Novel Food Processing Technologies

Edited by

Gustavo V. Barbosa-Cánovas
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To our families

Preface

Food processing has become more sophisticated and diverse in response to the growing demand for quality foods. Consumers today expect food products that provide, among other things, convenience, variety, adequate shelf life and caloric content, reasonable cost, and environmental soundness. Strategies to meet such demands include modification to existing food processing techniques and the adoption of novel processing technologies.

Innovation is a key factor in the sustained growth of the food industry, although the journey from concept to implementation is not trivial, and often quite painful. One reason the path can be so bumpy is that hurdles in the road to implementation are neither properly addressed nor fully understood. The chances for success, however, can be improved significantly through basic research covering a broad spectrum of disciplines prior to the commercialization of new products and technologies. At the same time, it is worth mentioning that consumers all around the world are learning more about the food products they eat, regulatory agencies are becoming more stringent and the food industry more liable. Therefore, in order to meet the demand for better quality food products, every effort should be made to understand the basic principles behind food processing, as well as to recognize new opportunities and to consider combined strategies. Today the world of food technology has a handful of options to explore that could make the food industry more diverse, competitive, and efficient. The aim of this book is to investigate some of the options available, namely the alternative technologies and strategies, and to address the new challenges facing the food industry by providing specific examples on how these alternatives could be applied to specific food products.

This book is the most comprehensive and ambitious undertaking we are aware of on the subject of emerging technologies, in that it covers most of the relevant novel technologies applicable in food processing. All chapters are written by key scientists with diverse backgrounds in either industry or academia, and all provide an update on emerging technologies as well as vision for the future. In addition, the most comprehensive support is offered. To aid in the understanding of novel technologies, a section on microbial prediction is included, a topic that parallels the technologies discussed throughout the book. Microbial prediction is included because we believe that new technologies have forced the issue of revisiting traditional (and sometimes obsolete) methods to describe microbial inactivation kinetics and the calculation of lethality.

At present, new food processing technologies are capturing the attention of many key scientists in academia and government, as well as food industries endeavoring to stay one step ahead in terms of technology. Consumers prefer high-quality foods with longer shelf life and, clearly, some of the new technologies can meet these demands. For these reasons, the number of books, conferences, workshops, and discussion groups centered on topics relevant to new technologies for quality foods is growing at an exponential rate. It is also worth mentioning, as an indicator of strong interest in the subject, that the U.S. Institute of Food Technologists (IFT) has a new division dealing with emerging technologies, and that there is also a new international journal, *Innovative Food Science and Emerging Technologies,* exclusively dedicated to covering novel technologies.

This book is the result of a two-step process. First, the EMERTEC Conference was held in Madrid, Spain, which was organized and sponsored by the *Ibero-American Program for Science and Technology (CYTED)* through one of its subprograms - "Treatment and Conservation of Foods," Project XI: "Development of Emerging Technologies of Interest to Ibero-America." The Project Leader was María S. Tapia and the EMERTEC Chair of the Organizing Committee was M. Pilar Cano-Dolado. Before and during the conference, in light of the quality of the presentations and the interest and enthusiasm generated from the audience, the editors and associated editors discussed putting a book together; one that would be based on the most relevant EMERTEC invited presentations and subsequently combined with other chapters identified as key to the book's theme. It has been quite apparent from the beginning that this book grew into a well-integrated unit organized in five sections. Consequently, it reads more as a single authored book with fully integrated chapters than as one compiled by editors, having benefited directly from the discussions by true experts in their fields. The five sections mentioned are: Ultra High Pressure, Pulsed Electric Fields, Other Methods of Nonthermal Processing of Foods, Alternative Thermal Treatments, and Impact of Predictive Microbiology in the Food Industry. We strongly believe this book will be embraced by the food science and food technology communities as a valuable—perhaps the most valuable—reference used for consultation on matters of novel food science and technology.

Gustavo V. Barbosa-Cánovas
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1

Present Status and the Future of PEF Technology

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I. INTRODUCTION

High-intensity pulsed electric field (PEF) technology is a nonthermal food preservation technology that is based on the use of electric fields to eradicate food-borne pathogens and to control spoilage microorganisms in foods. This technology is highly appreciated for its ability to extend the shelf life of food products without the application of heat, thus also preserving quality attributes such as sensory quality and nutritional value, as well as controlling the microbiological safety of food products. PEF technology is not limited by the propagation of lethal agents in the treated product, which occurs in the case of thermal processing through conduction or convection; however, electric fields have a volumetric effect, ensuring fast and homogeneous application of the lethal principle throughout the treated product. Successful application of PEF technology to liquid products such as fruit juices, liquid egg, and milk at laboratory and pilot plant levels suggests the potential of this technology as a substitute for traditional thermal pasteurization or, at the very least, as a complement.

A general review of PEF technology that includes a detailed description of the most relevant aspects related to its development and application is presented in this chapter.

A. Chronology

The use of electricity for food preservation processes has been explored since almost from the time that electricity was first made commercially available. At the end of the 19th century, the use of electric current to pasteurize milk, in a process known as the Electro-pure method, was an important topic of research (Anderson and Finkelstein, 1919; Fetterman, 1928; Getchell, 1935). Although the Electro-pure method was fundamentally a thermal process based on the use of heat generated from the electric current flowing through milk, some researchers posed the question of whether the electric current itself could have a bactericidal effect, while others claimed that the Electro-pure method was capable of destroying some varieties of bacteria unaffected by other thermal pasteurization methods (Getchell, 1935). The treatment applied by the Electro-pure process varied greatly among research groups; voltages ranging from 220 to 4200 V were employed, and only those

researchers using the highest voltages and rapidly alternating currents reported that the process destroyed bacteria under conditions below their thermal death point (Beattie, 1916; Beattie and Lewis, 1916, 1925).

Around 1949, Flaumenbaum reported the use of electric fields for food processing; however, this process was not related to the preservation of foods or inactivation of microorganisms, but instead was intended to increase the permeability of fruits to facilitate subsequent extraction of juice, which currently represents an important application of PEF technology as well (Heinz and Knorr, 2001).

In 1960, Doevenspeck filed a patent in which the existence of a nonthermal effect of pulsed electric fields on microbes was mentioned for the first time, followed by the publication of a scientific paper exploring the interaction between pulsed electric fields and cell walls (Doevenspeck, 1960, 1961).

Following this patent, Sale and Hamilton (1967, 1968) published a series of papers on the use of pulsed electric fields as a bacterial decontamination method; their work laid the foundation for pulsed electric field technology and most of their findings are still current today. These researchers demonstrated that direct current pulses cause a loss in the semipermeability properties of the bacterial membrane, and identified the permanent loss of these properties as the cause of cell death. They also determined that the effect of PEF was not due to heating or electrolysis, but was independent of the current density and energy input; the electric field strength, pulse duration, and size and shape of microbes were found to be the most relevant factors (Sale and Hamilton, 1967; Hamilton and Sale, 1967; Sale and Hamilton, 1968).

In the field of genetic engineering, Zimmermann et al. (1974) developed a method to promote in vitro cell-to-cell fusion, based on the use of pulsed electric fields. This resulted in a controlled increase of permeability in the localized zones of the membrane, a process later referred to as reversible electrical breakdown, or electropermeabilization or electroporation (Zimmermann et al., 1974). Although different in purpose and intensity, electroporation established the basis for studying the mechanisms of action in pulsed electric fields on bacterial cells. Several studies in the field of genetics have since focused on the principles under which pulsed electric fields operate to disrupt the cell membranes (Kinosita and Tsong, 1977; Dimitrov, 1984; Sugar and Neumann, 1984; Bryant and Wolfe, 1987; Glaser et al., 1988; Tsong 1990; Tsong, 1991; Weaver and Barnett, 1992; Ho and Mittal, 1996). The knowledge generated in this field has helped researchers of pulsed electric fields as a food preservation process to understand the nature of the technology being used to reduce bacterial populations in food products.

In the early 1980s, a research group led by Hülsheger continued the work of Sale and Hamilton, by publishing a series of papers that discussed the sensitivity of different kinds of bacteria to PEF. They also developed a mathematical expression that included field strength and treatment time to describe the effect of PEF on microorganisms (Hülsheger and Niemann, 1980; Hülsheger et al., 1981; Hülsheger et al., 1983).

Around the late 1980s, more research groups began studying the use of pulsed electric fields and several patents for food preservation processes were filed as a result (Table 1); interest in the topic also started to spread throughout the scientific community. Several food research groups began exploring the use of PEF technology as part of a group of novel food preservation technologies known as non-thermal preservation or emerging food preservation technologies (Gupta and Murray, 1988; Mizuno and Hori, 1988; Palaniappan and Sastry, 1990; Jayaram et al., 1992; Matsumoto et al., 1991; Grahl et al., 1992; Mertens and Knorr, 1992). Multidisciplinary groups, formed by microbiologists, food scientists and electrical engineers, developed the first food-oriented continuous systems and the first pilot plant systems around this time.

Table 1 List of U.S. Patents on Pulsed Electric Fields Technology

Reference	Patent
Held and Chauhan (2002)	Method for molecular destruction of waste-activated sludge using high electrical voltage
Morshuis et al. (2002)	Treatment apparatus and method for preserving pumpable food products by pulsed electric fields
De Jong and Van Heesch (2002)	Pulsed electric field treatment system
Robbins, 2001	Process and apparatus for reduction of microorganisms in a conductive medium using low-voltage, pulsed electrical energy
Lelieveld and Volanschi (2001)	Method and apparatus for preserving food products
Zhang and Qiu (2001)	High-voltage pulse generator
Mastwijk and Bartels (2001)	Integrated modular design of a pulsed electrical field treatment chamber
Bushnell et al. (2000)	High-strength electric field, pumpable food product treatment in a serial electrode treatment cell
Mittal et al. (2000)	Method and apparatus for electrically treating foodstuffs for preservation
Addeo (2000)	Process for use of pulsed electric fields, coupled with rotational retorting in processing MRE
Bushnell (2000)	Uniform product flow in a high electric field treatment cell
Qin et al. (2000)	Continuous-flow electrical treatment of flowable food products
Hayden (1998)	Method for killing microorganisms in liquids
Qin et al. (1998)	Continuous-flow electrical treatment of flowable food products
Yin et al. (1997)	High-voltage, pulsed electric field treatment chambers for the preservation of liquid food products
Qin et al. (1997)	Continuous-flow electrical treatment of flowable food products
Zhang et al. (1996)	Batch-mode food treatment using pulsed electric fields
Bushnell et al. (1996)	Process for reducing levels of microorganisms in pumpable food products using a high pulsed voltage system
Bushnell et al. (1995b)	Prevention of electrochemical and electrophoretic effects in high-strength electric field, pumpable food product treatment systems
Bushnell et al. (1995a)	Prevention of electrode fouling in high electric field systems for killing of microorganisms in food products
Bushnell et al. (1993)	High pulsed voltage systems for extending the shelf life of pumpable food products
Bushnell et al. (1991)	High pulsed voltage systems for extending the shelf life of pumpable food products
Dunn et al. (1991)	Methods for preservation of foodstuffs
Doevenspeck (1991)	Electric impulse method and device for treating substances
Dunn et al. (1989)	Methods for preservation of foodstuffs
Dunn and Pearlman (1989)	Apparatus for extending the shelf life of fluid food products
Dunn and Pearlman (1987)	Methods and apparatus for extending the shelf life of fluid food products

On July 7, 1995, the Food and Drug Administration (FDA) expressed no objection to the CoolPure® pulsed electric fields process developed by PurePulse Technologies for antimicrobial treatment of liquids and pumpable foods, representing the first regulatory effort to implement PEF technology at an industrial level (Anonymous, 1995). The growing interest in PEF as a viable technology to substitute or complement traditional preservation processes in the late 20th century and the beginning of the 21st century led to the creation of the first commercially available systems, ranging from small experimental bench top systems to industrial scale systems. It is estimated that the current number of research groups studying PEF technology easily surpasses 30, and is growing rapidly (Table 2). The coverage given to this technology by pioneering groups and the increasing availability of equipment has stimulated research groups all around the world to explore the potential of this technology as a food preservation method (Barbosa-Cánovas et al., 1999).

B. Basic Definitions

PEF food preservation is based on the ability of high-intensity pulsed electric fields to disrupt cell membranes, resulting in a lethal effect on the microorganisms. In this method,

Table 2 PEF Research Groups Around the World

Institution	Country
Catholic University of Leuven	Belgium
University of Guelph	Canada
AGIR	France
Pernod Ricard	France
Thomson	France
University of Bordeaux	France
University of Montpellier	France
CPC Europe	Germany
Technical University of Hamburg	Germany
Technical University of Berlin	Germany
ICE Tec	Iceland
ATO-DLO	The Netherlands
TNO	The Netherlands
Unilever Research Vlaardingen	The Netherlands
University of Aberdeen	Scotland
University of Lleida	Spain
University of Zaragoza	Spain
SIK Goteburg	Sweden
Tetra Pak	Sweden
University of Lund	Sweden
Nestle	Switzerland
Campden and Chorleywood Food Research Assoc.	United Kingdom
Natick Laboratories	USA
National Center for Food Safety and Technology	USA
Ohio State University	USA
PurePulse Technologies	USA
University of Wyoming	USA
Washington State University	USA

the food product is placed inside a treatment chamber wherein two conductive electrodes are mounted on a nonconductive material in which there is no electric flow from one electrode to the other through the chamber casing. A high-voltage pulse is then applied to the conductive electrodes to induce a high-intensity electric field pulse on the food product, which is located between the electrodes. The treated product is then removed or subjected to subsequent pulses until the treatment dose is complete.

An electric field E_r is the force F at a point identified by the position vector r, which is what a unit positive charge q would experience if located at that point and also if its placement did not alter the distribution of any charges in space, as shown in Eq. (1) (Blatt, 1989):

$$E_r = \frac{F_{qr}}{q} \tag{1}$$

From this definition, it follows that the units of an electric field are

$$E = \frac{\text{newton}}{\text{coulomb}} \tag{2}$$

By dimension analysis, as defined by the equivalence, it is possible to find that

$$\text{Volt} = \frac{\text{newton} \cdot \text{meter}}{\text{coulomb}} \tag{3}$$

The electric field can also be expressed as

$$E = \frac{\text{volt}}{\text{meter}} \tag{4}$$

From these manipulations, it is evident that by applying voltage across two points separated by a dielectric material, an electric field is generated in the region between the application points, with intensity (E) directly proportional to the potential difference (V) and inversely proportional to the distance between the application points (D), as stated in Eq. (5).

$$E = \frac{V}{D} \tag{5}$$

The Laplace equation (or potential equation) can then be used as a general expression to describe the generated electric field, depending on the voltage under different conditions within a boundary, where φ represents the electrical potential:

$$\nabla^2 \varphi = 0 \tag{6}$$

II. PULSED ELECTRIC FIELD TREATMENT UNIT

Electroporation of cells to promote in vitro cell-to-cell fusion was the first commercial application that took advantage of the effect of pulsed electric fields on bacterial cells. Many of the research groups working on food applications around the late 1980s and early 1990s used commercially available electroporators. This equipment, however, was quickly displaced by PEF systems designed especially for food processing, since the treatment conditions required for each of these processes are quite different (Ho and Mittal, 1996).

