MW. Based on the data given in Chapter 2, the costs of investment for such PEF systems can be estimated. For the power supply costs in the range of 0.5–1 k€/kW are commonly assumed, resulting in costs of 150–2000 k€ for power supply. Including an overhead for pulse modulation, capacitors, and control equipment in the range of twice the costs of the power supply the estimated total installation costs will be approx. 3 k€/kW or, €0.42 and €5.8 million for the two examples, respectively.

Not many data are available concerning equipment costs of large-scale PEF equipment, Braakman (2003) reported investment costs in range of €2 million for a flow capacity of 5 ton/h or €4 for 10 ton/h capacity, specific energy input is not given, but the cost range in good accordance to the previous estimation. As an industrial system is not available at present the costs can only be estimated based on data available from other pulsed power applications, or scaled up from pilot-scale equipment. Diversified Technologies (Gaudreau *et al.*, 2004) reported costs in the range of 150–200 k€ for pilot-scale design with an average power of 20 kW and a peak voltage of 20 kV. The investment costs for this system are in the range of 7.5 k€/kW average output power. The costs of investment

Table 7.3. Estimation of total costs of a PEF preservation at two different specific energy inputs at a production capacity of 10 ton/h and an operation time of 1875 h/a.

		Specific energy input	
Cost type	Unit	50 kJ/kg	700 kJ/kg
Production p.a.	ton	18,750	18,750
Investment	€	420,000	5,830,000
Residual value	€	—	
Replacement value	€	504,000	6,996,000
Expenditure	€	504,000	6,996,000
Depreciation range	years	7	7
Interest	%	7	7
Depreciation	€/a	72,000	999,429
Interest	€/a	35,280	489,720
Maintenance	€/a	28,000	280,000
Fixed costs p.a.	€/a	135,280	1,769,149
Variable costs p.a.	€/a	24,375	363,750
Total costs p.a.	€/a	159,655	2,132,899
Variable costs per ton	€/ton	1.3	19.4
Total costs per ton	€/ton	8.51	113.75

Investment costs are based on estimations given in the text, cooling system to maintain treatment temperature is not included.
a., annum; p.a., per annum.

can be regarded as quite significant, apart from the fact that no system has been proven to operate at sufficient reliability and lifetime. It has to be kept in mind that for the application operating at ambient temperature an active cooling device has to be added, being able to remove 700 kJ/kg of thermal energy, or scaled up to 10 ton/h a cooling capacity in range of 1.9 MJ/s.

Excepting doubts on technical feasibility of a pulse generator for an industrial scale system one can proceed with the investment cost estimations to set up a total cost balance including costs of operation and depreciation. An overview of the cost balance for the two variations of specific energy input can be found in Table 7.3. Supposing a depreciation time of 7 years and estimating the maintenance costs total production costs of 8.51 and 114 €/ton of product are obtained for the two examples selected. Costs for oil derived thermal pasteurization can be estimated to be in the range of 0.20 €/ton when applying heat recovery. Thermal pasteurization accounts, though requiring a significant amount of energy, only for a small amount of the total product costs. It can be easily seen that application of PEF will cause a significant increase from 0.20 to 8.51 or even up to 100 €/ton of product when operating at ambient temperature and high electric energy input. Braakman (2003) reported extra cost estimations in a range of 0.01–0.02 €/kg or 10–20 €/ton of product, which are in accordance with our estimations. These extra costs will have to be justified by sufficient margins or consumer benefits and most probably inhibit an industrial application as long as equipment availability and reliability, consumer benefit as well as consumer acceptance of these technique is not proven.

5. PROBLEMS AND CHALLENGES

It has been shown in the previous sections that the application of PEF in food and bioprocessing has potential for different applications, in particular for permeabilization of cellular tissue. Nevertheless some technological as well as economical pitfalls and challenges still remain and have to be

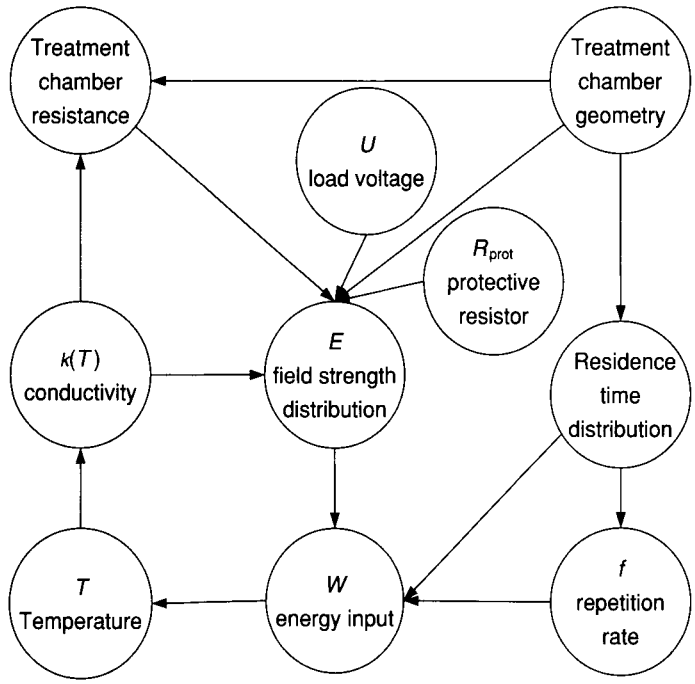

Figure 7.7. Scheme of coupling between different product, processing, and equipment parameters.

overcome prior to an industrial exploitation. Similar as for conventional, thermal processes a homogeneous treatment intensity distribution is crucial to ensure product safety and to avoid overprocessing and related detrimental effects on product quality. It has been shown that treatment chamber as well as electrode geometry determines the electric field distribution (Fiala *et al.*, 2001; Lindgren, 2001). Different treatment chamber geometries such as parallel plate, coaxial or colinear configurations of cylindrical electrodes have been used, each with their own, particular field distribution and homogeneity. It was shown that in addition the presence of particles, agglomerations or lipid drops can perturb the electric field homogeneity (Toepfl *et al.*, 2004b). These effects on the electric field strength experienced by the microorganisms and local deviations have to be taken into account when selecting processing parameters. In regions with higher electric field strength the ohmic heating will be pronounced. Flow pattern, residence time and field distribution are dependent on product temperature. An overview of the coupling between different processing parameters can be seen in Fig. 7.7, the electrical conductivity of the treated media along with treatment chamber geometry affects the electric field distribution. Flow pattern and residence distribution will additionally influence the distribution of electric energy input and joule heating, resulting in temperature gradients and changing product conductivity. It is obvious that the main processing parameters, electric field strength, treatment temperature, and specific energy input are not independent parameters but coupled via the product conductivity.

It has been reported that presence of air bubbles can cause a drop in electric field strength (Góngora-Nieto *et al.*, 2003b), in particular in boundary regions of bubbles the microorganisms might not be exposed to a sufficient electric field strength. Presence of bubbles, in case of sparkling products, dissolution of gas, or after electrolysis of water may therefore cause product safety problems. To overcome bubble formation the PEF system can be pressurized, but still electrochemical reactions might occur. Electrochemical reactions as well as electrode erosion and release of electrode material into the product found increasing attention within the last years (Morren *et al.*, 2003; Roodenburg

Table 7.4. Possible electrochemical reactions at the electrode/media interface.

