Lebensm.-Wiss. u.-Technol., 35, 375–388 (2002)

# Food Processing by Pulsed Electric Fields: Treatment Delivery, Inactivation Level, and Regulatory Aspects

**M. M. Góngora-Nieto, D. R. Sepúlveda, P. Pedrow, G. V. Barbosa-Cánovas* and B. G. Swanson**

M. M. Góngora-Nieto, D. R. Sepúlveda, G. V. Barbosa-Cánovas: Washington State University, Biological Systems Engineering Department, Pullman, WA (U.S.A.)
P. Pedrow: Washington State University, Food Science and Human Nutrition Department, Pullman, WA (U.S.A.)
B.G. Swanson: Washington State University, School of Electrical Engineering and Computer Science, Pullman, WA (U.S.A.)

*(Received June 21, 2001; accepted January 28, 2002)*

*Industrial implementation of pulsed electric field electro-technology (PEF) for food preservation has been rather slow, despite its potential to produce safe, nutritious and high-quality products. Several research groups around the world are in a race to validate and optimize the operation of PEF systems. Insufficient kinetic studies and inaccurate treatment delivery assessment are some of the main obstacles to the implementation of this technology. Equivalency among PEF systems, treatment delivery and control variables need to be clearly defined before actual industrial implementation takes place. Limited commercial availability of PEF systems, mainly due to the complexity and high cost of pulsers, and to technical and economical limitations of the scaling-up process, reflects into high initial investment and operation costs. This paper reviews the state of the art on the above-mentioned issues, and also discusses engineering, biological, and regulatory aspects that should be considered for optimization and commercial implementation of PEF as a food preservation technology.*



**Keywords:** pulsed electric fields; food preservation; commercialization; regulations; engineering principles

## Introduction

Pulsed electric field electro-technology (PEF) is an emerging technology in the field of food preservation. The origin of PEF can be found in electroporation, a biotechnology process used to promote bacterial DNA interchange by perforating microbial membranes with induced electric fields. The success of this technique depends on the intensity of the induced electric field. Induction of membrane potentials that exceed a threshold value often result in cell damage and death (Zimmermann, 1986). The main idea behind the use of electric fields as a food preservation method is then to take advantage of the lethal effect observed in electroporation to inactivate undesirable bacteria in food products. With an effect comparable to pasteurization, yet without the thermal component, PEF has the potential to nonthermally pasteurize several foods via exposure to high-voltage short pulses, while the material is between the electrodes of a treatment chamber. PEF has been most successful with fluid products; however, some semisolids and powders have also been treated (Zhang *et al*., 1994*b*, Keith *et al.,* 1997, 1998).

Inactivation of spoilage and pathogenic flora, as well as of enzymes of interest to the food industry, has been under intense study in the past decade, and with successful results, this technology is now striving for industrialization (Ho *et al*., 1997; Wouters and Smelt, 1997; De Jong and Van Heesh, 1998; Barsotti *et al*., 1999; Caplot and Cote, 1999; Yeom *et al*., 1999; Barbosa-Cánovas *et al*., 2000*a*; Giner *et al*., 2000; Kempkes *et al*., 2001).

## Engineering Principles

PEF technology is based on a pulsing power delivered to the product placed between a set of electrodes that confine the treatment gap of the PEF chamber. It is within the electrode gap that the food product experiences a force per unit charge, the so-called electric field. This electric field is responsible for the inactivation

*To whom correspondence should be addressed.

0023-6438/02/$35.00
doi:10.1006/fstl.2001.0880

or lethality of PEF against enzymes, microorganisms, and spores. The intensity of the applied electric field may be within a wide range (2–87 kV/cm). However, effective inactivation levels are generally obtained with electric field intensities in the range of 20–50 kV/cm (Barsotti *et al.,* 1999; Barbosa-Cánovas *et al*., 2000*b*; Heinz *et al*., 2002). Each one of the high-voltage pulses used in PEF technology usually lasts a very short period of time, in the order of microseconds. However, repetitive application of such pulses can result in treatment times ranging from several microseconds to milliseconds.