A typical PEF food processing unit is composed of a high-voltage pulse generator, treatment chamber, fluid-handling system, and control and monitoring devices (Fig. 1). The

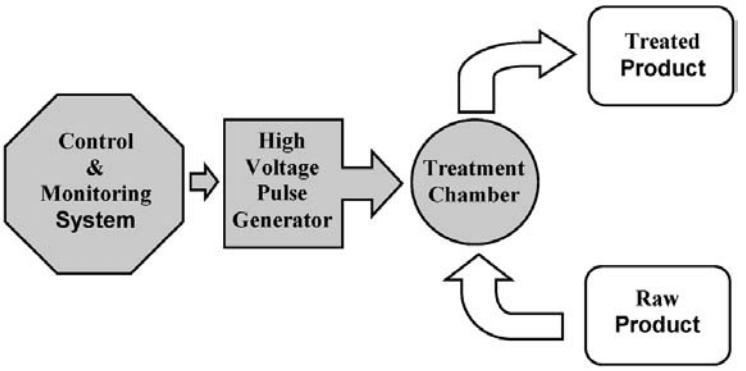

Figure 1 Schematic diagram of a pulsed electric field food preservation system showing the basic components.

construction and characteristics of each component vary from model to model and among research groups, but the basic principles remain the same.

A. High-Voltage Pulse Generator

The high-voltage pulse generator is the component in charge of supplying the high-voltage pulses of the shape, duration, and intensity required. The action of this equipment can be divided into three sections: the generation of direct current (d.c.) high voltage at the required intensity by a power supply, the storage of electrical energy in a capacitor or group of capacitors, and the release of high voltage in the form of a pulse with characteristic pulse shape and pulse width through a pulse-forming network (PFN) (Fig. 2).

The power supply converts the electricity from the utility voltage level (usually 220 or 440 V a.c.) to the required high-voltage d.c. power. The electric requirements of PEF technology usually range from 20 to 60 kV of mono- or bipolar voltage, depending on the specific characteristics of the systems. Depending on the required voltages and intensities, the generation of high d.c. voltage can be achieved through different strategies, the most common being the use of transformers and rectifiers. The total power rating of the power supply limits the maximum number of times the capacitor can be charged and discharged in a given time. The power required from the power supply to charge the capacitor will depend on the electrical resistance of the charging resistor and on the size and number of capacitors charged; a larger capacitor will take more time and/or more power to be charged than a smaller one. Similarly, a smaller charging resistor will speed up the charging process but also will increase the power requirement. Power supplies in the order of 1.5 kW of average power are commonly used for PEF laboratory-scale equipment, while power supplies of 75 kW or more can be used in semi-industrial or pilot plant equipment. The fast-developing area of pulsed power has made possible power supplies that are even larger than the ones mentioned and, currently, power supplies up to 3.2 MW are available, enormously increasing the pulsing frequencies that can be employed (Kelpies, personal communication, 2002).

Once the high voltage has been generated to the required level, a capacitor(s) is charged to store a specific amount of energy at a set level; a low capacitance capacitor can store less

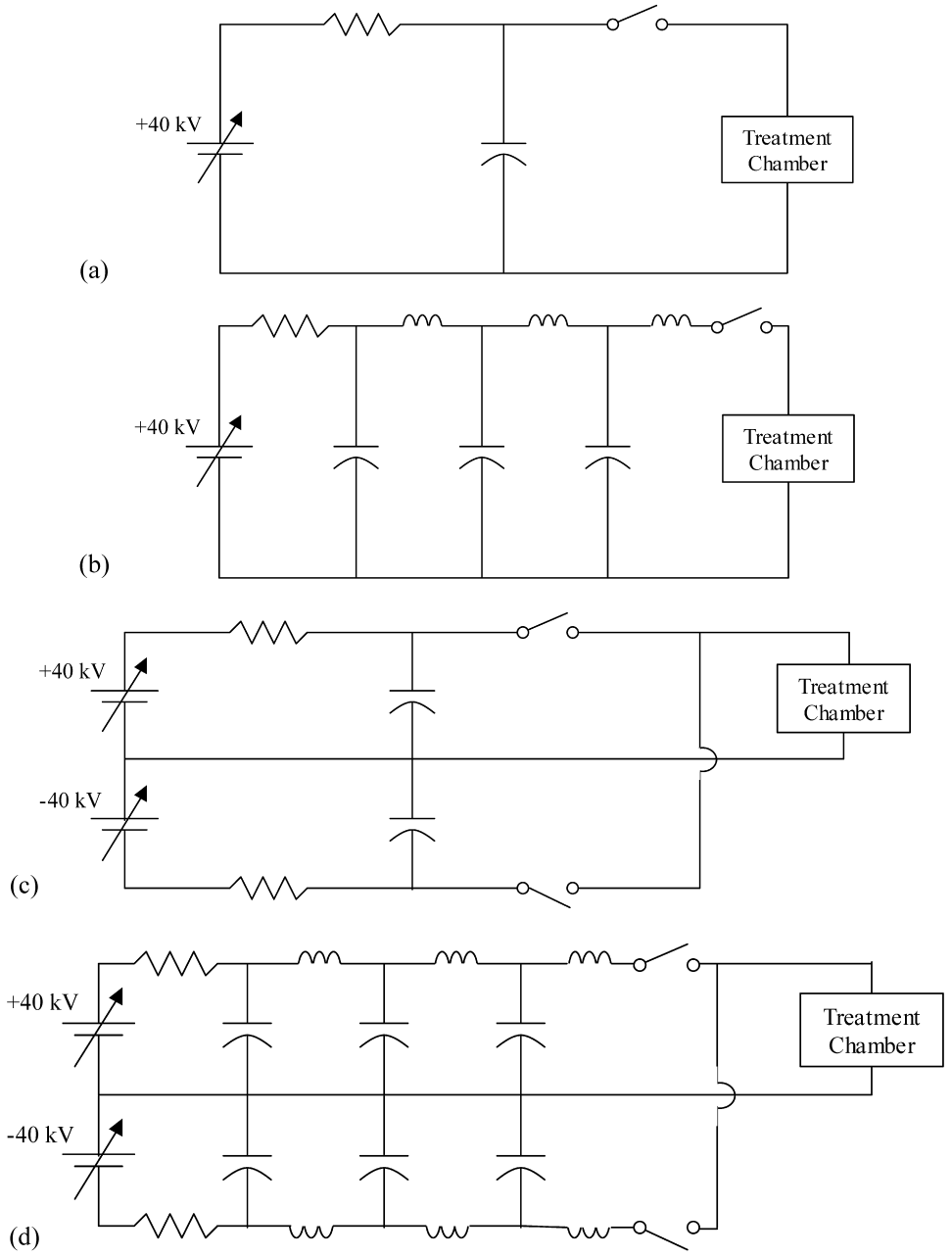

Figure 2 Simplified representation of electric circuits used in the generation of electric field pulses: (a) monopolar exponentially decaying, (b) bipolar exponentially decaying, (c) monopolar square, and (d) bipolar square.

energy than a larger one when both are charged at the same level. The energy stored in a capacitor is defined by the mathematical expression

$$Q = \frac{1}{2} CV^2 \tag{7}$$

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

After the electric energy is stored in the capacitor(s), a switching device releases the power as a high-voltage pulse through a pulse-forming network, where the pulse shape is defined. The switching device must be able to handle the high voltages present in the capacitor and the high currents required by the pulse-forming network and the treatment chamber. There is a variety of high-power switching devices currently on the market. They can be roughly classified as two main groups: ON switches and ON/OFF switches. ON switches are devices capable of establishing the connection between the discharging capacitor and the pulse-forming network, but which lack the capacity to interrupt the connection while the voltage level remains high; once the device has been turned ON, it is not possible to turn it OFF until the voltage drops below a certain voltage level. This kind of device is useful when complete discharge of a capacitor is desired. Generally speaking, ON devices can handle higher currents at higher voltages (~100 kV and up to 1 MA) at relatively low cost; however, low pulsing frequencies, a short life span, and the impossibility of being turned off are some of the limitations of this type of device. Most of these devices work by ionizing gas or vapor confined between two electrodes to promote conduction of the main current. Some examples are the Ignitron, Gas Spark Gap, Trigatron, and Thyratron.

ON/OFF switches, on the other hand, have the ability to be turned ON and OFF at will, which improves control over the pulse-generation process. This type of device allows for the direct generation of square pulses from a power supply, although it can also be used to partially or completely discharge capacitors through pulse-forming networks. Development of ON/OFF switches has advanced considerably in the last few years, thanks to advances in the area of solid-state pulsed power. Semiconductor solid-state switches are considered the most convenient option for future PEF technology. Solid-state switches have a very large operation life span when compared to other types of switches, have better performance, are easier to handle, do not require mechanical components (electrodes or gases), allow higher pulsing frequencies, and have low switching and conducting losses; their price also tends to drop, which is common with semiconductor operated equipment (Moore's law). A drawback to this type of switch is that it usually can handle only a limited amount of current at relatively low voltages (~1.2 kV, 1 kA), which makes necessary the use of several units connected in series and parallel to increase the switch capacity, causing a significant increase in the price of the unit. Examples of solid-state switches are the gate turn-off (GTO) thyristor, the insulated gate bipolar transistor (IGBT), and the symmetrical gate commutated thyristor (SGCT) (EPRI and Army, 1997; Barsotti et al., 1999; Barbosa-Cánovas et al., 1999; Kempkes et al., 2001; Góngora-Nieto et al., 2002).

As the switch discharges the voltage from the capacitor, a PFN composed mainly of capacitors, resistors, and inductors can be used to modify the shape and length of the pulses as required. The most commonly used pulse shapes in PEF technology are exponentially decaying pulses and monopolar and bipolar square pulses.

The simplest configuration of a PFN is the direct discharge of the capacitor to a treatment chamber with a purely resistive load and no other associated loads, which produces an exponentially decaying pulse defined as:

$$\tau = RC \tag{8}$$

where τ (sec) is a time constant corresponding to the time required for a given pulse to decay from its peak voltage to 37% of the peak voltage, R is the electrical resistance of the load, and C is the capacitance of the discharging capacitor.

The more complex pulse-forming networks can generate square pulses, bipolar pulses, and instantaneous reversal pulses, as illustrated in Fig. 2.

The generation of square pulses by a PFN involves matching the electrical characteristics of the equipment with those of the treated food, which makes it difficult to obtain nicely shaped pulses. Reduction of the peak voltage to half the charging voltage is another inconvenience found in generating this type of pulse by PFN, but the use of an ON/OFF switch instead can help solve this problem. Increased effectiveness and sustained treatment at peak intensity make square pulses an excellent pulse shape for PEF technology (EPRI and Army, 1997; Góngora-Nieto et al., 2002).

Besides pulse shape, pulse intensity and pulse duration are important parameters to define the characteristics of high-voltage pulses. Commonly used high-voltage pulses in PEF technology range from 2 to 20 μsec in duration and commonly have from 20 to 60 kV of peak voltage, depending on the desired electric field intensity and the size of the gap between the electrodes in the treatment chamber, as defined in Eq. (5). In the case of exponentially decaying pulses, the maximum voltage, or peak voltage, defines the maximum intensity of the treatment. Since the voltage is constantly decaying in this type of pulse, the treatment voltage varies during the pulse duration. The time constant characterizes the duration of an exponentially decaying pulse, but does not indicate where it ends. It is very important to clearly define the characteristics of the pulses used, because in order to obtain consistent and reproducible results it is necessary to verify that the same treatment is being applied. Because defining the duration of an exponential pulse is not as straightforward as defining the pulse width of square pulses, several different ways to define a pulse have been proposed and almost any of them can be used, as long as it is clearly explained how the characteristics are being measured and expressed. The full width at half maximum (FWHM), which is the width of the pulse at half the peak voltage, is a way that some authors use to characterize exponentially decaying pulses, whereas other authors prefer to define the total length of a pulse as the period of time composed of five time constants (τ) (Cogdell, 1999). Square pulses, on the other hand, are easier to characterize because pulse duration is clearly defined and the peak voltage is sustained across the whole pulse duration.

B. Treatment Chamber

The treatment chamber is a key element of the PEF system; several different designs have been developed through the years and a list of some of these designs is included in Table 3. In the treatment chamber the high voltage pulses generated are applied to a pair of electrodes, causing the generation of high-intensity pulsed electric fields in the region between the electrodes where the product being treated is placed.

The most basic function of the treatment chamber is to contain the treated product while the electric fields are applied. However, its design characteristics define not only the treatment capacity, but also influence some of the treatment characteristics, such as peak electric field and treatment uniformity. The basic design of a PEF treatment chamber includes two electrodes made of a conductive material (usually stainless steel, preferred for its sanitary characteristics) mounted on a container made of a nonconductive material, such as polycarbonate or some other plastic polymer with high electric resistance and dielectric strength. Electrodes can also be made of other conductive materials, such as graphite, metals like gold or platinum, and conductive polymers such as polyacetylene or polysulfur nitride

Table 3 Examples of Treatment Chambers Used in Pulsed Electric Field Technology

Treatment chamber	Reference
Static chamber with U-shaped spacer	Sale and Hamilton (1967)
Static parallel plate treatment chamber	Dunn and Pearlman (1987)
Continuous treatment chamber with coaxial conical electrodes	Bushnell et al. (1993)
Static parallel plate treatment chamber	Zhang et al. (1996)
Continuous treatment chamber with parallel electrodes coaxial cylindrical electrodes	Qin et al. (1997)
Continuous co-field treatment chamber	Yin et al. (1997)
Glass coil static chamber	Lubicki and Jayaram (1997)
Continuous treatment chamber with coaxial cylindrical electrodes	Qin et al. (1998)
Ring-cylinder continuous treatment chamber	Sato et al. (2001)
Needle-plate continuous treatment chamber	Sato et al. (2001)

(Bushnell et al., 1996; Qin et al., 1997). Depending on the electrical resistance of the treated product, the configuration of the treatment chamber, and the resistance of the conduction lines, the consumption of electric current can range from 100 A to 10 kA. Chambers with low electrical resistance demand higher currents; hence, heat dissipation needs to be considered as a design factor.