Anode	Cathode
$2H_2O \rightarrow 2HO^{\bullet} + 2H^+ + 2e^-$	$2H_2O + 4e^- \rightarrow 2H_2 + 4OH^-$
$2HO^{\bullet} \rightarrow H_2O_2$	
$4OH^- \rightarrow O_2(g) + 2H_2O + 4e^-$	
$2Cl^-(aq) \rightarrow Cl_2(g) + 2e^-$	
$Fe(s) \rightarrow Fe^{2+}(aq) + 2e^-$	
Oxidation	Reduction

et al., 2003; Toepfl *et al.*, 2004b; Roodenburg *et al.*, 2005a,b; Clark, 2006). At the electrode surfaces, which are in direct contact to the liquid food, electrochemical reactions will occur. An overview of possible electrode reactions is shown in Table 7.4. The current flow within the liquid is based on movement of charges particles in contrast to the free electron flow within the electrodes. It has been reported that also the application of acidic and alkaline electrolyzed water can reduce microbes on vegetables (Lin *et al.*, 2005), an effect which might also occur at the electrodes of a PEF treatment chamber. To maintain chemical food safety (in addition to microbial safety) the extent of electrochemical reactions has to be controlled to a minimum or stable electrode materials have to be selected. Apart from stainless steel also the application of other metals or carbon or coated electrodes has been suggested. The application of short pulses and avoiding leak current in between the pulses has been suggested to minimize electrode degradation (Morren *et al.*, 2003). Leak currents occur when a switch based on semiconductor architecture is applied, a leak current in the range of 1 mA was determined when applying a potential of 10 kV to an open thyristor switch, addition of neutral red indicated electrolysis by change in pH at the cathode (Toepfl *et al.*, 2005). At a treatment chamber resistance of 50 Ω this results in a voltage drop of 10 V across the chamber, causing continuous electrode degradation. Roodenburg *et al.* (2005a,b) reported a reduced electrode degradation when using a PEF system without leakage current by application of a pulse transformer. The concentrations of electrode components in the media found after PEF treatment did not exceed the EU drinking water requirements; the electrode lifetime was estimated to be 760 h or approx. 1 month in continuous use. These results indicate that changes of the pulse modulator setup can help to reduce release of toxic metal particles, replacing electrodes once per month appears to be acceptable if the treatment chamber is easily accessible.

6. RESEARCH NEEDS, CONCLUSIONS, AND OUTLOOK

Though in lab and pilot scale the applicability and efficiency of pulsed electric field treatment as cell permeabilization technique has been proven up to present only one commercial exploitation (Clark, 2006) has been achieved. From an engineering point of view its feasibility in industrial scale remains to be proven, the development of pulse modulators and turn-key systems with high average power as well as reliability will be a challenging task. There is a clear need for user-friendly PEF systems, but in particular the investment costs need to be reduced to allow their utilization in food industry. The development of modular and scalable power supply and switching modules based on standard components as used in motor drives or other pulsed power applications could help to reduce the costs and allow to easily setup modulators with different specifications. To find an industrial application the costs for investment and operation should either be significantly reduced or a clear consumer benefit and added value needs to be created to surrogate conventional

processing techniques. For beverage pasteurization, the costs for investment as well as operation will be drastically increased by PEF application. Thermal preservation has been improved by application of short time—high temperature processing, the thermal load, and product deterioration have been reduced, the technique is well established and accepted by the customer. In fruit juice industry the margins appear too small to justify to replace thermal preservation by PEF, in particular against the disadvantages and pitfalls still remaining. Up to now the interactions between product and process and possible undesired changes as well as long-term reliability, service and maintenance costs, and legislative situation in Europe remain uncertain. When operating at low electric field strength and energy input, as reported for permeabilization of plant or animal tissue, the cost balance as well as availability of respective pulse modulators are significantly improved. As energy transfer across electrode-media interface is comparably low at the same time the occurrence of electrochemical reactions and undesired impact on the product will be reduced. From this point of view the application as cell disintegration technique provides the highest potential in terms of technical feasibility as well as economical relevance. Extraction of highly valuable substances to be used as health ingredients or for cosmetic formulations or disintegration of cellular tissue with comparable high value and production rates in a range of a few tons per hour such as meat products appear to promising to promote the development of this technique, an industrial exploitation could be obtained within short-term in contrast to large-scale beverage preservation. A successful, first industrial application for an appropriate product and in sound production scale will be necessary to show the feasibility, to produce interest of food and electrical engineering companies and to convince potential end-users of the feasibility of these techniques.

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CHAPTER 8

PULSED POWER SYSTEMS FOR APPLICATION OF PULSED ELECTRIC FIELDS IN THE FOOD INDUSTRY

Hennie Mastwijk

1. INTRODUCTION

In this chapter the main characteristics of pulsed power systems are discussed. Background information on the different kind of switches and electronics is provided and is related to the design of fluid handling equipment. The electronic design and construction of pulsed power systems used in industrial scale operations is a specialized task. Expertise on high voltage engineering and knowledge on the safety on handling of power electronics is required. In practice, food producers and manufacturers of hygienic fluid handling equipment do not have the kind of expertise that is needed to design and construct pulsed power systems. On the other hand, the knowledge and expertise that is required to specify and design fluid handling systems that can be used for food applications is not available among electronic engineers.

The main goal of this chapter is therefore to provide background information to pulsed power engineers, food scientist, and process engineers of fluid handling systems. The major concern is to identify minimal requirements and trade-offs that can be made in the design and construction of safe and reliable PEF systems for the production of foodstuffs.

2. PEAK POWER REQUIREMENTS

The operation of PEF systems rely on the application of electrical pulsed power (Angersbach and Knorr, 1997; Barbosa-Cánovas *et al.*, 1998; Barbosa-Cánovas and Zhang, 2001; Heinz *et al.*, 2002). The aim of a treatment is to achieve high field strengths into products for a short period of time. Usually, the duration of a pulse lies in the range of microseconds to milliseconds. The electrical field strength that has to be reached usually lies in the range of 5–30 kV/cm. The electrical field strength is defined by the voltage across the electrodes, the dimensions, and specific construction of treatment device.

The electrical power requirement of a system is primarily based on the required electrical peak power that is given by