*System components*

*Pulse-forming networks*. Generation of pulsed electric fields requires a fast discharge of electrical energy within a short period of time. This is accomplished by using a pulse-forming network (PFN). In general, a PFN (**Fig. 1c** and **d**) is an electrical circuit consisting of one or more power supplies (charging voltages up to 60 kV), switches (ignitron, thyratron, tetrode, spark gap, semiconductors), capacitors (0.1–10 $\mu$F), inductors (30 $\mu$H), resistors (2 Ω–10 MΩ), and treatment chambers (parallel plate, coaxial, co-field) (Zhang *et al.,* 1995; Evrendileck *et al*., 2000; Mac Gregor *et al*., 2000; Mittal *et al*., 2000; Raso *et al.,* 2000). The relative electric values of each component in the PFN determine the shape of the pulse (**Fig. 1a** and **b**).

The simplest PFN is an RC (resistance–capacitance) circuit in which a power supply charges a capacitor, which can deliver its stored energy to a resistive load (treatment chamber) in a couple of microseconds, by activation of a switch. The pulse generated is exponentially decaying (**Fig. 1a** and **c**). In this system the voltage

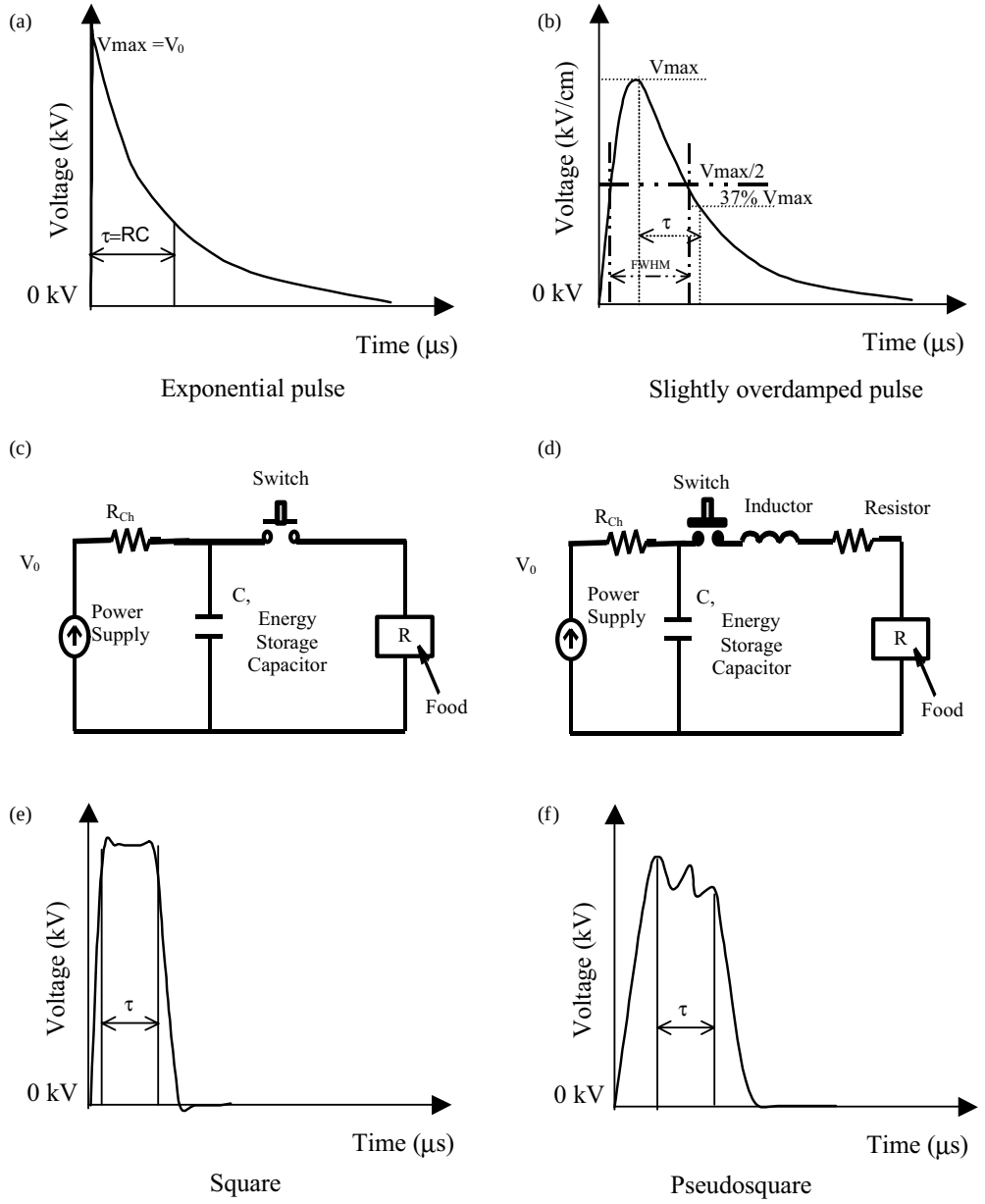


**Fig. 1** Typical pulse wave shapes used in PEF. Pulse wave shapes in a and b are generated with systems shown in c and d, respectively. $V_0$, is the charging voltage of the power supply; $C$, capacitor; $R_{Ch}$, charging resistor; $R$, PEF chamber resistance; and $\tau$, time constant. FWHM is the full-width of the highest-maximum. e and f show a square pulse and a pseudosquare pulse, respectively

across the resistive load (PEF chamber) as a function of time is described by

$$V(t) = V_0 e^{-t/\tau} \qquad \text{Eqn [1]}$$

where $V_0$ is the voltage charged in the capacitor of the PFN, $t$ is the pulse duration time, and $\tau = RC$ is the time constant. In an $RC$ circuit the pulse duration is approximately five time constants (Cogdell, 1999). However, practically all the energy of the pulse is delivered during the first time constant, thus some