The simplest classification of a treatment chamber distinguishes between the batch and continuous treatment chambers. Batch treatment chamber designs can be found in early experimental models and in chambers intended for treatment of solid or semisolid products. Parallel-electrode treatment chambers are the most common example of batch or "static" treatment chambers (Fig. 3). When using this kind of treatment chamber, it is necessary to mount and dismount the treatment chamber every time a new batch of product is processed, which is very inconvenient for industrial process operation, especially when the treatment volume of the chamber is limited by electrical constraints.

Continuous treatment chambers, on the other hand, contain a flow path that allows liquid and semiliquid products to be pumped through the chamber; examples of these chambers are the concentric cylinder, concentric cone, converged electric field, and co-field treatment chambers (Fig. 3). Continuous treatment chambers are appropriate for industrial applications, and most of the development in PEF technology has been around continuous treatment chambers. The systems of several continuous treatment chambers, interconnected either in parallel or series fashion, play a central role in some of the most successful systems available.

There are a number of design criteria that should be taken into account when designing a treatment chamber: intrinsic electrical resistance, electric field homogeneity, and reduction or generation of enhanced field areas are some of the most important. The intrinsic electrical resistance of the treatment chamber defines the pulse width, the peak electric field, and the power per pulse delivered to the treated product. The total resistance of the circuit (R_T), which includes treatment chamber resistance (R_{Ch}), transmission line resistance (R_t), switch resistance (R_s), and any other resistance present in the series circuit, in combination with the capacitance of the charging capacitor, define the pulse width, as stated in Eq. (8).

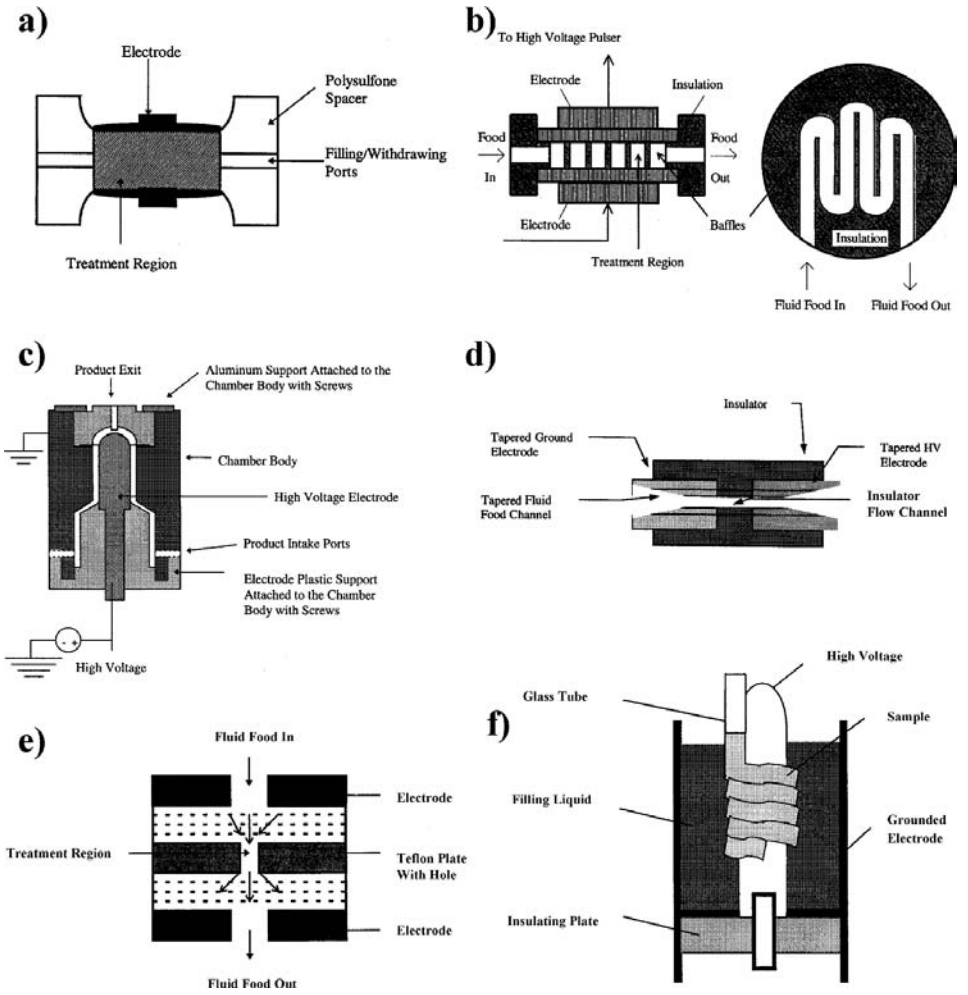

Figure 3 Schematic representation of some treatment chambers used in PEF technology: (a) parallel plate batch treatment chamber, (b) parallel plate continuous treatment chamber, (c) concentric cylinder continuous treatment chamber, (d) co-field continuous treatment chamber, (e) converged electric fields continuous treatment chamber, and (f) glass coil static chamber.

The resistance of a treatment chamber can be determined analytically provided the effective electrode area (A), the distance between the electrodes (d), and the electrical conductivity of the treated product (σ) are known:

$$R_{\mathrm{Ch}} = \frac{d}{\sigma A} \tag{9}$$

Using the simplified discharge circuit illustrated in Fig. 4 as example, the total resistance can be defined as

$$R_{\mathrm{T}} = R_{\mathrm{s}} + R_{\mathrm{t}} + R_{\mathrm{Ch}} \tag{10}$$

This circuit can be viewed as a voltage divider; hence, the larger the chamber resistance in comparison with the resistance of the rest of the system, the higher the peak voltage

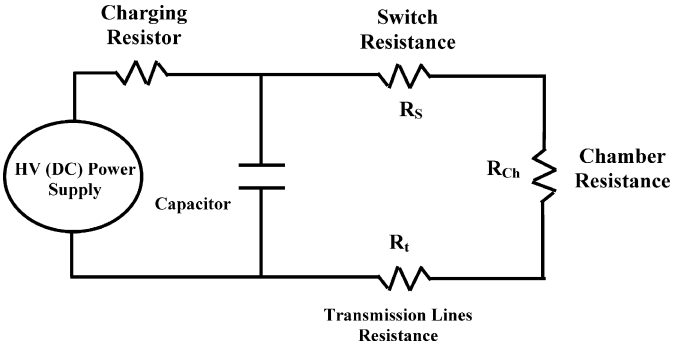

Figure 4 Simplified diagram of the discharge section of a PEF system.

reached at the chamber electrodes. The design of chambers with high intrinsic electric resistance, besides increasing the peak voltage at the electrodes, also signifies a reduction of the peak electric current flowing through the treated product, as defined in Ohm's law:

$$I = \frac{V}{R_T} \tag{11}$$

where I is the electric current flowing through the treatment chamber and the rest of the series system and V is the charging voltage.

It is obvious that if the electrical resistance of the switch and transmission lines is constant, and similar for all kinds of systems, that the particular design and intrinsic electric resistance of the treatment chamber governs the performance of the system.

A fraction of the total energy stored in the capacitor [defined in Eq. (7)] is delivered to the food product depending on the electric resistance of the treatment chamber and the rest of the components of the pulse-forming network. The voltage in the treatment chamber (V_{Ch}) defined by the ratio of electric resistance of the treatment chamber to the total resistance of the system, and multiplied by the charging voltage (V), is:

$$V_{Ch} = V \cdot \frac{R_{Ch}}{R_T} \tag{12}$$

In combination with the electric current flowing through the system, the power (P) delivered to the food product is defined as:

$$P = V_{Ch} I \tag{13}$$

It is important to consider that since energy is being applied in the form of pulses, the power delivered in every pulse (P_{pulse}), integrated throughout the duration of the pulse (t), would yield the total energy per pulse (Q_{pulse}):

$$Q_{pulse} = \int_{t_0}^{t} V_{Ch(t)} I_{(t)} \, dt \tag{14}$$

The average power ($P_{Average}$) delivered can be calculated based on the number of pulses applied per second (N):

$$P_{Average} = N Q_{pulse} \tag{15}$$

If the pulse is generated from the discharge of a capacitor, the energy per pulse delivered to the treatment chamber can be described as

$$Q_{\text{pulse}} = \left(\frac{R_{\text{Ch}}}{R_{\text{T}}}\right)\frac{CV^2}{2} \tag{16}$$

where C is the capacitance of the charging capacitor and V is the charging voltage. A system with a treatment chamber showing high electrical resistance and low losses through the switch and conduction lines will deliver practically all of the stored energy to the food product with every pulse, delivering high voltage levels (due to the high ratio of R_{Ch} to R_{T}) and low currents (due to the high resistance of the system caused by the high R_{Ch}). This peculiarity of treatment chambers with high electrical resistance allows for the use of smaller capacitors and lower charging voltages, which produce lower energy pulses in equivalent treatments as compared to systems operating with treatment chambers of low resistance.

Low-energy pulses, besides signifying a less expensive treatment and less demanding requirements for the power supply, also reflect less heating of the treated product. As electrical energy, acting as an electrical resistance, is delivered to the food product, this energy is dissipated mainly in the form of heat. Heating of the product is defined as

$$\Delta T = \frac{Q}{mC_{\text{p}}} \tag{17}$$

where ΔT is the temperature increase, Q is the total energy delivered to the treated product, m is the mass of the food product being treated, and C_{p} is the specific heat of the product. In a continuous process, the total delivered energy (Q) can be calculated as

$$Q = \frac{fvQ_{\text{pulse}}}{F} \tag{18}$$

where f is the pulsing frequency, v is the chamber volume, and F is the flow rate. The mass of the treated product can also be easily calculated in continuous systems as

$$m = v\rho \tag{19}$$

where ρ is the density of the product being treated. From these expressions, Eq. (17) becomes:

$$\Delta T = \frac{fQ_{\text{pulse}}}{F\rho C_{\text{p}}} \tag{20}$$

The ratio between the pulsing frequency (f) and the flow rate (F) defines the number of applied pulses (n) and is a characteristic value that can be set by the processor to reach the desired degree of inactivation, and is defined as

$$n = \frac{fv}{F} \tag{21}$$

In summary, it can be stated that in terms of energy consumption and low product heating, treatment chambers with high electrical resistance are the most desirable designs. All possible measures should be taken to increase chamber resistance as much as possible. From Eq. (9), it is possible to observe that the electrical resistance of the treatment chamber (R_{Ch}) increases in direct proportion to the distance between the electrodes (d) and in inverse proportion to the electrodes' effective area (A) and food product conductivity (σ). In general, the electrical conductivity of the treated product is a parameter that cannot be controlled, unless development of a product especially designed for treatment by PEF is

involved. The two remaining parameters, the distance between the electrodes and the electrode surface, on the other hand, can be modified to change the intrinsic resistance of the treatment chamber. Increasing the distance between the electrodes certainly increases the treatment chamber's electrical resistance; however, this action also decreases the intensity of the electric field in the same proportion [Eq. (5)], thus reducing the effectiveness of the process. Reducing the electrode surface is another possibility to increase the intrinsic resistance of the treatment chamber, although the cost in this case is the reduction of the volume of the treatment chamber.

Depending on the design and treated media, coaxial and parallel plate treatment chambers have intrinsic resistances in the range of 3 to 30 Ω, whereas co-field chambers have resistances on the order of 50 to 300 Ω (Góngora-Nieto et al., 2002). This increased resistance of the co-field treatment chambers due to the reduced electrode area also reduces the chamber volume, requiring higher pulsing frequencies.

One other design parameter that needs to be considered in the design of a treatment chamber is the homogeneity of the generated electric field. Homogeneity of the electric field throughout the gap between the electrodes ensures homogeneous treatment. When different field intensities are present between the electrodes, some of the product may be subtreated, while other fractions of the product may be overtreated. Careful attention must be placed on deciding whether average treatment or minimum treatment is to be considered as the monitoring parameter. Parallel plate treatment chambers have a constant electric field throughout the chamber volume. Electric field vectors in this type of chamber are perpendicular to the electrodes and, therefore, present the same density throughout the region between the electrodes, with intensity defined in Eq. (5). However, this design is technically disadvantageous due to its large electrode surface, high electrical resistance, relatively small treatment volume, and the complexity of turning it into a continuous treatment chamber. Concentric electrode treatment chambers, on the other hand, present a radial distribution of the electric fields, with a decrease of the electric field vector density from the inner high-voltage electrode to the outer low-voltage electrode. The intensity of the electric field ($E_{(r)}$) at any point (r) between the electrodes in a concentric electrode treatment chamber is defined as

$$E_{(r)} = \frac{V_{\mathrm{Ch}}}{r\ln(r_{\mathrm{LV}}/r_{\mathrm{HV}})} \qquad r_{\mathrm{HV}} \le r \le r_{\mathrm{LV}} \tag{22}$$

where r_{LV} is the radius of the low-voltage electrode and r_{HV} is the radius of the high-voltage electrode. This type of treatment chamber, although it also has a low intrinsic electrical resistance and somewhat heterogeneous radial distribution of electric field intensity, has better characteristics for operation in continuous fashion than do parallel plate treatment chambers. Equation (22) illustrates that as the radii of both electrodes is increased while maintaining the same distance between them, the difference in electric field intensity at the surface of the two electrodes becomes smaller.

The distribution of the electric field in co-field chambers is variable depending on the exact configuration of the electrodes and their relative position to insulating elements. No precise expression that defines the electric field intensity in the area between the electrodes of such chambers has been developed yet, although Eq. (5) is commonly employed. Efforts in the modeling of the distribution of electric field intensities in this type of chamber by finite element methods have yielded interesting results; however, more study is needed (Lindgren, 2001; Fiala et al., 2001). Co-field electric chambers provide the best flow dynamics and have high intrinsic resistance, which permits the use of several chambers in series supplied by a single high-voltage pulse generator.

A final consideration during treatment-chamber design is the enhancement of the generated electric fields by the presence of dielectric materials, due to the contact between three different materials inside the treatment chamber (triple points) or at the edges of the electrodes. This enhancement of the electric field is undesirable when it occurs at the electrode edges or at the points of contact between the insulating housing and the electrodes, because it produces arcing and localized increase in temperature, which can cause sparking and damage to the treatment chamber. To avoid such damage, the design of treatment chambers should include rounding of the electrodes and location of the triple points outside the area of influence of the electric fields. Electric field enhancement, however, plays a fundamental role in the design of some treatment chambers. Systems, such as the "converged electric fields" designed by Matsumoto et al. (1991) or the "concentrated field electrode system" designed by Sato et al. (2001), work by introducing a perforated sheet of dielectric material into the area where the electric field is being induced, resulting in an enhancement of the electric field in the regions where the dielectric material is perforated. However, the use of such treatment chambers needs to be explored further.