$$P_{\text{peak}} = \sigma \cdot E^2 \cdot V,$$

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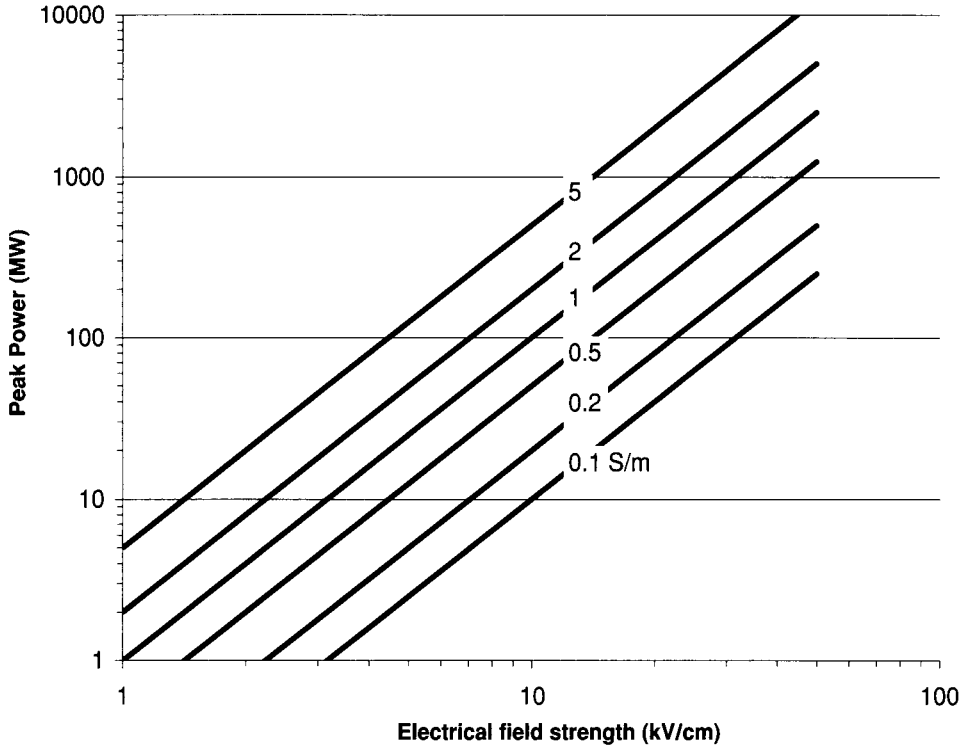

Figure 8.1. Required peak power for a treatment device at different combinations of electrical field strength and electrical conductivity.

where σ is the electrical conductivity of the product, E is the electrical field strength, and V is the volume of the device in which treatment is received.

For typical foods the electrical conductivity lies in the range of 0.5–3 S/m. If we assume industrial scale applications there is a minimum volume of the device that should be considered. For continuous flow operation at a throughput of 10,000 L/h the diameter of piping in a typical food factory is 2″ (50.8) to 3″ (76.2) (mm). This diameter is needed to pump a diversity of foods at the required throughput at reasonable velocities within the range of 1–8 m/s. These processing conditions and dimensions determine the minimum required dimensions of a treatment device. Other requirements that should be met simultaneously, is that the treatment received in the process is homogeneous. Secondly, there are usually geometrical constrictions that have to be met. The treatment device should fit into the fluid line, particles of specified dimensions should be able to pass the device. These requirements determine that the minimum volume of a treatment device lies in the range of 50–175 mL.

For industrial scale operation the required peak power ranges from 10 kW to 10 MW depending on the specific product under consideration and required field strength for processing (Fig. 8.1). In order to deliver these high levels of peak power, electrical energy should be stored in the system. This is in practice realized by adding electrical storage capacitors in the electrical system (see next paragraph).

3. CONTINUOUS POWER REQUIREMENTS

Although a PEF process is defined by extremely high peak powers, the continuous power requirements are considerably less. The continuous power rating depends on the required intensity

of the treatment and is defined by duration of the pulse and on the repetition rate (frequency) of the pulses:

$$P_{\text{cont}} = P_{\text{peak}} \cdot \tau \cdot f_{\text{rep}},$$

where τ is the duration of a single pulse and f_{rep} is the repetition rate.

Both the electric field strength and the time of exposure to these conditions have to be defined prior to the design of the pulsed power system. The total time of exposure of the product to high field conditions is given by product of the residence time inside the device (V/Φ), p the repetition rate (f_{rep}), and the duration of a single pulse (τ). The required repetition rate is given, which depends on the volume of the treatment device, the throughput (Φ), and the average number of pulses (N) that is specified for a treatment

$$f_{\text{rep}} = N \cdot \Phi / V.$$

In order to guarantee that on average all product transversing the device is treated, the repetition rate should exceed the corresponding residence time, i.e.,

$$f_{\text{rep}} > \Phi / V.$$

As an example, the repetition rate should exceed 28 Hz for a device of 100 mL volume at a rated throughput of 10 m^3/h.

To summarize: after the specifications of the coarse dimensions the treatment device is known, together with required intensity of the treatment, the basic electronic specifications have been obtained. These basic specifications are the minimum requirements before a more detailed design or a first cost-estimate can be made of the pulsed power system.

4. POWER SUPPLIES, SWITCHES, AND TREATMENT DEVICES

After the coarse specifications have been obtained, a more detailed design can be made. Essentially, this means that a choice has to be made for high voltage power supplies, switches, and storage capacitors. In order to be able to deliver the levels of peak powers indicated under conditions of high field strength, energy is stored in high voltage capacitors. The switch is required to deliver the electrical pulses in a controlled way.

There are basically two schemes of electronic circuitry—or topologies—for pulsed power applications. The first is where energy stored into the capacitor is much more than required for a single pulse. In this case the electrical pulse is initiated by switching the system "on." After a predetermined period of time, turning the switch "off" terminates the current. Thus the function of the switch is threefolds:

1. it can hold off the high voltage
2. it can rapidly open to initiate the pulse
3. it can rapidly close under high current loads.

The scheme is referred to as on–off configuration and is characterized by the voltage of the storage capacitor during operation is maintained within 95% of the peak voltage (Fig. 8.2). In this topology the storage capacitor is continuously charged by a constant high voltage power supply. The duration of the pulse is controlled by the time that the switch is closed. The pulse shape does not depend on the impedance of treatment device as long as the charge and voltage on the storage capacitor is maintained during operation.

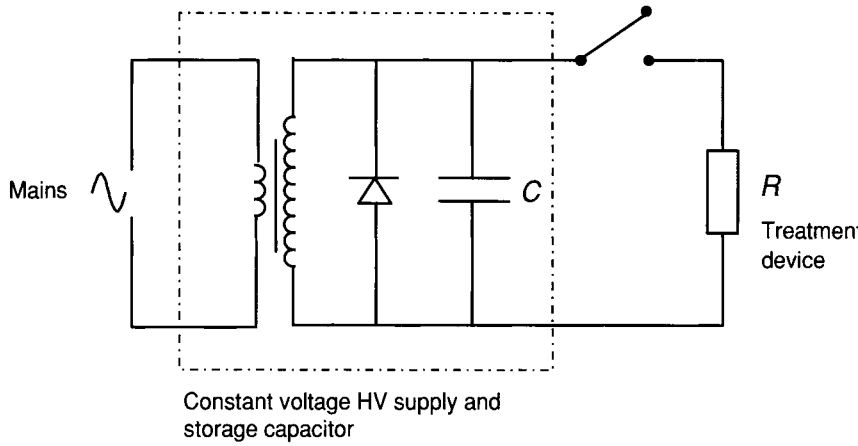

Figure 8.2. On/off configuration.

A second configuration is characterized by a system where sufficient energy is stored for a single pulse only (Fig. 8.3). In this case, the number of requirements for the switch is less. It should be able to hold off the high voltage and has the ability to close rapidly. After closing, the storage capacitor is completely discharged across the treatment device. After that the electrical current has vanished, the switch is closed under conditions of zero voltage and zero current. As this system is often combined with network of capacitors and inductors (Pulse forming network) to shape the electrical pulse, this topology is referred to as a PFN design. In a PFN design the actual shape of the pulse is realized after closing the switch. This is the result of a coherent superposition of multiple reflections at the load of running waves along the network of capacitor and inductors. For a predefined shape of the pulse the impedance of the treatment device should be matched to impedance of the PFN. Changes in the electrical conductivity and processing conditions in general require different configurations of the PFN.