research groups have adopted $\tau$ as the effective pulse width, which is calculated as the time needed for the voltage to decay to $1/e$ (0.37) of its maximum value (Zhang *et al*., 1995; Grahl and Markl, 1996; Wouters and Smelt, 1997; De Jong and Van Heesch, 1998; Barsotti *et al*., 1999). There are other researchers who prefer to use the total pulse duration (Knorr and Angersbach, 1998).

Since there is always some resistance and self-inductance produced by the connections of main components, the ideal $RC$ circuit is not likely to be a true representation of a high voltage PFN. Instead, an RLC (resistance–inductance–capacitance) circuit must be assumed, and a slightly over or underdamped pulse is produced (**Fig. 1b** and **d**). An RLC circuit can also generate an instant reverse pulse. In the case of instant reverse pulses, the pulse width is the whole duration of the pulse.

As discussed before, some researchers consider the effective pulse width of slightly over or underdamped pulses as the time difference between the peak voltage and the decay up to 37% of the peak voltage (time constant). However, since slightly over or underdamped pulses have a rise time that can be almost 1/5 of the total pulse time, using the full-width of the highest-maximum (FWHM; time the applied voltage remains at a level superior to half the peak voltage), could be a better way to describe effective pulse duration (**Fig. 1b**).

Use of any of the mentioned strategies to characterize pulse duration (time constant, total pulse time or FWHM) may be effectively used to compare the relative effectiveness of different treatments, as long as a consensus is reached to use the same characterization strategy. Letting aside the issue of finding 'the best' way to quantify pulse duration or whether the whole pulse or only part of the pulse causes the microbial inactivation, a strategy to unequivocally characterize pulse shape and intensity is needed, and thorough adoption of such strategy by the whole scientific community is required.