C. Fluid-Handling System

Continuous PEF systems are equipped with the piping and pumps needed to bring liquid or semiliquid product being treated from the raw-product tank to the treated-product tank. Positive displacement pumps or peristaltic pumps are commonly used in PEF systems, although no restrictions exist for the use of other types of pumps. Continuous or pulseless pumps are preferred to ensure treatment homogeneity. Stainless steel piping is preferred for sanitary reasons, although plastic tubing is required in some areas for electrical reasons. Because pumps and lines are electrically connected to the power source through the liquid media, it is very important that all devices and installations are properly grounded for operative and safety reasons. Valves and bypasses are installed at several points of the circuit to ensure that recirculation or diversion of streams is possible at any time. Cleaning in place systems (CIP) or sterilizing in place systems (SIP) are commonly present in PEF pilot plant systems. Heat exchangers used to heat or cool the product as desired are commonly placed at the entrance and exit of the treatment chamber. Aseptic packaging units are frequently used to ensure proper packaging of the product, and to avoid posttreatment contamination (Zhang et al., 1997).

D. Control and Monitoring Devices

PEF systems are usually hooked up to a central computer that controls the operation of the high-voltage pulse generator, setting the proper conditions of voltage and pulsing frequency, and also controlling the operation of pumps and electric valves in the system. This computer also records data logs that include information such as the temperature at several points in the system during operation of equipment, the flow rate of the product, and the voltage, current, and power curves of the applied pulses. Temperature data are gathered through PID devices directly connected to the central computer while electric parameters are measured by high-intensity voltage and current probes fed into the central computer through an oscilloscope card. Available software such as HP V Lab® or Lab View® can be used in a PC to administer the control and data-gathering process. Some PEF systems, especially bench top lab models, are equipped with a dedicated central processor unit programmed from the factory to accomplish such tasks as its exclusive function. Detection of missed or weak pulses and soft protection against arcing are also valuable features

included in the latest versions of control equipment for PEF technology (EPRI, 1998; Kempkes, personal communication, 2002).

III. MICROBIAL INACTIVATION MECHANISM

Most food preservation processes are based on the use of a specific energy source to destroy or inactivate unwanted bacteria present in food products. Thermal preservation processes use energy in the form of heat to inactivate microorganisms; nonthermal preservation processes depend on energy sources other than heat, such as high-intensity pulsed electric fields, to ensure the microbiological safety of foods. The exact mechanism by which high-intensity pulsed electric fields inactivate microorganisms is not yet completely understood; however, much of the research in the field points toward damage of the cell membrane as the principal factor responsible for microbial inactivation (Heinz et al., 2002). Other effects resulting from the application of high-intensity pulsed electric fields, such as DNA damage and generation of toxic compounds, have been suggested, although posterior studies have rejected these hypotheses (Dunn, 2001).

Cell membranes play an important role in maintaining bacterial cells contained and isolated from the external environment. The peculiar semipermeability properties of the cell membrane allow the cell to interact with its environment by uptaking the nutrients and releasing waste products in a selective manner, thus maintaining the homeostasis of the bacterial cell. Studies conducted by Sale and Hamilton in the 1960s demonstrated that the application of direct-current high-voltage pulses caused the loss of the semipermeability properties of the cell membrane, altering homeostasis and causing death. Although this statement could not be visually confirmed by electron microscopy, tracing of extracellular and intracellular metabolites demonstrated the altered state of the semipermeable cell membrane (Hamilton and Sale, 1967). Since the porous area of the cell membrane represents less than 0.1% of the total surface area (Ho and Mittal, 1996), it is not surprising that visual evidence may be difficult to obtain.

An electromechanical model explaining the formation of pores on the cell membrane by the application of pulsed electric fields was developed by Zimmermann et al. (1974). In this model, the cell membrane is considered a dielectric material that separates ionic species and free charges on both sides (inside and outside the microbial cell). The differential concentration of charges on both sides of the membrane causes an intrinsic transmembrane potential to be naturally present in the microbial cell. Induction of an external electric field causes free charges to accumulate artificially at both sides of the membrane, oriented in such a way that opposite charges are only separated by the membrane, causing the addition of an external membrane potential to the preexisting intrinsic transmembrane potential, which increases in proportion to the intensity of the applied external electric field. The charge movement generated by the external electric field causes compression of the membrane separating the charges, which is further enhanced by the mutual attraction experienced by the charges at both sides of the membrane as established by Coulomb's law:

$$F = k\frac{q_1 q_2}{r^2} \tag{23}$$

where F is the magnitude of the attraction force between the two charges, k is a proportionality constant, q_1 and q_2 are the magnitudes of charge 1 and charge 2, and r is the distance separating the charges (membrane thickness). This equation shows that the intensity of the compressive force increases exponentially as the membrane becomes thinner.

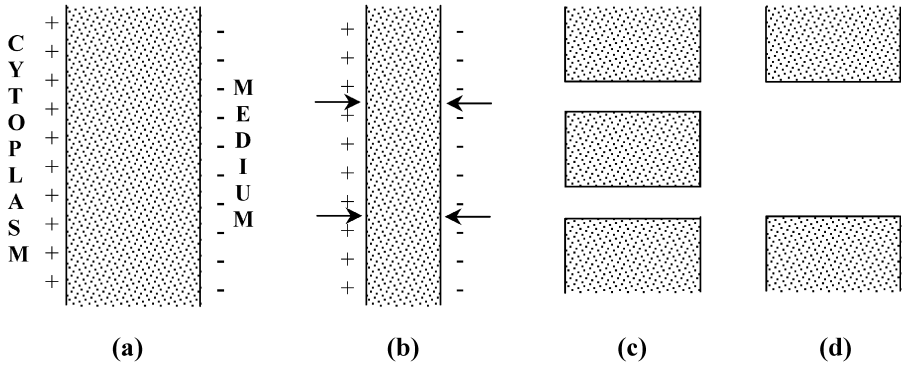

Figure 5 Schematic diagram of membrane breakdown, showing: (a) the cell membrane action as a dielectric barrier, (b) charging of the membrane by application of an electric field, (c) formation of pores after the critical electric field intensity has been reached, followed by (d) the expansion of the pore. (From Zimmermann, 1986.)

The rheological characteristics of the membrane determine its ability to "flow" under compression or to deform elastically. Since the applied compression is exponentially increasing, the membrane will reach a point where its ability to be elastically restored is surpassed and a local failure known as electrical breakdown of the membrane will occur (Fig. 5).

It is believed that electrical breakdown occurs when a transmembrane potential of around 1 V is induced in a microbial cell. Depending on the cell orientation relative to the external electric field, membrane disruption will most likely occur on preexisting local perturbations of the cell membrane that can be enlarged by the compression caused by the electric field. Formation of pores will lead to an increase in the permeability of the cell membrane and allow the interchange of intra- and extracellular materials, causing osmotic imbalance and further disruption of the cell (Fig. 6) (Zimmermann, 1986). It has been reported that the rupture of the cell membrane in erythrocytes occurs when the swelling of cells due to osmotic imbalance approaches around 155% of the normal cell volume (Tsong, 1990).

Alternative theories relate the changes in membrane permeability to other factors such as phase transitions in the lipid phase (Sugar and Neumann, 1984), increased transbilayer mobility of the lipid molecules (Deuticke et al., 1983), evolution of small hydrophobic pores (Chernomordik, 1992), induced transition of hydrophobic to hydrophilic

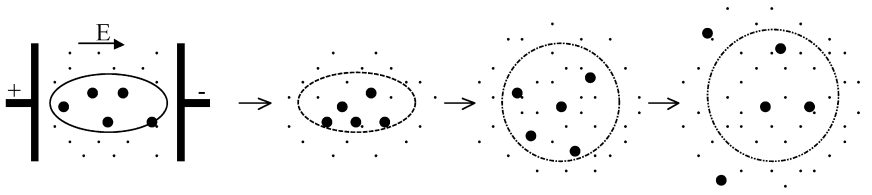

Figure 6 Destruction of bacterial cells through application of PEF, illustrating the formation of pores, the exchange of intra- and extracellular fluids followed by swelling caused by osmotic imbalance, and, finally, the permanent loss of intracellular material and organelles. (From Tsong, 1991.)

pores (Weaver and Barnett, 1992; Glaser et al., 1988), and opening and denaturation of sensitive protein channels (Tsong, 1992). Most theories involving the modification of the lipid phase are based on the rearrangement of the lipid bilayer after the pulse has been applied and a pore has been formed. These theories argue that if the pore is open long enough, conformational changes and dipole reorientation would cause a change in the characteristics of the pore from a hydrophobic pore to a hydrophilic pore, which is more stable, allowing for free traffic of fluids and growing indefinitely after a critical diameter has been exceeded. On the other hand, theories based on disruption of protein channels argue that voltage-operated protein channels would open after the required potential has been induced, followed by channel denaturation due to the high currents and ohmic heating caused by the applied high voltages. Such denaturation would cause the channels to remain permanently open, thus altering the cell's normal operation (Tsong, 1990).

It is worth mentioning that not one of these theories has proven to be a major mechanism in membrane permeabilization; and it is possible that some or all of these processes are responsible for cell damage during the application of pulsed electric fields.

IV. DETERMINANT FACTORS IN PEF TECHNOLOGY

The effectiveness of pulsed electric field technology as a microbial-inactivation process depends on several factors related to the type of equipment used, such as the setting of the treatment parameters, the type of media processed, and the target microorganism, among others. Although all factors mentioned here undoubtedly have an important influence on the effectiveness of PEF as a microbial inactivation agent, they do not always account for the total observed effects; there is a need for more study in this area to understand the nature of the multiple interactions between all the involved factors and to explore the possibility of additional factors.

A. Technical Factors

Technical factors are normally of an extrinsic nature. They can be modified at will and dosed as required to provide the desired treatment. Some of the technical factors involved in PEF technology are not independent of each other and therefore cannot be adjusted without also modifying the related factor(s). Equipment design and operation characteristics usually define the technical factors; however, constraints imposed by intrinsic factors of the product or the process are also relevant.

1. Electric Field Intensity

Electric field intensity has been identified as the most relevant factor defining microbial inactivation by pulsed electric fields. Hamilton and Sale (1967) demonstrated that the disruption effect of PEF depended only on the intensity of the electric field and the total treatment time, and that localized heating, electrolysis, current density, or energy input did not play a role in membrane disruption. Subsequent studies also demonstrated that a critical electric field intensity level must be reached to have any effect at all on microbial cells, and that electric fields above this threshold level have an exponential effect on microbial inactivation (Hülsheger et al., 1981). The critical electric field intensity corresponds to the external electric field intensity capable of inducing a transmembrane potential of around 1 V, which is the threshold for membrane disruption. In practice, electric field intensity must

be kept as low as possible for the treatment to be effective, since high electric fields can also cause dielectric breakdown of the liquid being processed and are conducive to arcing and undesirable reactions (Zhang et al., 1994b; Zhang et al., 1995a).

2. Treatment Time

PEF treatments are applied in the form of short pulses to avoid excessive heating or undesirable electrolytic reactions. The pulse width multiplied by the number of applied pulses defines the total treatment time. Pulse width is intimately related to the effectiveness of electric fields in disrupting the cell membranes (Neumann et al., 1992; Gaskova et al., 1996). It has been found that pulses between 1 and 5 μsec produce the best results for microbial inactivation. It is hypothesized that a period of time of around 10 nsec, after the electric field is applied, is required to establish a transmembrane potential (charge the membrane). After pore formation, an electric field over the critical threshold level sustained for a period of 1 to 5 μsec is required to allow the expansion of pores to a critical diameter so that bacterial cells are not able to repair the damage, hence causing cell death and increasing treatment effectiveness (Schoenbach et al., 2000). Longer pulses tend to cause electrolytic reactions and electrodeposition at the electrodes' surface, and therefore must be avoided (Zhang et al., 1994b, 1995a).

It is not clear how the repetitive application of pulses acts at the cellular level to increase inactivation; however, it has been established that inactivation increases linearly with treatment time (Hülsheger et al., 1981). Repetitive pulse application has no effect on microbial cells whose membranes have already been disrupted, because a free flow of intra- and extracellular content prevents the reestablishment of a transmembrane potential until the formed pore is sealed (Knorr et al., 2001). Repetitive pulse application, however, probably increases treatment effectiveness by creating repetitive tension on the membrane until breakdown occurs due to (a) repeated stress, (b) proper orientation of preexisting flaws in the electric field, or (c) individual cells within the treatment chamber where the surrounding conditions, such as the presence of nutrients, electrolytes, or higher electric fields, promote membrane disruption.

3. Pulse Shape

There are two waveshapes of practical relevance in PEF technology: exponentially decaying pulses and square pulses. Both pulse shapes can be applied in a mono- or bipolar fashion, which means only positive pulses (with respect to ground level) or alternating positive and negative pulses can be applied (Fig. 7).