Both configurations have been demonstrated as a realistic alternative as a pulsed power system in large-scale pilot plants based on PEF treatment (Wouters and Smelt, 1997; Min *et al.*, 2003). As indicated, these two configurations have different features. This means that choices have to be made with respect to the design of high voltage power supplies, storage capacitors, and other electrical components used for the switch.

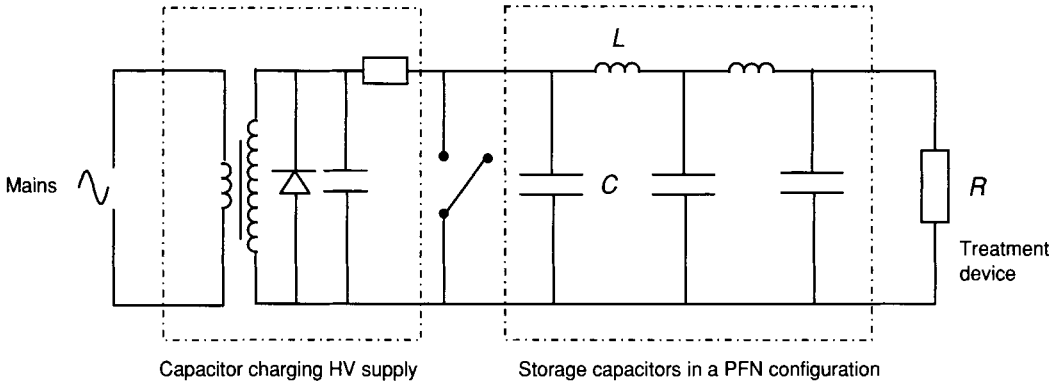

Figure 8.3. Pulse forming network (PFN) configuration.

4.1. DC Power Supplies

The difference of the on–off configuration in comparison with the PFN configuration is the amount of energy that is stored in the capacitor. In case of the on–off configuration an excess amount of energy is stored such that after each pulse the voltage has not dropped significantly. During operation the capacitor is loaded at a constant voltage level. This feature is of importance as it simplifies the design and construction of the required high voltage power supply. A high voltage power supply is a major economical and reliable concern in a pulsed power system. The trade-off is that the switch should both have closing and opening capabilities. In particular, the requirement that the switch can be opened while a steady-state electrical current is maintained, implies a more critical design of the switch.

In the PFN configuration sufficient energy is stored in the capacitor for a single pulse. After delivering the capacitor is completely discharged and has to be reloaded from the level of zero voltage to peak voltage. This scheme therefore requires capacitor loading high voltage power supply. From a technical point of view this means that a more complex high voltage power supply has to be considered. On the other hand, the technical requirements are less stringent as the switch does not require capabilities for terminating currents.

4.2. Switches

The switches that have been reported in different pulsed power applications are of various designs and in general many different components have been used. These include gas switches as thyratrons and solid-state switches based on semiconductor technology (IGBT). Gas-based switches have been used in pulsed power applications (particle accelerators, radar systems, etc.) for more than 30 years. Whereas solid-state switches for pulsed power applications that are based on semiconductor components have been developed only recently (Gaudreau *et al.*, 1998). However, the performance of single components in terms of peak power and reliability has increased within the last few years. It is therefore generally expected that the performance of semiconductor pulsed power components will increase even more while the costs are expected to drop due to mass production. Eventually, semiconductor switches are expected to gradually replace gas-type switches.

The choice and construction of a particular switch depend on the peak power and continuous power requirements, the required repetition rate, and electrical topology of the circuitry (Gaudreau *et al.*, 2004). Thyratron switches can be operated as a single component switch in industrial scale applications as the peak power rating of a single component exceeds 1 GW (Table 8.1).

The basic component of a solid-state switch is an IGBT, which stands for insulated gate bipolar transistor. Typical ratings of these commercially available semiconductor components are included in Table 8.1. For a switch based on those components, multiple IGBTs are required to construct a switch of the specified peak power.

Table 8.1. Comparison of typical electric power ratings of common used, commercially available components used in pulsed power applications.

	Thytratron (ESV Technologies Ltd.)	IGBT module (ABB Ltd.)
Peak voltage	100 kV	1.7 kV
Peak current	10 kA	450 A
Continuous current	3 A	225 A
Peak power	1000 MW	765 kW
Continuous power	300 kW	383 kW

In case of pulsed power applications in the food industry there are additional specifications for the switch concerning reliability and maintenance-free operation. For a food production lines repetition rates of 100–1000 Hz are required for extended period of times. Typically, production runs of 15 h duration should be supported without failure of a single pulse. Service intervals should exceed 5000 h of operation. This means a guaranteed lifetime of the switch in excess of 1.8×10^9 pulses.

Lifetime data for electronic components are usually expressed in terms of mean time between failure (MTBF). Guaranteed lifetimes and recommended times between servicing of various switches to achieve maintenance-free operation over a period of time are scarcely available. Lifetime specifications of switches derived from the specifications of single components are in general not reliable. In the case of IGBTs the lifetime of single components exceeds MBTF of 50,000 h.

However, as indicated by Table 8.1, a switch used in an industrial scale pulsed power system will contain many of these components in series and in parallel to meet the power requirements as discussed in the previous paragraph. As the lifetime of a composite switch is usually determined by its design rather than the lifetime of individual components the reliability and lifetime expectations should be evaluated in more detail.

In case of thyratrons, performance and lifetime data are available when operated as a single component switch in a PFN configuration. It is known that the conditions of operation is a important factor that determines the actual lifetime of the switch. In state of-the-art thyratron systems, a lifetime in excess of 17,000 h has been demonstrated (Parmenter *et al.*, 2001).

4.3. Electrical Properties of Treatment Devices

With regard to the treatment device many comments can be made concerning hydrodynamic properties and the homogeneity of the electrical field. In this paragraph the attention is focused on the properties of the device as an electrical component in a pulsed power circuit and on the electrode materials. As the treatment device is a part of the electrical circuitry, it is important to understand the response of the device under pulsed power conditions.

4.3.1. Electrical Impedance

In order to describe the electrical behavior of electrode structures over a broad range of frequencies the electrical response is usually modeled a network of idealized resistors, capacitors, and inductors. In a pulsed power applications as PEF the response of a treatment device should be accurately known for frequencies ranging from DC to 100 MHz. The high frequency limit corresponds to temporal responses within intervals of 10 ns to describe the rise time of the pulse. In addition the model should be accurate under high field conditions up to 60 kV/cm.

From experimental observations it has turned out that the electrical properties of many different devices within this range of conditions is described by a single resistor (R). The value of the resistor depends on the conductivity of the product, the type electrode configuration that is used, and on the average temperature through

$$R(T) = c/\sigma(T),$$

where c is the so-called cell constant with dimension of an inverse length (m^{-1}). The value of the cell constant depends on the electrode geometry of the PEF device. For some basic geometries the cell constant can be expressed in terms of the design parameters. The cell constant of a PEF device may

be obtained experimentally using a low voltage signal generator under DC and AC conditions or may be obtained by using the data of the voltage and current traces under operation. As mentioned this holds for pulsed power conditions at applied field strengths up to 100 kV/cm (see next paragraph) for many high water content products. However, in all cases it should be verified whether or not this simplification is justified. Cases have been reported where this approximation certainly does not hold (Knorr and Angersbach, 1998).