Square pulses are considered superior to exponentially decaying pulses (Qin et al., 1994; Zhang et al., 1994a; Pothakamury et al., 1996), because in the case of square pulses, the applied treatment is sustained at a constant intensity for the total duration of the pulse, whereas in exponentially decaying pulses, the intensity of the treatment varies from a peak electric field passing through the critical electric field level to a series of sublethal electric field intensities until it reaches ground level. The effectiveness of the treatment applied with exponentially decaying pulses decreases linearly from a maximum at peak electric field to a minimum when the critical electric field level is reached. The rest of the pulse energy delivered after the critical electric field has been left behind only contributes to the heating of the treated liquid and, therefore, besides being wasted (increasing energy expenditure), negatively affects the conditions of the nonthermal treatment (EPRI and Army, 1997; EPRI, 1998). Qin et al. (1994) reported 60% more inactivation with square pulses than with exponentially decaying pulses (with pulse width equivalent to τ). Although theoretically true, the

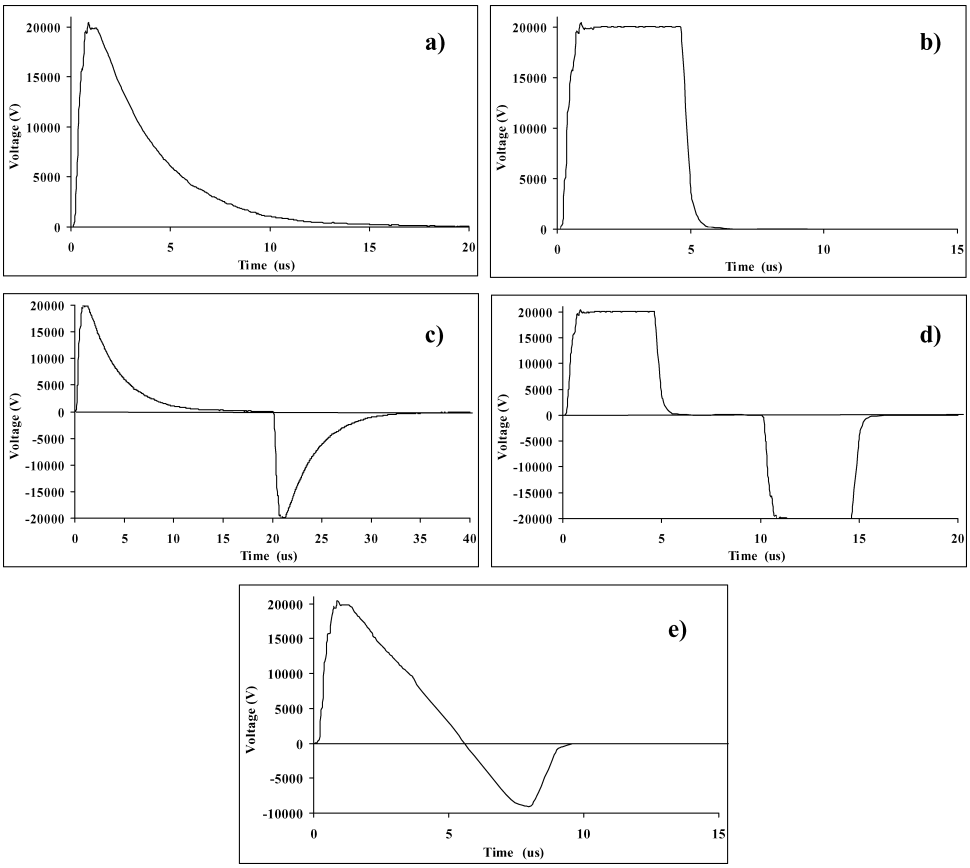

Figure 7 Pulse shapes commonly used in PEF technology: (a) exponentially decaying pulse, (b) square pulse, (c) bipolar exponential, (d) bipolar square, and (e) instant charge reversal.

superiority of square pulses over exponentially decaying pulses is difficult to quantify in practice, because there is no clear way to define the equivalence between square and exponentially decaying pulses. A common approach to this problem is to consider any exponentially decaying pulse "equivalent" that has the same peak electric field as a square pulse, and whose time constant (τ) is the same as the total pulse width of the square pulse. Other researchers prefer to use the FWHM, or five times the constant of the exponentially decaying pulse as the total pulse duration. Probably a sounder approach, from the engineering and thermodynamic points of view, would be to compare isoenergetic pulses; however, none of these possibilities can be claimed as the "right way" to conduct such comparison, and the quantitative superiority of square pulses remains partially unknown.

Application of bipolar pulses is more effective than application of monopolar pulses for both square and exponentially decaying pulses (Qin et al., 1994). The repeated change in polarity causes movement of charges inside and outside the membrane to switch directions alternatively at the same time that charged groups on the membrane change orientation (Chang, 1989), causing a mechanical oscillation of the membrane, which results in alternating stress, structural fatigue, and enhanced susceptibility to electric breakdown (Barbosa-Cánovas et al., 1999). Moreover, bipolar pulses also offer the advantage of reducing the

deposition of solids on the electrodes of the treatment chamber and limiting the possibility of electrolytic reactions, while offering as much as twice the effectiveness as monopolar pulses (Qin et al., 1994).

A special case of bipolar pulses is called "instant-charge-reversal" pulses, where the positive and negative sections of the bipolar pulse are immediately contiguous instead of having a "relaxation time" between positive and negative sections. It has been claimed that such pulses have an enhanced effectiveness and that high inactivation levels are easily obtained with fewer pulses, even at subcritical electric field levels (Ho et al., 1995); however, this has not been confirmed by independent research groups, and more study is required (EPRI, 1998). Zhang et al. (1997) extended the shelf life of orange juice stored at 37°C for 8 days by applying square pulses for 5 days, using instant-charge-reversal pulses, and for 4 days, using exponentially decaying pulses, which points to a similar effect between instant-charge-reversal pulses and exponentially decaying pulses.

4. Applied Energy

It is not clear at this point whether the total amount of applied energy per se has an effect on the effectiveness of PEF processes. As explained in Section 2.2, the applied energy directly depends on other factors, such as electric field intensity, treatment chamber design, product conductivity, and treatment time, and cannot be chosen independently of those factors. Further complications arise from the fact that the same applied energy can be delivered by different combinations of voltage, current, and pulse width or pulse shape, hence making it necessary to fix at least one of these factors before attempting any comparisons, as demonstrated by Qin et al. (1994). Although Hamilton and Sale (1967) claimed that energy input did not play a relevant role in membrane disruption, other authors such as Muraji et al. (1993) and Tatebe et al. (1995) found that electrical energy has a major impact on microbial survival rate. Both claims, while apparently contradictory, could be true; although not having a direct effect on membrane disruption, the applied energy (e.g., through heat production) could modify the treatment conditions to inhibit recovery or enhance the effect of the main inactivation factors. Defining the relevance of this parameter has important practical implications, because the ability to reduce energy delivery without compromising the effectiveness of PEF processes would significantly lower operational and equipment costs, while at the same time reduce product heating.

The consensus is that a preservation process similar to thermal pasteurization can be accomplished by PEF with treatment applications of around 100 J/mL (Zhang et al., 1995b), depending on the type of equipment used, pulse shape, conductivity of the treated media, and required level of inactivation. Extreme practical situations can result in highly efficient treatments of less than 80 J/mL or high-energy-consuming processes with energy levels of around 400 J/mL (Barsotti and Cheftel, 1999; Schoenbach et al., 2000; EPRI, 1998). It has been reported that the use of instant-charge-reversal pulses reduces energy expenditure to around 5 J/mL; however, as mentioned before, such claims have not been corroborated by independent research groups and need to be further studied (EPRI, 1998).

B. Biological Factors

Individual characteristics of target microorganisms are a determining factor in defining the inactivation effectiveness of PEF. Characteristics such as genre, species, size, shape, or physiological state define the susceptibility of microorganisms to be inactivated by PEF. These factors are of intrinsic nature; they are a characteristic of the product being treated

and cannot be controlled or modified by the processor before processing. It is of great relevance to understand that food products often are accompanied by a complex variety of microorganisms and that the sensitivity to pulsed electric fields may vary from organism to organism. Therefore, PEF treatments should be planned and conducted, taking into consideration the most resistant organisms potentially present in a given product to ensure adequate processing and effective control of all existing microorganisms.

1. Size and Shape of Microorganisms

Size and shape of microorganisms define the required external electric field intensity required to cause disruption of the cell membrane. As explained before, a transmembrane potential of around 1 V is required to cause permeabilization of the membrane (Zimmermann, 1986). The electric field intensity required to induce a given transmembrane potential into a cell can be calculated (Schoenbach et al., 1997) by the integrated Laplace equation

$$V_c = fE_c a \cos \vartheta \tag{24}$$

were V_c is the breakdown voltage, a is the cell radius, E_c is the required critical field strength, ϑ is the angle between a given membrane site and field direction, and f is a form factor equal to 1.5 for spheres and related to length (l) and diameter (d) of cylinder-like cells, defined as

$$f = \frac{l}{l - d/3} \tag{25}$$

These equations show that external electric fields induce higher transmembrane potentials in larger cells (Fig. 8) and that the highest potential is localized in the membrane areas perpendicular to the electric field. The influence of cell size on the effectiveness of PEF treatments has been addressed by several research groups and abundant examples are available in the literature (Sale and Hamilton, 1967; Hülsheger et al., 1983; Zhang et al., 1994c).

2. Type of Microorganism

A description of the effects of PEF on microorganisms in broad terms gives a general idea of the common behavior of microorganisms under the influence of PEF. Nevertheless, these guidelines should not be considered as strict rules, because particular cases may transgress

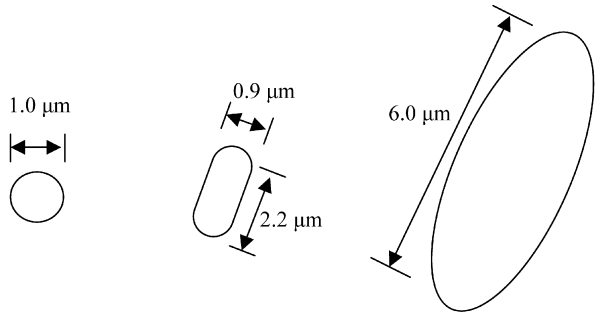

Figure 8 Cell size comparison between different types of microorganisms. (From Barbosa-Cánovas et al., 1999.)

the principles stated here. Even when a specific genre and species behaves within the boundaries defined here, especially sensitive or resistant individuals or strains within that population may depart from these general guidelines.

Generally speaking, yeasts can be considered to be more sensitive to PEF treatments than bacteria. Such greater sensitivity is probably due to the large size of yeasts when compared to bacteria, as explained in the previous section (Jacob et al., 1981; Hülsheger et al., 1983; Gaskova et al., 1996). Among bacteria, gram-negative bacteria are more sensitive than gram-positive bacteria. The presence of a cell wall with thick mucopeptide backbone layers in gram-positive bacteria in contrast with a thin middle membrane in gram-negative bacteria (Ray, 1996) may be part of the explanation for the increased resistance of gram-positive over gram-negative bacteria. Reports of superior resistance of gram-negative bacteria when compared with gram-positive bacteria can be found throughout the literature (Hülsheger et al., 1983; Pothakamury et al., 1995; Lubicki and Jayaram, 1997).

3. Physiological State of Microorganisms

Concerning the physiological state of microorganisms, it has been shown that within a single type of bacteria, individual cells are more or less sensitive to electric fields depending on the growth stage of its population at the time of PEF application. Growth of microbial populations is characterized by an initial lag phase where the population remains unchanged; microorganisms are getting used to the environment, assimilating nutrients, and growing in size. In a second stage, known as the exponential or logarithmic phase, cells start to reproduce, first just some, then all of them, and the population increases rapidly. After the size of the population reaches its maximum defined by nutrient availability, concentration of toxic metabolic by-products, and other similar factors, the populations enters a stationary phase in which some individuals die and some reproduce themselves, hence maintaining a stable population. Finally, in the death phase, environmental conditions worsen and the population decays (Ray, 1996).

It has been demonstrated that microorganisms are more sensitive to PEF in the exponential phase than in the lag or stationary phases (Pothakamury et al., 1996; Gaskova et al., 1996), and it is hypothesized that this increased sensitivity is caused by the presence of "fresh scars" or sensitive areas in the membrane due to recent cell division. The maximum resistance of cell populations is found in the early stationary phase, when the population is not engaged in rapid reproduction, and environmental conditions have not reached a point where cells are significantly damaged or inhibited.

Another particularly important physiological state of microorganisms in PEF technology is sporulation. Formation of spores is a reproductive method for molds and yeasts and a protective strategy for bacteria. Formation of spores consists of the encapsulation of genetic material in highly resistant envelopes formed by several layers of refractory materials. The coating of bacterial spores is more resistant than the coating of mold and yeast spores (Ray, 1996). It is known that vegetative cells are more sensitive to PEF than spores. The susceptibility of spores to be disrupted by the application of PEF is a polemic subject, and whereas some studies show that spores are not affected by PEF at all, other researchers claim at least partial success inactivating spores, so the issue remains to be clarified (Su et al., 1996; Marquez et al., 1997; Jin et al., 1998; Pagan et al., 1998). A hurdle approach involving a previous processing step where germination of spores is promoted (such as application of moderate heating) has been suggested as a viable strategy to deal with spores in food products treated by PEF (Pol et al., 2001; Simpson et al., 1995). The inability of PEF to inactivate spores might constitute an obstacle for PEF technology in becoming a

suitable substitute for commercial sterilization; however, milder processes such as pasteurization do not require spore inactivation, and PEF technology remains a good candidate to substitute or complement such processes.

C. Factors Related to Treated Products

Studies on processing of several products ranging from simple laboratory-simulated foods to complex real-world products such as milk or fruit juices have been conducted with PEF technology. A list of references on published studies for a variety of products is given in Table 4.

Table 4 List of Some of the Products Processed by Pulsed Electric Fields

Treated medium	Reference
0.1% NaCl solution	Sale and Hamilton (1967), Gupta and Murray (1989)
17.1 mM saline solution	Hülsheger and Niemann (1980)
0.9% NaCl solution	Jacob et al. (1981), Yonemoto et al. (1993)
Phosphate buffer, pH 7.0	Hülsheger et al. (1983), Matsumoto et al, (1991), Jayaram et al. (1992)
Milk	Dunn and Pearlman (1987), Gupta and Murray (1989), Reina et al. (1998), Bendicho et al. (2002a)
Yogurt	Dunn and Pearlman (1987)
Deionized water	Mizuno and Hori (1988)
Sodium alginate	Grahl et al. (1992)
Orange juice	Grahl et al. (1992), Qiu et al. (1998), Hodgins et al. (2002), Liang et al. (2002), Zhang et al. (2002)
UHT milk (1.5% fat)	Grahl et al. (1992)
Potato dextrose agar	Zhang et al. (1994a)
Apple juice	Qin et al. (1994), Zhang et al. (1994b), Qin et al. (1995), Evrendilek et al. (1999)
Simulated milk ultrafiltrate (SMUF)	Qin et al. (1994), Zhang et al. (1994c), Pothakamury et al. (1995)
Skim milk	Zhang et al. (1994a), Martin et al. (1997), Calderon-Miranda et al. (1999)
Sucrose and xanthan solution	Ho et al. (1995)
Pea soup	Vega-Mercado et al. (1996a)
Liquid egg	Martin-Belloso et al. (1997), Calderon-Miranda et al. (1999), Góngora-Nieto et al. (2001)
Cranberry juice	Raso et al. (1998), Jin and Zhang (1999)
Dry spices	Keith et al. (1997)
Wheat flour	Keith et al. (1998)
Liquid egg white	Jeantet et al. (1999)
Rice wine	Mok and Lee (2000)
Orange–carrot juice	Rodrigo et al. (2001)
Rice pudding	Ratanatriwong et al. (2001)
Apple cider	Iu et al. (2001)
Cheese sauce	Ruhlman et al. (2001a)
Beef burgers	Bolton et al. (2002)
Horchata	Góngora-Nieto et al. (2002)

The chemical and physical characteristics of treated products play an important role in defining the applied treatment and its effectiveness. Most characteristics of treated products are inherent attributes that generally cannot be modified without changing the characteristics of the product and must be dealt with as is. Examples of such intrinsic factors related to the treated product are its electrical conductivity, dielectric strength, pH, ionic strength, nutrient content, presence of suspended solids, and viscosity. The temperature of the treated product is an important characteristic that can be adjusted during treatment to the most convenient level, and probably constitutes the only extrinsic factor related to the treated product. Specifically designed products formulated for processing by PEF are a special case, where intrinsic factors can be manipulated (within a restricted range) to meet the best conditions for PEF processing and represent an important area for future study of PEF technology.