4.3.2. Comparison of Cell Resistance by Calculation and Measurement

The static electrical field has been determined for a specific electrode configuration using a finite element analysis. A voltage difference of 30 kV at the electrodes is assumed. The electrical conductivity of the orange juice at 20°C is 0.43 S/m. The electrical field is derived from the calculated voltage distribution as below. From the static electrical field distribution the total current for this geometry is obtained by integration of the current density given by over the plane of symmetry of the device. By doing this a total current of 195 A is obtained yielding a device resistance of 30 kV/195 A = 154Ω.

This result has been checked by actual pulsed power experiments using this device filled with orange juice. The pulse traces of the voltage and current were acquired under conditions of 5 Hz pulse repetition rate, at an inlet temperature of 20.0°C and an outlet temperature of 20.5°C. At these conditions the small temperature increment can be neglected. Thus it has been verified that static thermal conditions can be assumed. By using these data a device resistance of $150 \pm 10\Omega$ is determined under pulsed conditions at a temperature of 20°C. This value should be compared to the results obtained by finite element analysis of the system and corresponds well.

4.4. Pulse Shapes

In literature many different pulse shapes have been used. Basic forms of pulse shapes include exponential, rectangular and damped oscillating, and bipolar pulses (Zhang *et al.*, 1995; Barbosa-cánovas *et al.*, 1998). A pulse shape itself has many characteristics and features that can be described by a rise time, hold time, droop, decay time, overshoot, charge reversal, etc. However, it is not practical nor accurate to try to establish a pulse definition by words. The best practice in specifying a particular process is to consider a basic—idealized—pulse shape. In a more detailed design of the system more details should be given on the actual voltage and current traces that has been obtained. Finally, actual pulse trace data should be measured under steady-state processing conditions. Accurate data over the entire frequency range of operation are required as the pulses obtain information on the electrical power consumption and the occurrence of electrochemical reactions.

4.5. Measurement Under Pulsed Conditions

Accurate measurement of the actual pulse shapes is specialized task. Detection of high voltages requires not only practical experience and a thorough knowledge on high voltage safety but requires special equipment as well. For the accurate measurements of high voltage traces, the use of broadband high voltage dividers (such as, e.g., Tektronix P6015A) is a necessity. High voltage dividers allow safe measurement of high voltages up to 100 kV when installed correctly. The accuracy of the level of high voltage pulses to an absolute scale is limited. Comparison of two calibrated Tektronix P6015A probes at the same pulsed high voltage source has shown that the peak voltage of the traces is accurate within 10% only (van Emmerik, 1999).

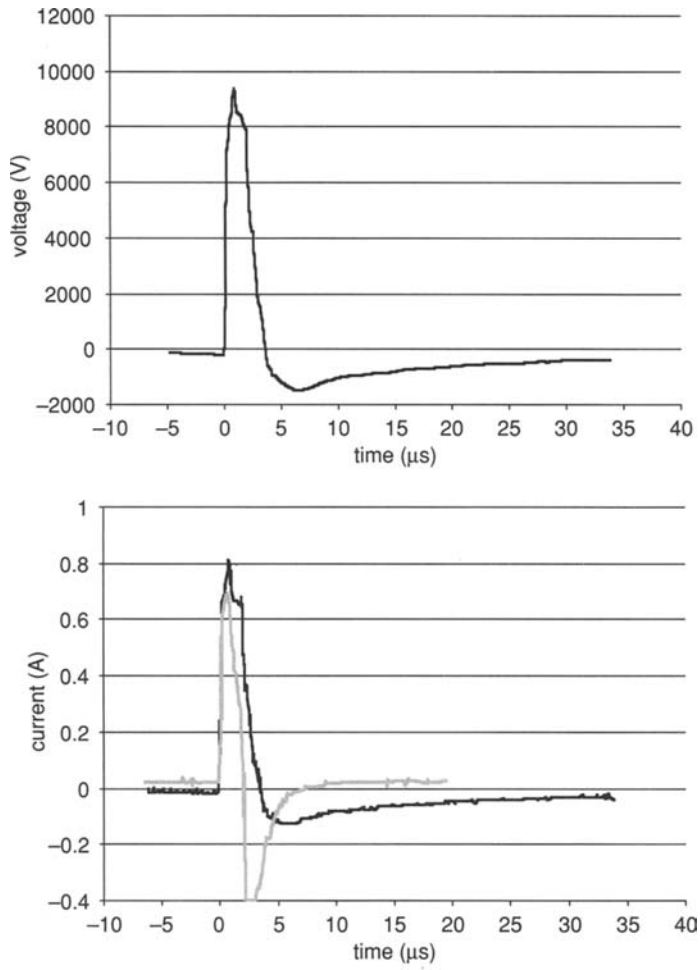

Figure 8.4. Voltage and current measurements of NaCl solution in a co-linear device. Up) High voltage pulse trace. Below) Current measurements using a current transformer (grey) and shunt resistance (solid black). The current transformer data suggests non-ohmic response. However, this is not valid. When the current measurement is carried out by a shunt resistance it is demonstrated that the device behaves as an ohmic resistance as expected.

Safe measurement of electrical currents in high voltage applications is realized by application of current transformers. These detectors are in general installed in the grounded lines of the high voltage systems. However, care must be taken as current coils operate on the principle on detection of changes in the magnetic field by alternating currents. The low frequency response of current transformers is therefore limited. This leads to deformation of pulse shapes in the low frequency regime. The actual and measured pulse shapes detected by current coils are in general not the same as illustrated in Fig. 8.4.

For pulse widths as short as 1 μs in duration and with a typical rise time of 10 ns, most treatment devices can be described by a truly ohmic resistance. The validity of this assumption is checked by comparison of the actual current and voltage traces. If these have identical shapes and if the pulses are not delayed in time with respect to one another the device can be considered an ohmic resistance.

4.6. Partial Discharges, Electrical Breakdown, and Arcing

The problem of arcing in PEF systems occurs when operating conditions lead to uncontrolled discharge. Discharges lead to very high local current densities and high temperatures at the electrode surfaces resulting in evaporation and release of metal. Arcing should be considered as a deviation of standard operation as result of electrical breakdown of the medium at excessive field strengths. The electrical breakdown is dry air typically occurs at 30 kV/cm whereas the electrical breakdown in water may exceed a value of 1000 kV/cm. In PEF experiments arcing is primarily caused by electrical field inhomogeneities in combination with air bubbles. At locations of high electrical field strength, high temperatures can be reached. At high temperature the air dissolved in products is released. This leads to bubble formation if the vapor pressure of dissolved gases exceeds the backing pressure.

Arcing should be avoided at all times and is prevented by two measures. The first is proper design and shaping of electrodes to eliminate field inhomogeneities and regions of excessive high field strength significantly (Fiala *et al.*, 2001). Secondly, the appearance of air bubbles should be suppressed by applying a static backing pressure (Fig. 8.5).

Observation of partial discharges can be used to identify the range of normal operation of PEF systems. Partial discharge is the phenomenon where UV light is emitted under conditions of high electrical field strength before a complete electrical breakdown occurs. Partial discharges are the precursor for electrical breakdown that results in arcing. Arcing is the event when a short-circuit is obtained after complete breakdown of the dielectric. Severe arcing in industrial scale PEF systems leads to short-circuit currents under a loud bursts of noise (>120 dB) and may destroy the electronic pulsed power circuitry and the treatment device completely. It goes without saying that arcing should be prevented at all times.

Solid-state switches that operate in an on–off configuration have advanced capabilities to prevent breakdown and arcing. Unlike gas switches, semiconductor switches can be turned on and off within a few nanoseconds allowing full control in opening the switch in the event of electrical breakdown. For gas switches operated in a PFN cannot be interrupted during the pulse. Once the total stored energy in the capacitor banks is released measures can be taken. These measures prevent damage to electronics and the electrodes in the system. However, this kind of protection is not an alternative to

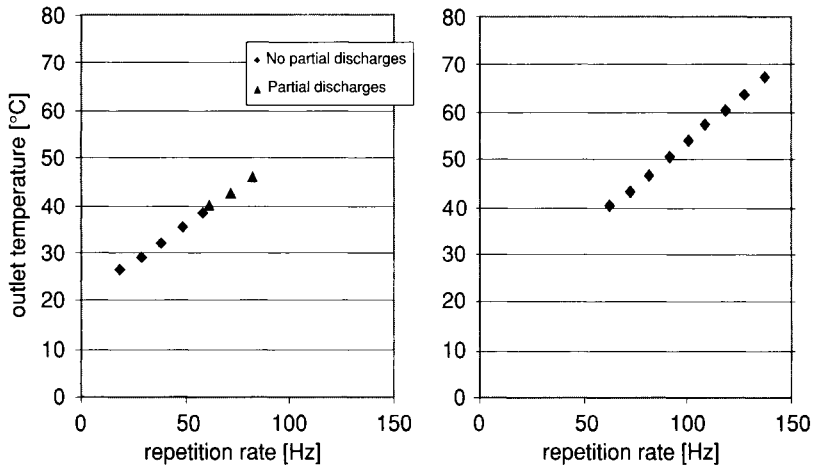

Figure 8.5. Occurrence of partial discharges under pulsed power conditions at 20 kV/cm. Left) No backing pressure. Partial discharges are observed at conditions (triangles) at moderate energy input corresponding to a 10°C differential temperature across the PEF device. Right) Backing pressure of 2.5 bar. No partial discharges could be observed up to a level of 50°C differential temperature.

proper electrode design and application of backing pressure. In the event of arcing and subsequent shut down of the electronic system or delay of pulses, the liquid handling process should be interrupted as treatment of the product according to preset conditions is no longer guaranteed.

5. ELECTROCHEMICAL PROPERTIES OF PULSED POWER SYSTEMS

A treatment device that contains a high water content product in electrical contact with metal electrode will inevitably suffer from electrochemical reactions. Effects of electrochemical yield under pulsed power conditions have been recognized and discussed from the first start until recently (Hamilton and Sale, 1967; Tomov and Tsoneva, 2000; Lelieveld *et al.*, 2001; Heinz *et al.*, 2002; Morren *et al.*, 2003; Roodenburg, 2003; Mastwijk and Bartels, 2004). However, in recent work on pulsed power treatment scientific data are still presented using systems that are known subjects severe to electrochemical degradation (Evrendilek *et al.*, 2004; Reyns *et al.*, 2004).

Chemical-induced changes in foods and metal release are a major food safety concern (Lelieveld *et al.*, 2001; Mastwijk and Bartels, 2004). The key question is: which parameters control electrochemical reactions? In a systematic study it has been found that many design parameters in pulsed power systems are related to electrochemical behavior (www.eet.nl/projecten/). From this work it has been concluded that electrochemical effects in pulsed power systems can and should be evaluated in each the stage of its design. In the next paragraph the main sources of electrochemical reactions are identified and discussed.

5.1. Electrochemical Reactions

Little data on metal release from electrodes are available for the range of conditions that is required for PEF treatment of foodstuffs. It is assumed that pulses of sufficient short duration eliminates electrode degradation. The explanation is that an electrochemical reaction requires a threshold voltage. If this threshold value is not reached, reactions cannot occur. For pulses of short duration used in PEF systems no time is available to build an electrical potential near the liquid–metal region where electrochemical reactions take place. It is therefore assumed that reactions do not occur. However, the design of electrode systems and the specific processing conditions of PEF-based systems are quite different from systems that have been used in electrochemical processes known to date.

Electrochemical effects in pulsed power systems have been observed (Hamilton and Sale, 1967; Evrendilek *et al.*, 2004) and have been investigated in more detail (Tomov and Tsoneva, 2000; Lelieveld *et al.*, 2001; Heinz *et al.*, 2002; Morren *et al.*, 2003; Roodenburg, 2003). In view of the unintended production of toxic compounds, pulse power systems have to be assessed before it can be used for the production of foods.

5.2. Electrode Degradation by DC Offset

Recently, multiple sources of electrode degradation in pulsed power systems have been identified that will be discussed in this chapter. A major source is related to the presence of DC leak currents through the treatment device. These leak currents occur when the switch in a pulsed power system is open, especially, when the switch configured by semiconductor components. It is well known that semiconductor materials are known poor electrical insulators under high voltage conditions. Solid-state devices as IGBTs are based on semiconductor technology. IGBT modules typically have leak currents of 1.5 mA at 1.7 kV operation. This means that a solid-state switch configured by five

Table 8.2. Overview of electrochemical processes relevant to chemical changes of foods and emissions of metals.

Electrochemical reaction	Threshold potential (V)
Electrochemical-induced changes in foodstuffs	
$O_2(g) + 4H^+ + 4e^- \leftrightarrow 2H_2O$	+1.23
$H_2O_2 + 2e^- \leftrightarrow 2OH^-$	+0.94
$O_2(g) + 2H^+ + 2e^- \leftrightarrow H_2O_2$	+0.68
$O_2(g) + 2H_2O + 4e^- \leftrightarrow 4OH^-(g)$	+0.40
$2H^+ + 2e^- \leftrightarrow H_2(g)$	0.00
$2H_2O + 2e^- \leftrightarrow H_2(g) + 2OH^-$	−0.83
Metal release from electrodes	
$Fe^{3+} + e^- \leftrightarrow Fe^{2+}$	+0.77
$Cu^+ + e^- \leftrightarrow Cu(s)$	+0.52
$Cu^{2+} + 2e^- \leftrightarrow Cu(s)$	+0.34
$Cu^{2+} + e^- \leftrightarrow Cu^+$	+0.15
$Ni^{2+} + 2e^- \leftrightarrow Ni(s)$	−0.25
$Fe^{2+} + 2e^- \leftrightarrow Fe(s)$	−0.44
$Cr^{3+} + 3e^- \leftrightarrow Cr(s)$	−0.74
$Al^{3+} + 3e^- \leftrightarrow Al(s)$	−1.67
$Mg^{2+} + 2e^- \leftrightarrow Mg(s)$	−2.34

of these components in parallel series will have total a leakage current of 7.5 mA when open (idle). In case of a 100 Ω cell resistance this leads to a 750 mV DC voltage across the PEF chamber. This is well above the threshold values of many electrochemical reactions relevant for food applications (Table 8.2).