1. Composition

The composition of treated media may influence the effectiveness of PEF treatment and must be considered when defining processing strategy. Although some relevant factors related to composition, such as pH or the presence of naturally occurring antimicrobials, are not directly involved in the process of inactivation by PEF, they contribute to the inactivation process through their own bactericidal properties, working as hurdles (either additively or synergistically) to increase the effectiveness of the treatment (Liu et al., 1997). On the other hand, product characteristics such as temperature, ionic strength, or electrical conductivity are factors deeply involved in the process of inactivation by PEF. Probably the most important factor is ionic strength, which is responsible for the electrical conductivity of the treated media. As described in previous chapters, the conductivity of the treated media defines the resistance of the treatment chamber. As a general rule, lowering the conductivity reduces the temperature and applied power, thus increasing the electric field intensity and overall effectiveness. However, one must not forget that although reducing the concentration of ions in solution lowers the conductivity with all its described advantages, ions are needed in the treated media to establish a transmembrane potential (Hülsheger et al., 1981; Jayaram et al., 1993; Vega-Mercado et al., 1996b). It is important to note that the electrical conductivity of ionic solutions strongly depends on the kinetic state of the solution, and as such is highly dependent on temperature. An increase in temperature increases the mobility of ions throughout the solution, causing an increase in the conductivity (Heinz et al., 2002). As a general rule, the higher the ionic concentration, the stronger the temperature dependence (steeper slope); nevertheless, some specific electrolytes or systems may behave in a different manner. Some examples of food products and their electrical conductivities are given in Table 5.

Another relevant factor related to the treated product is its dielectric strength. This attribute defines the maximum electric field a product can withstand without undergoing dielectric breakdown, where an uncontrolled discharge of electric current flows through the product, causing intense heating and damage to the equipment and product. The dielectric strength of the product limits the maximum electric field intensity that can be used for preservation purposes, and therefore an intensity level must be chosen so as to achieve the desired inactivation effect without reaching the breakdown of the product (Lindgren, 2001). Suspended particles or trapped air bubbles can have a different dielectric strength from the rest of the treated media; therefore, their presence further limits the maximum treatment intensity that can be applied. When products of different dielectric strengths are mixed at a macroscopic level (suspensions or emulsions), the one with the lowest dielectric strength

Table 5 Electrical Conductivity of Selected Food Products

Food product	Conductivity (S/m)	Temperature (°C)
Apple juice[a]	0.175	15
Beer[b]	0.143	22
Carrot juice[b]	1.147	22
Cranberry juice[b]	0.090	22
Coffee (black)[b]	0.182	22
Distilled water[a]	0.00011	—
Egg white[a]	0.645	15
Egg (whole)[a]	0.588	21
Grape juice[b]	0.083	22
Lemonade[b]	0.123	22
Milk (whole)[a]	0.385	15
Milk (skim)[a]	0.323	15
Milk (whole w/chocolate)[b]	0.433	22
Orange juice[a]	0.427	42
Orange juice concentrate[a]	0.333	15
Pea soup[a]	0.263	15
Tomato juice	1.697	22
Tomato ketchup[a]	2.38	15
Vegetable juice	1.556	22
Yogurt[a]	0.592	23

[a] Barbosa-Cánovas et al. (1999).
[b] Ruhlman et al. (2001b).

defines the maximum applicable treatment intensity. Generally, dielectric solids and liquids have higher dielectric strength than dielectric gases, whereas vacuum has infinite dielectric strength (Lide, 2002).

Differences in electrical permittivity between components of a food product affect the behavior of nonhomogeneous products as well. As stated by Gauss' law, the presence of substances with high permittivity in a mixture with lower permittivity enhances the electric field intensity of substances in the low permittivity mixture to levels over that of the externally applied electric field, hence promoting dielectric breakdown in conditions where it does not normally happen (Bruhn, 1997; Crowley, 1986). Mixtures at the molecular and atomic levels tend to behave as homogeneous products and develop their own bulk electrical properties (i.e., dielectric strength, conductivity, and permittivity).

The presence of components such as fat and proteins has been related to a protective effect on microorganisms against PEF, and the inactivation of microorganisms in complex foods represents a major challenge when compared to simple suspension systems (Zhang et al., 1994a; Ho et al., 1995; Grahl and Märkl, 1996; Martin et al., 1997). Although some hypotheses in this regard can be developed, for example, considering the capacity of some substances to "shield" microorganisms from the applied electric field (i.e., microorganisms suspended in substances with high electrical permittivity within a nonhomogeneous product) or assuming the ability of some chemical species to stabilize or prevent ion migration (i.e., by interaction with charged groups in a rigid structure), this protective effect has not been clearly demonstrated. There are contradictive findings, and more evidence is needed to define the effect of such components on the effectiveness of PEF treatment (Mañas et al., 2001).

2. Temperature

Pulsed electric field technology is considered a nonthermal preservation method in view of the fact that the electric field itself is responsible for microbial inactivation, not a variation in the product's temperature. However, this is not a reason to believe that processing temperature has no impact on the effectiveness of the process. It is important to understand that nonthermal preservation methods are referred to as such due to the alternative origin of their inactivating agent, not the complete absence of thermal effects. In fact, processing temperature is probably one of the most relevant processing parameters in PEF technology, surpassed in importance only by the intensity of the applied electric field and the treatment time.

It has been demonstrated that mild thermal treatments (i.e., sublethal combinations of temperature and processing time) combined with pulsed electric fields increase the effectiveness of PEF when compared to PEF treatments conducted at room temperature (Jayaram et al., 1992; Zhang et al., 1995b; Sepúlveda et al., 2002). As the temperature of the product processed by PEF increases, the effectiveness of the process increases. The thermal enhancement of PEF treatments can be considered a synergistic interaction within the boundaries of the commonly considered nonlethal thermal conditions (up to around 65°C for less than a few seconds). After this limit is surpassed, it is difficult to distinguish between the thermal and nonthermal effects of the process, and it is believed that the thermal effect governs the preservation process after this point (Barsotti and Cheftel, 1999; Dunn, 2001).

The increased effectiveness of PEF at higher temperatures is probably due to the dependence of the rheological properties of the microbial membranes on temperature, which causes membranes to lose their elastic properties as temperature increases (becoming more fluidlike), and therefore are more easily disrupted by the application of PEF. At the same time, modification of the properties of the cell membrane decreases the critical electric field required to cause disruptive effects on microbial cells and increases the effective treatment time of exponentially decaying pulses (Jayaram et al., 1992). Experimental results have demonstrated that the breakdown voltage of the lipid–protein membranes ranges from 2 V at 4°C to 1 V at 20°C and 500 mV at 30–40°C (Zimmermann, 1986). The enhanced ion mobility promoted by higher temperatures reduces the required time needed to induce critical transmembrane potentials and increases the chances for permanent membrane damage; however, it has been demonstrated that this effect is negligible for pulses over 1 μs long and is probably not directly related to the enhancement of PEF's effectiveness by increased temperatures (Heinz et al., 2002).

As explained in previous sections, the application of pulsed electric fields causes an increase in the product's temperature depending on the delivered energy per pulse. The electrical conductivity of the product defines the required energy per pulse under a given set of conditions; to achieve the same desired treatment time, highly conductive products require more pulses or higher energy per pulse than low conductive products. Processing temperature can be controlled by adjusting the product's temperature prior to PEF application to reach the desired temperature inside the treatment chamber after the product has been heated through application of the pulses. Although some chamber designs include built-in heat exchangers, the instantaneous and volumetric nature of the generated ohmic heating makes it almost impossible to avoid a temperature increase, and the only viable strategy to reduce heating (and required energy) in the treatment chamber is to make the ratio between the distance within the electrodes and the electrode effective surface as large as possible. In practice, it is desirable to limit treatment temperatures that modify nutritional or sensory properties to below levels, which could defeat the entire purpose of a nonthermal

treatment; however, a middle ground is desirable wherever possible to take advantage of the synergistic interaction between PEF and mild thermal treatments.

3. Physical Attributes

The physical attributes of the treated media have practical implications for defining the intensity or effectiveness of the applied treatment. The attributes of greatest relevance in PEF technology and their influence on process effectiveness are summarized below:

- Density and specific heat of a product define the temperature increase caused by a given amount of energy.
- Electrical conductivity defines the absorbed energy and treatment chamber electrical resistance.
- Rheological factors, such as viscosity, in combination with operational parameters, such as flow rate and chamber geometry, define the flow regime of the product through the treatment chamber, defining the homogeneity of the applied treatment, and the extent of over- or undertreated product at the zones of maximum and minimum flow velocity. Viscosity and flow regime play an important role in controlling cell orientation as well; as described earlier, the possibility of cells reorienting within the electric fields increases the chances for membrane damage when orientation causes application of the maximum membrane compression to a preexisting flaw. Laminar vs. turbulent flow can also cause a difference in the effectiveness of the applied treatment for the same reasons.
- Thermal conductivity and thermal diffusivity define how heat distributes throughout the product and how fast the product can be heated or cooled.
- The physical structure of the product defines the homogeneity of the treatment as well. Presence of suspended particles or differentiated phases causes enhancement or reduction of treatment intensity in certain areas of the product, which may lead to over- or undertreatment conditions.

V. MODELING PEF MICROBIAL INACTIVATION

The development of mathematical expressions to model preservation processes is an important task aimed at defining and quantifying the influence of processing parameters (independent variables) on treatment effectiveness (dependent variables). Mathematical models can be used to gain insight into possible mechanisms of action or to predict the microbial concentration and shelf life of processed products (Lund, 1983).

The response variable or dependent variable that is typically quantified in PEF preservation processes is the reduction in the population of an undesirable biological agent attained by a given treatment. The first step toward developing a useful mathematical model, after defining a response variable, involves a thorough identification of the most relevant processing parameters (independent variables) influencing such a response variable. Erroneous or incomplete identification of these factors leads to ill-fitted models and poor predictive capacity. Accurate and reliable experimental data are needed to properly identify all relevant factors affecting the response variable of a process; however, proper interpretation of the observations plays an important role as well, because the presence of correlated factors can easily lead to misinterpretations (i.e., confusing the effect of electric current flowing through a resistor with the effect of heat produced by electric current); incorrect inferences can even be made with completely independent factors (e.g., assuming that lead

poisoning is responsible for the lethal effect of lead bullets). To avoid such misinterpretations, fundamental knowledge is required to establish causality for proper interpretation of observations, because purely empirical correlations do not unequivocally indicate a cause–effect situation (Lund, 1983). Development of unsupported, purely mathematical models that "mimic" the behavior of the response variable under a given set of conditions, and often do not react to changes in processing conditions, is of limited value.

In special situations, when fundamental knowledge is not available, empirical models can be used as tools to test assumptions in the process to generate new knowledge. This is probably the case in the development of models for PEF preservation processes, since a complete understanding of the mechanisms underlying the inactivation process is not yet available. The assumptions drawn from these empirical models are useful in describing the response of entire microbial populations to the treatment conditions; they do not necessarily elucidate the precise reason that some individuals in a population survive a given treatment.

As study of PEF technology as a food preservation method has evolved over the years, some mathematical empirical models have been proposed to describe microbial inactivation by PEF, but no one has been completely successful and the search is still on to identify a suitable expression.

The obvious first approach to model PEF processes would be to borrow the classic mathematical expression used to describe microbial inactivation by thermal treatments:

$$\frac{dN}{dt} = -kN \tag{26}$$

This expression corresponds to a first-order kinetics equation where the microorganism population (N) varies throughout processing time (t) in a constant direct proportion (k) depending on its size. Integration of this expression over a finite period of time becomes

$$\ln \frac{N}{N_0} = -kt \tag{27}$$

where N_0 is the initial microbial population; hence, $\ln(N/N_0)$ corresponds to the natural log of the survival fraction and yields a straight line when plotted against treatment time in semilog charts. Microbial inactivation curves in PEF technology, however, rarely follow this exponential decrease in population with respect to treatment time at a given electric field intensity (as described by first-order kinetics), but may present an initial portion of the curve where no considerable effect is found (shoulder region), followed by an exponential decrease section (linear region), and ending with a gradual reduction in the effectiveness of the treatment until no further reduction is attained (tail region), as illustrated in Fig. 9 (Qin et al., 1994; Lubicki and Jayaram, 1997; Alvarez et al., 2000; Knorr et al., 2001). Some researchers have used first-order kinetic expressions to describe the linear part of the inactivation curves (Sensoy et al., 1997; Martín-Belloso et al., 1997); although this approach applies only to a segment of the curve, it omits the regions of maximum and minimum effectiveness, limiting its practical utility.

The nonlinear behavior of PEF inactivation curves calls either for more complex mathematical expressions to account for these sigmoid curves (to relate inactivation with treatment time) or for the inclusion of other relevant factors in the model to find different types of relationships. Available experimental data generated by several research groups have made apparent the large influence of specific experimental conditions on the effectiveness of PEF treatments (Hülsheger et al., 1983; Martín-Belloso et al., 1997; Barbosa-Cánovas et al., 1999; Alvarez et al., 2000). A comparison of results from various groups

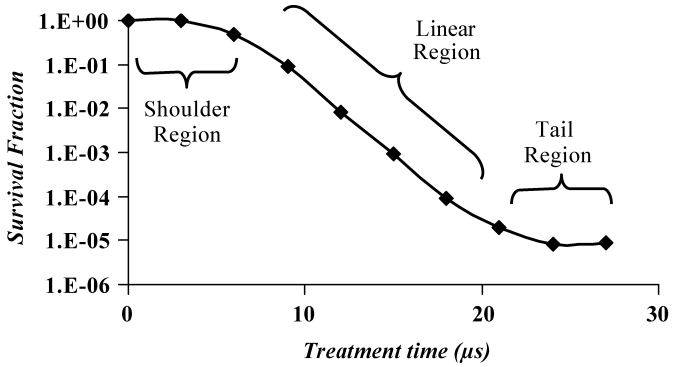

Figure 9 Example of an inactivation curve obtained during PEF processing, illustrating the shoulder, linear, and tail regions.

shows large discrepancies, probably due to differences in unaccounted for experimental conditions.

Although there is no clear understanding of the influence that all processing parameters have on PEF technology, there is a consensus that microbial inactivation is exponentially increased by increasing the electric field intensity and linearly increased by increasing the treatment time (Peleg, 1995; Schoenbach et al., 1997).

Hülsheger et al. (1981) developed a mathematical model to describe microbial inactivation by PEF, based on first-order kinetics and on the empirically known relationship between the population survival fraction and the electric field intensity and treatment time. First, the natural log of the survival fraction is related to the electric field intensity (E) and critical electric field (E_c) through a proportionality constant (b_E) in a first-order kinetics-like fashion:

$$\ln \frac{N}{N_0} = -b_E(E - E_c) \tag{28}$$

The proportionality constant (b_E) is dependent on experimental conditions such as treated media and target microorganisms, and may be affected by more than one processing factor. The critical electric field (E_c) is a value that can be empirically found by extrapolating a survival curve toward zero inactivation or calculated theoretically, based on the characteristics of the media and cell size and shape, as explained in previous sections. The effect of treatment time on the survival fraction is then estimated:

$$\ln \frac{N}{N_0} = -b_t \ln\left(\frac{t}{t_c}\right) \tag{29}$$

where the empirically known linear relationship between survival fraction and treatment time is corrected by a proportionality constant (b_t), and a critical time factor (t_c), which is the extrapolated time at zero inactivation.

A general expression including both treatment time and electric field intensity is then assembled from these elemental expressions:

$$s = \left(\frac{t}{t_c}\right)^{-\left(\frac{E-E_c}{k}\right)} \tag{30}$$

where k is an independent constant factor. Hülsheger et al. (1981) acknowledge that departing from the general experimental conditions used to develop this model (1–30 pulses of 8 to 20 kV/cm, discharging 0.05- to 2.5-μF capacitors on *E. coli*, suspended on electrolytic solution of specific resistance 576 Ω cm at 20°C) could reduce the predictive effectiveness of the model; nevertheless, several researchers have modeled survival curves under different conditions using this model with moderate to good success ($r^2 > 0.9$) (Zhang et al., 1994a; Martín-Belloso et al., 1997; Sensoy et al., 1997).

Current approaches to modeling gaining acceptance involve looking for new theories that explain the departure of PEF survival curves from linear kinetics (Peleg, 1995; Peleg, 1999) and new models that consider the possibility of individual resistance variability within microbial populations as related to a population probabilistic distribution (Peleg and Cole, 1998; Cerf, 1977). Peleg (1995) developed a mathematical expression capable of modeling the sigmoid shape of survival curves relating survival fraction to electric field intensity (E) and number of applied pulses (n), based on Fermi's distribution.

$$\frac{N}{N_0} = \frac{1}{1 + e^{[(E - E_{C50}(n)]/k(n)}} \tag{31}$$

Here, the critical electric field [$E_{C50}(n)$] corresponds to the electric field intensity, reducing the initial population to 50% after the application of n pulses; $k(n)$ is a proportionality constant dependent on the number of applied pulses. Variations in the pulse width and other processing parameters may also modify the values of the proportionality constants and, hence, need to be maintained constant as with other models. Experimental data have shown good agreement with values predicted by this model with correlation values between 0.97 and 0.99 (Peleg, 1995).

Other probabilistic distributions that are being explored as suitable candidates for modeling PEF inactivation kinetics include the following:

Weibull distribution:

$$\frac{N}{N_0} = e^{-(bt)^a} \tag{32}$$

where a and b are shape and scale constants, respectively (Peleg and Cole, 1998).

Log-logistic distribution:

$$\log\left(\frac{N}{N_0}\right) = \alpha + \frac{\omega - \alpha}{1 + e^{[4\sigma(\tau - \log\ t)]/\omega - \alpha}} \tag{33}$$

where α and ω are the upper and lower asymptotes, respectively, σ is the maximum slope of the inactivation curve, and τ is the logarithm of the time at which the maximum slope is reached (Cole et al., 1993).

Modified Gompertz equation:

$$\log\frac{N}{N_0} = Ce^{-e^{BM}} - Ce^{-e^{B(t-M)}} \tag{34}$$

where t is time and the rest of the parameters are distribution constants.

Baranyi model:

$$\log\frac{N}{N_0} = \log(q_B + (1 - q_B)e^{-k_{\max}[1 - B(t)]} \tag{35}$$

where t is time and the rest of the parameters are distribution constants.

Most of the models presented here are kinetic models, which by definition describe "movement" or, better stated, "changes with respect to time," and therefore exclusively include time as an independent variable. Not all models need to be exclusively kinetic, and other independent variables can be included, as is the case of Hülsheger et al.'s (1981) and Peleg's (1995) models, where electric field intensity is included as well.

Experimental data show that the inactivation effectiveness of PEF processes is influenced by several other experimental conditions, such as treatment temperature, type of microorganism, and type of treated media, as discussed earlier; hence, an alternative approach to modeling PEF processes is to include more factors instead of more complex models having only one factor (e.g., treatment time), which could lead to more powerful models.

An example of this alternative approach is the work by Sensoy et al. (1997) who suggest that the influence of treatment temperature on PEF effectiveness can be determined by an Arrhenius-type equation, modifying the value of k in Eq. (27) depending on the temperature (T):

$$k = k_{Eo} e^{-\frac{E_A}{RT}} \tag{36}$$

where k_{Eo} is a constant factor, R is the universal gas constant, and E_A the activation energy. This attempt to include the effect of treatment temperature, although appealing, assumes that microbial inactivation by PEF follows a first-order kinetic model [Eq. (27)] and, therefore, even if it effectively accounts for changes induced by temperature variation, it applies only to the linear portion of the inactivation curves.

To explore the influence of other factors on PEF technology, researchers around the world should standardize research protocols and reach a consensus on the relevant factors that need to be controlled and monitored to generate reliable and accurate data for independent testing of new models. Almost all models developed to date are based on the findings of Sale and Hamilton in 1967, who found that the most important factors in the inactivation of a given microorganism were intensity of the electric field and pulse width or treatment time. Other processing parameters might play an important role in the inactivation process by PEF and more attention is needed, if for no other reason, to discard them as possible relevant factors.

VI. ALTERNATIVE APPLICATIONS OF PEF TECHNOLOGY

Although the study of PEF technology has been centered on its ability to inactivate microorganisms in liquid food products at low temperatures, some other applications in the food industry have been explored as well. Improvement of intracellular metabolite extraction, enhancement of drying efficiency, modification of enzymatic activity, preservation of solid and semisolid food products, decontamination of liquid waste, and modification of functional properties of food ingredients can be mentioned, among others. A remarkable feature of the use of PEF technology for alternative purposes is that depending on the intensity of the applied treatment, if desired, it is possible to attain the alternative desired effect (i.e., enhancing extraction or drying rates) and the bactericidal effect at the same time, which translates into cleaner processes and lessens the risk of cross-contamination or bacterial proliferation during such operations.

A. PEF-Assisted Cell Expression and Extraction

Animal and plant cells are affected by the application of pulsed electric fields in the same way that bacterial cells are. Processes where extraction or expression of intracellular contents is required are enhanced by the pretreatment of cells with PEF. Pores formed on the cell membrane enhance mass transfer out of the cells. The larger size of animal and plant cells, compared to bacterial cells, makes it easier to induce the transmembrane potentials required to cause permeabilization of the membrane, as defined in Eq. (24), and normally lower electric field intensities are employed to permeabilize these types of cell membranes, which is reflected in lower energy consumption (6–10 J/g) (Knorr et al., 2001). Permeabilization of plant cells can be used to increase yield in the production of fruit juices, enhance the extraction of intracellular metabolites (of commercial interest, such as pigments or flavors), and to increase the extraction efficiency of processes such as sugar beet extraction (Brodelius et al., 1988; Eshtiaghi and Knorr, 1999; Knorr and Angersbach, 1998; Bouzrara and Vorobiev, 2003). Sugar beet extraction by PEF-assisted processes with application of electric fields around 1.2 to 2.5 kV/cm increases the solid concentration twofold in the obtained extract when compared to traditional methods, reducing the costs incurred for drying of the extracted solutions (Eshtiaghi and Knorr, 2002). Extraction of apple juice from apple mash has been enhanced by application of PEF, thus increasing yield from 67% to 73% and producing clearer color (Barsotti and Cheftel, 1999). PEF-assisted extraction of fruit juices has been shown to produce similar yields to those obtained in extraction processes enhanced by the use of commercial enzymes; nevertheless, use of PEF results in a faster continuous process and products more similar to freshly pressed products when compared to enzyme-assisted products (Eshtiaghi and Knorr, 2002). PEF-assisted processes also improve extraction and preservation of the natural characteristics of heat-sensitive products in fruit juices. Extraction yield of carrot juice increases from 51% to 67% when using PEF, attaining higher availability of β-carotene as well when compared to juice produced by traditional methods (Knorr et al., 1994).

B. PEF-Assisted Drying

Pulsed electric fields induce an increase in mass and heat transfer rates between plant or animal cells and their surroundings due to permeabilization of the cell membranes, making this process also suitable for enhancing drying process efficiency (Knorr et al., 2001). Pretreatment of cubed potato tissue prior to drying has shown that whereas the untreated product does not release water by centrifugation, PEF-treated tissue releases 29% liquid by centrifugation after the application of 6.4 to 16.2 J/g at an intensity of 0.9 to 2 kV/cm, yielding dehydrated products of comparable quality to traditionally processed products (Angersbach and Knorr, 1997). In the same way, preconditioning of coconut by PEF increases its drying rate by about 22% (Ade-Omowaye et al., 2000). Osmotic dehydration is a process involving immersion of treated products in solutions of higher osmotic pressure that can be used prior to conventional drying, or freezing to enhance mass transfer without modifying the characteristics of fruit products while reducing energy consumption. This process can be further enhanced by the simultaneous application of PEF, which improves the water and solution diffusion from and to the tissue (Ade-Omowaye et al., 2001). Successful results pretreating carrots (Rastogi et al., 1999), apple slices (Taiwo et al., 2002), mango (Tedjo et al., 2002), and bell peppers (Ade-Omowaye et al., 2002) have been obtained.

C. Enzyme Inactivation by PEF

Enzymes present in food products are naturally occurring proteins with catalytic activity responsible for the regulation of internal biological processes. Enzymes are highly specialized proteins with a complex structure on which its catalytic activity and high specificity are based. The structure of the active site of an enzyme allows it to act selectively, catalyzing very specific reactions with certain substrates. Inactivation or reduction of catalytic activity may be desirable in some cases where it leads to deterioration or undesired changes such as senescence or discoloration. On the other hand, some enzymes in food products play a positive role such as inhibition of microbial attack or can carry potential health benefits; hence, their presence is desirable (Proctor and Cunningham, 1988).

Inhibition of enzymatic activity by PEF is a polemic subject. Whereas some research groups claim important inactivation of some enzymes, others report no effect of PEF on enzymatic activity (Van Loey, 2001; Van Loey et al., 2002). These discrepancies may be due to differences in the applied treatments or differences in the studied enzymes. As described in the case of microbial inactivation, more parameters need to be controlled and monitored to describe a PEF treatment (i.e., temperature, electric current, etc.) more precisely. On the other hand, the complex structure and extended variety of enzymes in food products suggest that some enzymes are more sensitive to PEF than others, depending on the stability of their chemical structure. As enzymes are commonly classified by their activity and substrate, it is even possible that two enzymes considered similar may actually be structurally different depending on their origin. Some examples of enzyme inactivation results obtained by different research groups are shown in Table 6.

D. Preservation of Solid and Semisolid Foods by PEF

Pulsed electric field technology has been mainly focused on the preservation of liquid food products. The limited size of the treatment chamber required to obtain high electric field intensities in combination with high electrical resistance limits the use of this technology to pumpable products, since batch operation of small chambers results in expensive and inefficient operation. Another limitation when dealing with solid products comes from the fact that solid products are usually in the form of powders or chunks and, hence, are mixed with air, which has a low dielectric strength, thus limiting the maximum applicable electric field intensity. Despite these complications, some researchers have tried to process solid and semisolid products with different degrees of success. Laboratory tests have successfully used model solid foods (potato dextrose agar, or plate count agar, completely filling the static treatment chamber) to assess treatment homogeneity within the treatment chamber (Zhang et al., 1994a; Mañas et al., 2001). Real food products such as spices or flour have been processed without success, attaining inactivation levels of less than one log cycle, purportedly due to the low water content of such products (Keith et al., 1997, 1998; Mañas et al., 2001). Although these trials were unsuccessful from the preservation point of view, it is important to acknowledge that in these trials the limitation on the maximum electric field imposed by the presence of air in the treatment chamber was overcome by increasing the pressure in the system and/or using alternative gases in the system (O_2, N_2, and Ar), which represents important progress. As additional information, 600 mJ/pulse was identified as the energy limit for processing of powders to avoid dust explosions in the event of dielectric breakdown within the treatment chamber.

Recent attempts to control *E. coli* O157:H7 in beef burgers by the application of PEF in combination with organic acids, then freezing, were not successful either (Bolton et al.,

Table 6 Reported Enzyme Inactivation by PEF

Enzyme	Maximum inactivation	Reference
NADH dehydrogenase	None	Hamilton and Sale (1967)
Succinic dehydrogenase	None	Hamilton and Sale (1967)
Hexokinase	None	Hamilton and Sale (1967)
Acetylcholinesterase	None	Hamilton and Sale (1967)
Lipase	None	Hamilton and Sale (1967)
Alpha amylase	None	Hamilton and Sale (1967)
Alkaline phosphatase	59–65%	Castro (1994)
Plasmin	90%	Vega et al. (1995a)
Protease	25–70%	Vega et al. (1995b)
Lipase	65%	Grahl and Märkl (1996)
Peroxidase	25%	Grahl and Märkl (1996)
Alkaline phosphatase	<5%	Grahl and Märkl (1996)
Alkaline phosphatase	96%	Barbosa-Cánovas et al. (1996a)
Peroxidase	30%	Ho et al. (1997)
Alkaline phosphatase	5%	Ho et al. (1997)
Alpha amylase	85%	Ho et al. (1997)
Lipase	85%	Ho et al. (1997)
Lysozyme	10–60%	Ho et al. (1997)
Glucose oxidase	75%	Ho et al. (1997)
Polyphenol oxidase	40%	Ho et al. (1997)
Pepsin	150% increase	Ho et al. (1997)
Alkaline phosphatase	60%	Barbosa-Cánovas et al. (1998)
Papain	50–90%	Yeom et al. (1999)
Polyphenol oxidase	62–70%	Giner et al. (1999)
Pectinmethylesterase	93.8%	Giner et al. (2000)
Pectinmethylesterase	88%	Yeom et al. (2000)
Lactate dehydrogenase	None	Barsotti et al. (2001)
Protease	100%	Palomeque et al. (2001)
Lipase	13–62%	Bendicho et al. (2002b)

2002). Semisolid or viscous liquid products can be successfully processed as long as they can be pumped. Yogurt and rice pudding are examples of products successfully processed by PEF (Dunn and Pearlman, 1987; Ratanatriwong et al., 2001). Liquid containing large suspended particles has been successfully processed as well. Caviar suspended in electrolytic solutions, pea soup, and plastic beads suspended in model solutions are some examples (Mañas et al., 2001; Vega-Mercado et al., 1996a; Dutreux et al., 2000).