Under these conditions the electrical current that is present will result in electrochemical reactions. The treatment device will act as an electrochemical cell (Fig. 8.6) where gasses are produced

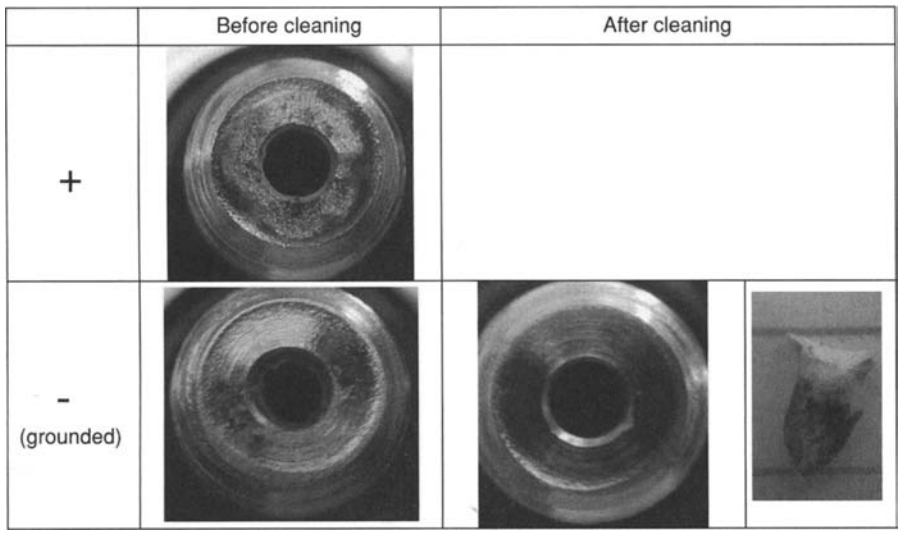

Figure 8.6. Electrode degradation by electrochemical reactions at 2.5 VDC/2 mA after 24 hours of exposure. Above) The surface of the positive electrode is oxidized and is therefore designated as anode. Below) Metalic compounds are precipitated on the surface of the grounded electrode and could be removed by a cloth (see inset).

at the liquid–metal interface and metals are released from the electrodes into the product stream. The release rate of metals—even at low current low voltage conditions—can be surprisingly high: for stainless steel 316 electrodes at 2.5 VDC/2 mA the rate of metal release exceeds 2 mg/h.

5.3. Metal Release Under Pulsed Conditions

A second source of unintended electrochemical reactions is related to the presence of low frequency AC voltages in the electrical circuitry. In pulsed power applications the products are exposed to a broad frequency range of alternating voltages and currents. For a better understanding of the class of electrochemical processes that are related to AC operation we evaluate the emissions by each individual frequency component. For this we evaluate the Fourier expansion of an idealized repetitive pulse of magnitude V_0, duration τ, and repetition rate $f = 1/T$.

$$V(t) = V_0 \cdot \frac{\tau}{T} + 2 \cdot V_0 \frac{\tau}{T} \left[\sum_{n=1} \cos(n \cdot \omega_n \cdot t) \right],$$

where $\omega_n = \frac{2\pi \cdot n}{T}$ is the frequency of nth harmonic compound.

For a 100 kV pulse across a treatment device of 10 Ω with a duration of 1 μs at a repetition rate of 100 Hz ($T = 10$ ms) the harmonic compounds of voltage and currents can be expressed as:

$$\begin{aligned} V(t) = {} & 10\,\text{VDC} + 20V \cdot [\cos(2\pi \cdot 100\ \text{Hz}) + \cos(2\pi \cdot 200\ \text{Hz}) + \cos(2\pi \cdot 300\ \text{Hz}) \\ & + \cos(2\pi \cdot 400\ \text{Hz}) + \cdots] \\ I(t) = {} & 1\,\text{ADC} + 2A \cdot [\cos(2\pi \cdot 100\ \text{Hz}) + \cos(2\pi \cdot 200\ \text{Hz}) + \cos(2\pi \cdot 300\ \text{Hz}) \\ & + \cos(2\pi \cdot 400\ \text{Hz}) + \cdots]. \end{aligned}$$

This means that for this idealized pulse shape a continuous DC voltage is present that exceeds all of the electrochemical threshold potentials in Table 8.1. However, in practice this DC component is eliminated by using pulse transformers at the expense of deviations from an ideal pulse shape. Another effective measure is to apply a DC offset compensation during pulsed operation. In some pulsed power schemes bipolar pulses are used to eliminate the DC component. However, balancing a low voltage DC component by two high voltage pulses of different polarity is relatively difficult to achieve and certainly not cost-effective as two high voltage power supplies are needed in this set-up.

The major concern in this paragraph is the presence of the low frequency AC components that are a source of electrochemical yield. Like DC offset voltages, low frequency AC voltages also lead to electrochemical reactions when the voltage exceeds the threshold potentials given in Table 8.2.

Because of the short pulse time in combination with the relative low repetition frequency of 100 Hz in principle more than 100,000 terms in the Fourier expansion are required for an accurate description of an idealised pulse sequence as in Fig. 8.7. Fortunately, for an accurate forecast of electrochemical yield in industrial scale PEF systems considered here only the lowest 10 terms in

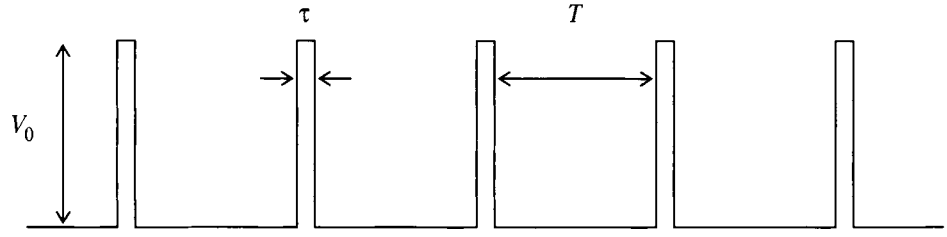

Figure 8.7. Idealized pulse sequence in pulsed power systems.

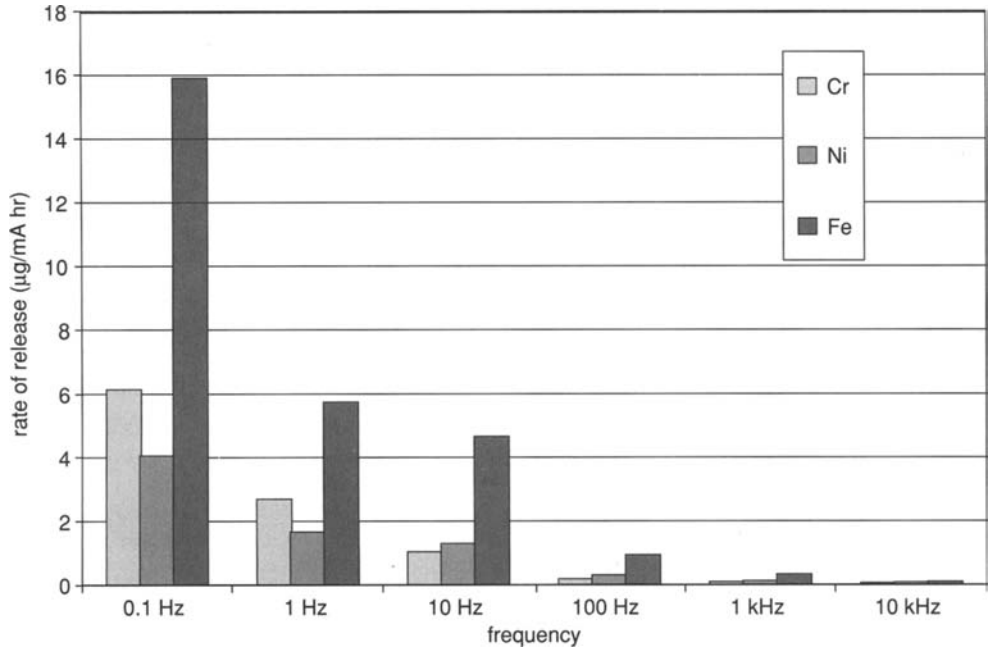

Figure 8.8. Specific release of metals from SS 316 electrodes for various harmonic frequency compounds determined at 150 VAC/420 mA.

the expansion are of importance. Morren *et al.* has determined the release of metal compounds in a model PEF cell by sinusoidal voltages over a broad frequency range. The release of electrode material has been studied under continuous operation at 15 VAC/30 mA and 150 VAC/420 mA in the range of 1 mHz to 1 MHz.