E. Other Uses for PEF Technology

Use of PEF technology for other purposes than those already described has been suggested in several areas. All alternative uses of PEF technology attempt to take advantage of either the induced membrane permeabilization of cells or the ability of this technology to cause microbial inactivation at low temperatures. Examples of these alternatives processes are the following:

- PEF-stress-induced synthesis of microbial metabolites of commercial interest (Knorr et al., 2001)

- Facilitated infusion of solutes into biological tissues (Barsotti and Cheftel, 1999)
- Decontamination of wastewater and slaughterhouse waste management (Castro et al., 1993)
- Decontamination of waste brine solutions (Mittal and Choudhry, 1997)
- Pretreatment of milk for cheese-making (Sepulveda-Ahumada et al., 2000)
- Cleaning water from cooling towers (Abou-Ghazala and Schoenbach, 2000)

VII. CONCLUSIONS

Use of pulsed electric fields for food preservation purposes has been studied for more than 40 years now. Although great advances in the understanding and application of this technology on food products have been achieved, there are still a number of technical, economical, and regulatory hurdles that need to be solved before commercial implementation is possible. The challenge to increase the capacity of PEF equipment has been met by the use of higher pulsing frequencies and systems with multiple chambers. Use of solid-state pulse generators has also played an important role in the scale-up of PEF technology, with square mono- or bipolar pulses being the most common choice. The availability of equipment has increased substantially in the last 10 years, and there are currently a relatively large number of suppliers that build and commercialize PEF equipment, from bench-top to pilot plant models. The price of such systems ranges from $40,000 to $500,000, and operating cost is estimated at around $0.02 per liter (Góngora-Nieto et al., 2002). Although technology is already available to build and operate PEF equipment at an industrial level, commercial food processors have not yet adopted this technology. Liquid food products of high acidity like fruit juices are identified as the most suitable products for processing by PEF technology; however, no known commercial efforts are currently being taken in that direction (EPRI, 1998; Morris, 1997). Legal restrictions placed by the FDA on novel preservation processes need to be lifted before PEF can be applied at an industrial level for marketing and product consumption; a clear demonstration of the safety and effectiveness of PEF technology is still needed (Cole, 1997; Góngora-Nieto et al., 2002; EPRI, 1998). Pioneer steps in the regulation and acceptance of PEF technology have been taken, as demonstrated by a nonobjection letter issued by the FDA to the CoolPure cold pasteurization process on July 7, 1995, regarding the application of PEF technology for antimicrobial treatment of high-acidity liquids and pumpable foods under specific treatment conditions and in accordance with appropriate GMPs (Anonymous, 1995).

It is expected that continued research on the use of pulsed electric fields for food preservation purposes, accompanied by the development of better and less expensive pulsed power semiconductors, will lift legal restrictions and reduce the cost of PEF technology, making it available for commercial processing and preservation of liquid foods in the near future.

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2
Microbial Inactivation by Pulsed Electric Fields

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I. INTRODUCTION

Pulsed electric fields (PEF) treatment is one of the most relevant nonthermal processes available for food preservation because of its potential to inactivate microorganisms without altering the organoleptic and nutritional properties of foods (Barbosa-Cánovas et al., 1999).

The design of an effective PEF treatment that also ensures the safety and stability of foods requires: (a) knowledge of the mechanism of microbial inactivation, (b) identification of factors affecting inactivation of microorganisms, and (c) a mathematical model capable of accurately describing the kinetics of microbial inactivation by PEF.

A better understanding of the way that PEF destroys microorganisms would help to define PEF treatment that, alone and in combination with other physical treatments or antimicrobial agents, is an alternative to traditional heat preservation. Moreover, knowledge of the mechanism of microbial inactivation would also allow the discovery of a biological base for the mathematical models describing microbial inactivation by PEF. These findings could explain the kinetics of microbial inactivation under the influence of different factors and help find the causes of possible deviations. Additionally, to extend the range of products processed with PEF, a thorough knowledge of the factors affecting microbial PEF resistance will be required.

The present chapter gives an overview of the state of the art in microbial inactivation by PEF. Mechanisms of inactivation of microorganisms and factors affecting microbial inactivation by PEF are discussed.

II. MECHANISMS OF INACTIVATION OF MICROORGANISMS BY PEF

Sale and Hamilton (1967) and Hamilton and Sale (1967) reported the first systematic studies on the effects of PEF treatments on the inactivation of microorganisms. They proved that microbial inactivation was due to the direct effect of PEF treatment rather than the products of electrolysis or temperature increase. They finally proposed that PEF treatment caused an irreversible loss of membrane function as the semipermeable barrier between the bacterial

cell and its environment, and that this was the cause of cell death. Several decades later, the mechanism of microbial inactivation by PEF still has not been fully elucidated.

The use of PEF in cell biology, biotechnology, and medicine has attracted great interest. Therefore the effects of PEF on cells, mainly on eukaryotic cells, have been studied. PEF can cause electroporation or electrofusion in cells. Electroporation, which can be defined as the formation of pores of cells and organelles (electropermeabilization), is a valuable tool for injecting such foreign substances as drugs, proteins, or DNA into cells without causing deterioration of cellular functions. On the other hand, the electrofusion 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 (Zimmermann, 1986; Palaniappan and Sastry, 1990).

The effects of PEF treatment on microorganisms have not yet been deeply investigated. Probably, the mechanism of microbial inactivation is intimately related to the formation of pores in the membrane, as most authors have reported (Hamilton and Sale, 1967; Tsong, 1991; Ho and Mittal, 1996; Weaver and Chimadzhev, 1996; Barbosa-Cánovas et al., 1999; Heinz et al., 2002; Wouters et al., 1999, 2001). However, microorganisms are very different, mainly within their envelopes. Thereby, differences might exist in the way that PEF treatment destroys them. Other phenomena may also be involved.

Several theories have been proposed to explain the mechanism of electropermeabilization and microbial inactivation. Nevertheless, most theories are based on the experiments carried out on model systems such as liposomes or protoplasts instead of on microorganisms.

The most accepted theory, proposed by Zimmermann et al. (1974), considers the cell membrane as a capacitor filled with a dielectric material of a very low dielectric constant compared to inside the cell and the surrounding environment. Due to the difference in dielectric constants, free charges can accumulate at both membrane surfaces, generating a transmembrane potential of about 10 mV. Maintaining the transmembrane potential is vital to the cell since it forms part, in addition to the difference in proton concentration, of a type of potential energy called protonmotive force, which can be used to drive a variety of energy-linked processes, including 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; and generating ATP (Neidhardt et al., 1990).

When an external electrical field is applied, transmembrane potential increases because of the additional free charges that accumulate at both 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 membrane breakdown occurs (Fig. 1). Both 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 the threshold or critical electric field. During application, when the electric field reaches values close to the critical electric field or when the treatment time is short, both the number and size of generated pores are low. Under these conditions, permeabilization of the membrane is reversible since the cell membrane restores its structure and functionality when the PEF treatment ceases. However, when more intense PEF treatments are applied, both the number and size of pores increase, resulting in irreversible permeabilization or mechanical disruption of the cell (Zimmermann, 1986).

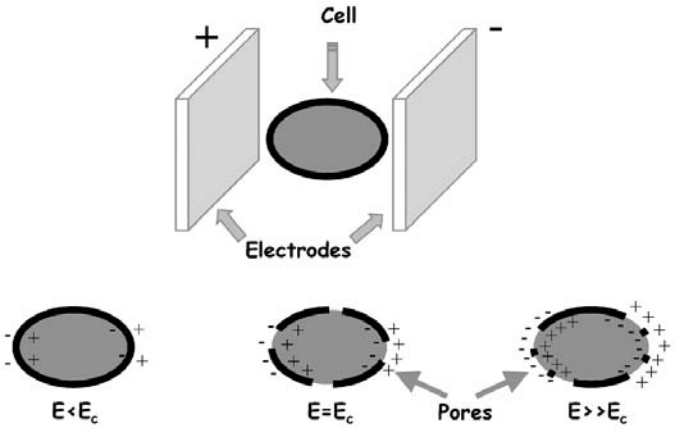

Figure 1 Mechanism of microbial inactivation with PEF treatment. Electroporation of cell membrane when exposed to PEF treatment. E: electric field strength; E_c: critical electric field strength (Zimmerman's theory).

Other theories consider permeabilization as the consequence of a dipolar reorientation of the phospholipids within the two monolayers of the membrane under electric field application. 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). 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).

According to Tsong (1991), exposure of the membrane 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 transition of the lipid bilayer from a rigid gel structure to a liquid crystalline structure, which might also impair the semipermeable nature of the cell membrane. Protein channels, pores, and pumps are also present in cell membranes. Their functionality, as 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 less than that of the critical transmembrane potential. Therefore when PEF treatment is applied, most of the protein channels are opened. Again, a Joule heating may occur or other electric modifications, and protein channels might become irreversibly denatured resulting in the formation of pores.

Most of the theories described above explain the formation of pores in the cell envelopes. Nevertheless, there is no clear evidence on the underlying mechanism of membrane permeabilization at the molecular level.

Inactivation of microorganisms by PEF is believed to be due to the mechanical instability of the cell membrane. However, it is not so clear regarding the inactivation of microorganisms whether only the formation of some pores is necessary or if the complete mechanical disruption of the membrane or other stresses associated with alteration of membrane functions is implied. Mechanical disruption of the cell might be a consequence of pore formation. For example, it has been considered that the mechanical disruption of erythrocyte membranes might be based on the colloidal osmotic swelling of the cell. The pores formed during the electric field are small enough to block the passage of molecules

responsible for the internal osmotic pressure, but they allow the entrance of water. In these circumstances, cell volume would increase until cell membrane rupture occurs (Tsong, 1991).

Many attempts have been made to discover whether pore formation is related to microbial inactivation. Maintenance of integrity and functionality of cell envelopes is vital to 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; it 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 plays an important role in the control of DNA synthesis. Basically, the cell membrane controls the cell's metabolic activities by maintaining an effective osmotic boundary between the cell and the surrounding environment. Any membrane damage, which impairs one or more of these activities, could result in cell death.

Membrane damage includes the loss of membrane integrity and membrane functionality. Physical damage to the bacterial cell membrane as a consequence of PEF treatment has been demonstrated in the leakage of ATP or UV-absorbing material from bacterial cells subjected to PEF (Hamilton and Sale, 1967; Simpson et al., 1999), in the release of β-galactosidase activity in a permease-negative mutant of *Escherichia coli* (Hamilton and Sale, 1967), in the loss of the ability of *E. coli* to plasmolyze in a hypertonic medium (Hamilton and Sale, 1967), and in the increased uptake of fluorescent dyes such as propidium iodide (that do not normally penetrate the membranes of healthy cells) in *Lactobacillus plantarum* (Wouters et al., 2001). Loss of membrane functionality has also been considered since the ability to maintain internal pH decreases under PEF treatment. However, a correlation was not found between the inhibition of H^+-ATPase activity and PEF treatment (Simpson et al., 1999).

Sublethal injury measured using a selective medium plating technique is supposed to be a consequence of loss of membrane integrity and functionality. Sublethal injury was not detected in *Listeria monocytogenes* and *Salmonella typhimurium* after several PEF treatments when a selective medium plating technique consisting of adding 3% sodium chloride to the recovery medium was used. These findings indicate that bacterial inactivation by PEF may be an "all or nothing" event (Simpson et al., 1999). The same was the case in PEF-treated cells of *Micrococcus luteus*, *E. coli*, and *Listeria innocua* (Dutreux et al., 2000a,b).

Electron microscopy has also been used to look for morphological changes in cells after PEF treatment. Most studies on the morphology of different bacteria and yeast treated by PEF have shown an increase in surface roughness, craters, elongation, disruption of organelles, cell wall breakage, and pore formation (Jayaram and Castle, 1992; Pothakamury et al., 1996, 1997; Harrison et al., 1997; Calderón-Miranda et al., 1999c). Pothakamury et al. (1997) observed using scanning electron microscopy (SEM) that cells subjected to PEF treatment lost smoothness and uniformity, and that some cells were shrunken. Using transmission electron microscopy (TEM), they 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 under maximum PEF treatment conditions. However, an extension of changes was not noticeable at lower PEF treatment, although most of cells were also inactivated. Therefore a relationship was not found between membrane damage and loss of viability following PEF treatment. However, this cannot be taken as proof that PEF treatments do not result in a structural disorganization of the membrane.

From a practical point of view, there are two aspects of the mechanism of inactivation by PEF that deserve special attention: (a) the presence of reversible pores during PEF treatment and (b) the occurrence of sublethal injury. Both circumstances will prove valuable in developing appropriate combination processes using PEF treatment. The use of several hurdles in combination or in a successive manner may act additively or synergistically and make the survival of spoilage and food-poisoning microorganisms difficult (Leistner, 1992).

A. Reversible Pores

Electropermeabilization can be reversible or irreversible, depending on the degree of membrane damage (Zimmermann, 1986; Rols et al., 1990; Tsong, 1991; Weaver and Chimadzhev 1996). A proportion of cell membranes, depending on the intensity of the PEF treatment, become leaky during PEF treatment but reseal to a greater or lesser extent after. Appropriate techniques are needed to evaluate the magnitude of pore reversibility after PEF treatment. Pagán and Mackey (2000) reported the use of fluorescent dye propidium iodide added to the treatment medium as a marker of permanent or non-permanent loss of membrane integrity after high hydrostatic pressure treatment. Based on the same approach, our preliminary results have shown that when propidium iodide is present in the suspending medium during PEF treatment, the degree of staining of *Salmonella senftenberg* cell population is approximately twofold greater than when added after PEF treatment. These results would indicate that approximately 50% of the damaged cells resealed their pores just after PEF treatment (Pagán et al., unpublished data). If microbial inactivation by PEF is an "all or nothing" event, cells unable to reseal their membranes will die. Thereby, combined processes using PEF treatment might require conditions that avoid resealing of pores.

On the other hand, the occurrence of pores in part of the cell population suggests the possibility of combining PEF treatment 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 treatment 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 that destroys high PEF-resistant gram-positive organisms at low temperature. The capability of permeabilizing cells suggests that PEF treatments might even improve the action spectrum of natural antimicrobials. The possible combination of PEF treatments and organic acids at neutral pHs might also be considered. The more abundant organic acid dissociated molecules at neutral pHs that do not normally penetrate cell membranes might enter the cell under PEF.

B. Sublethal Injury

The occurrence of sublethal injury is the other interesting aspect of the mechanism of inactivation. Figure 2 shows that although sublethal injury could not be detected in *Yersinia enterocolitica* and *S. senftenberg*, which were PEF-treated in pH 7 McIlvaine buffer and recovered in a selective medium with sodium chloride added, more than 90% of the number of survivors of *L. monocytogenes* and *Bacillus subtilis* were inactivated due to the selective recovery medium conditions. According to these results, microbial inactivation by PEF is not an "all or nothing" event and would depend on the microorganisms and the treatment conditions investigated. Our preliminary results have shown that the degree of sublethal injury depends on the microbial characteristics, such as cell envelope structure, growth