Note that the range of voltages and frequencies in the work of Morren *et al.* corresponds to the industrial scale of pulsed operation for PEF systems where pulses are applied with a duration of 1–10 µs and with peak voltages ranging from 10 to 100 kV peak voltage.

The emissions from electrodes for different compounds are given in Fig. 8.8. In this graph the emissions are given in terms of specific rates of metal release. The actual release of metal ions from SS316 electrode surfaces can be obtained by multiplication by the total current and time of exposure. In this way the data can be used to estimate the metal release for a specific pulsed power process that is designed.

Evidently, the absence of low frequency components is of key importance to minimize the release of metals during pulsed operation. The relevance of this work is the demonstration that the release of metals into product is mainly due to the harmonics present in the frequency range of 0 Hz to approximately 1 kHz. Secondly, the repetition rate in the pulsed power process defines the components of the lowest frequency, which is responsible for the highest emission yield. This suggests that during the process of engineering and design of pulsed power applications optimal pulse conditions can be formulated.

5.4. More Sources of AC Components

Low frequency currents across the treatment chamber are introduced by finite duration of the voltage and current pulses. In addition low frequency components may be induced during the charging

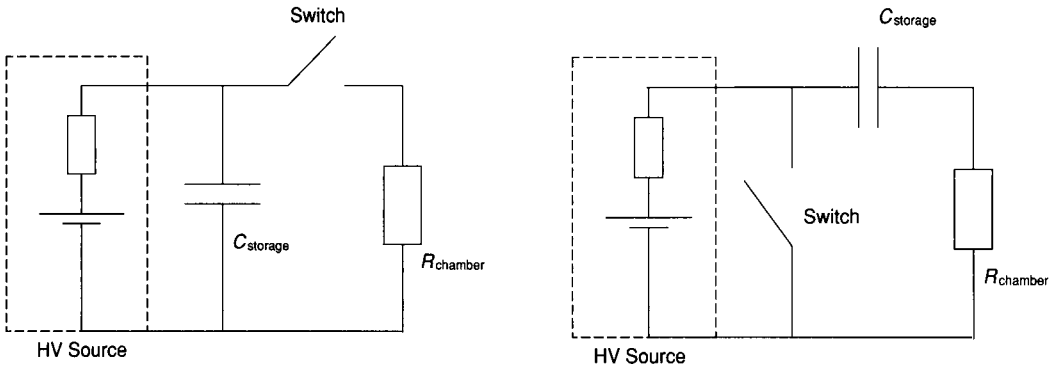

Figure 8.9. Two basic electrical configurations for charging storage capacitors in pulsed power applications. Right: In this configuration an electrical current is passed through the treatment chamber ($R_{chamber}$) during charging. Left: In this case a current through the device during charging is eliminated.

of the capacitors in the PEF system. The charging time is determined by the repetition frequency of the process and typically ranges from 10 to 1000 Hz. This means that capacitors are reloaded every 1–100 ms. During this time currents will flow through the system under conditions where electrochemical degradation of electrodes have been demonstrated. In some electrical designs of PEF systems the storage capacitors are configured such that during the event of charging a current is passed through the treatment device (Fig. 8.9). As the charging and of the capacitors typically occur at frequencies from 10 to 1000 Hz times per second this leads to the presence of low frequency currents across the PEF chamber. In this case the electrodes are (unintentionally) subject to electrochemical degradation.

Quite often the use of different electrode materials are mentioned as an alternative route to reduce the amount of electrode degradation (Góngora-Nieto *et al.*, 2004). In this respect alternatives as carbon or metals of special metallurgical content are proposed. If the common known sources of electrode degradation discussed in this chapter are not eliminated, the use of different metals should not be considered. Specialized electrode materials may reduce the release of metal compounds. However, electrochemical reactions that induce chemical changes in the foods are not controlled by this measure. The best practice is therefore to fight causes of electrochemical reactions rather than eliminate the symptoms of degrading electrodes. Within the framework of the precautionary principle, any electrochemical reaction is undesirable for a process that is intended for preservation of foods. This is connected to the ALARA principle (as low as reasonable possible) commonly adapted in general risk assessment used to guarantee the safety of foods. This principle merely states that all known solutions and technical measures should be considered to eliminate unintended emissions as discussed.

6. PROCESS ASSESSMENT

In order to control PEF operations diagnostic tools should be available to verify whether sufficient treatment under the right conditions has been received. A convenient method is to check the consistency of the energy balance by comparison of the electrical energy at the input to flow of calorimetric heat out of the system.

6.1. Energy Input

In case of PEF operation the bulk temperature of the fluid increases due to ohmic heating. The temperature increment is related to the input power and is the result of conversion of electrical energy

into heat. The input power is given by

$$P_{el}(t) = V(t) \cdot I(t),$$

which is the product of pulsed voltage and current. The input power is the temporal rate of dissipated electrical power. The continuous power that is injected in the product stream under steady-state conditions is given by

$$P_{in} = \nu \cdot \int_0^T V(t) \cdot I(t) \mathrm{d}t,$$

where $\nu = 1/T$ is the repetition rate and the integral is over the duration of a single pulse. The product takes this electrical energy as heat. The temperature increment of the bulk is determined by the specific heat characteristics of the product and results in either a rise in the temperature or is used in phase transitions as melting of fats, denaturing of starches, and proteins.

6.2. Calorimetric Heat Output

The total amount of heat that is injected should balance the calorimetric heat that is leaving the system. The amount of heat that is leaving the system per unit of time is given by P_{out} (W) which can be expressed by the temperature increment of the bulk, the throughput (Φ) and the specific heat of the product:

$$P_{out} = \Phi \cdot \int_{T_{in}}^{T_{out}} C_p(T) \mathrm{d}T.$$

For products, which have a specific heat that is temperature independent, this simplifies to

$$P_{out} = \Phi \cdot (T_{out} - T_{in}) \cdot C_p.$$

In some concepts of pulsed power processes intermediate cooling of the product takes place (Min *et al.*, 2003). In these systems, the treatment is realized by a number of devices placed one after another. After treatment has been received, in the first or first two devices, intermediate cooling takes place before entering the subsequent next device(s). Part of the heat that is generated during PEF treatment is removed in this way. For comparison of the electrical input power to the calorimetric heat contained in the product flow it is required that the temperature is measured and recorded before and after each device to eliminate losses by cooling.

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