Application of exponential decaying pulses on food implies that the food is exposed to a spectrum of electric fields with intensity proportional to the voltage potential ($V(t)$) received by the load. Pulse-forming networks, generating square pulses (**Fig. 1e**), can apply a relatively constant electric field to the food, provided the impedance of the network (defined as the square root of the inductance of the system divided by the capacitance of the system) is well matched with the impedance of the food product (**Fig. 1e**). However, proper matching of the chamber impedance with the PFN impedance becomes very challenging for chambers with low resistance ($< 50\,\Omega$). Poorly matched impedance will degrade the signal, and therefore also generate a spectrum of electric fields (**Fig. 1f**) (Zhang *et al*., 1994*a*, 1995). Under well-matched conditions, the pulse width of this transient is close to the time constant of the pulse, which simplifies assumptions, and minimizes the energy waste in ohmic heating of the product. Multiple switches can be used to generate bipolar pulses, with the same basic PFN configurations.

A third type of PFN, designed to generate instant reverse pulses, is also composed of a power supply and switch, capacitors, resistors, and a load with relative values, such that the resultant voltage across the load has positive pulse polarity followed by negative pulse polarity. Electric circuit theory defines these pulses as severely underdamped transients. These pulses are the result of inductance in the PFN via the connections of the main elements, which are combined with the small resistance in the load. Instant reverse pulses are the most controversial of all pulse wave shapes, yielding total inactivation in some cases or very low inactivation in others, but with very low energy density ranging from 1 to 25 J/mL (other pulse wave shapes range from 90 to 600 J/mL).

Differences between ideal and actual transient behavior of PEF pulses, and the lack of consensus in the way in which the pulse width and total treatment time are defined, are some of the reasons limiting the usefulness of conclusions drawn on the effectiveness of a particular piece of equipment or a specific set of process conditions, and the kinetic data generated as a result.

*PEF treatment chambers and electrical considerations.* The treatment chamber houses the discharging electrodes and holds them in position with an insulating material. It also encloses the food material within the gap between the electrodes, during the PEF process. There are three more commonly used chamber configurations, defined by chamber placement and geometry of its electrodes. The parallel plate chambers are generally static chambers with gaps (from less than 0.001–0.01 m) considerably smaller than the electrodes surface (usually less than 0.0050 $m^2$) (Dunn and Pearlman, 1987; Ho *et al*., 1995; Grahl and Markl, 1996; MacGregor *et al*., 2000). In the coaxial category, the cylinders and cones are the more common electrode geometries. These continuous chambers have gaps between 0.003 and 0.009 m (Bushnell *et al*., 1993; Qin *et al*., 1997). One of the most recent chamber designs has the two electrodes arranged in a co-field configuration. This chamber consists of two metallic pipe tubes (about 0.005 m of internal diameter), one charged to a high voltage (20–40 kV) and the other to a ground potential ($\sim 0$ V) interconnected by an insulating tube (Yin *et al*., 1997). Such configuration of the co-field chamber has advantageous fluid dynamics, highly desirable for food processing and convenient for cleaning in place. The dimensions and geometry of the chamber electrodes as well as the conductivity of the food product being treated define the resistance of the chamber. Ohm's law (Eqn [2]) describes the resistance of a purely resistive chamber as the voltage across it divided by the current

through it. An increase in resistance will limit the current, achieving higher peak voltages within the electrodes and minimizing the energy per pulse.

$$R = V/I \qquad \text{Eqn [2]}$$

If the conduction current through a certain cross section of the electrode is defined as

$$I = |J_c|(1)A \qquad \text{Eqn [3]}$$

where $A$ is the effective area and $J_c$ the conduction current density defined as

$$J_c = \sigma E_f \qquad \text{Eqn [4]}$$

where $\sigma$ is the conductivity of the food, and $E_f$ the electric field developed within the gap, then the electric field can be obtained solving the Laplace equation for each particular geometry. It is worth mentioning that if accurate electric field values are to be reported, careful solving of the electric field equation is required.

For a coaxial chamber, Eqn. [5] defines the area, Eqn [6] the electric field, and Eqn [7] the gap:

$$A = 2\pi R_{HV} L \qquad \text{Eqn [5]}$$

$$E_f(r_i) = \frac{V_0}{r \ln(R_{LV}/R_{HV})} \quad R_{HV} \le r_i \le R_{LV} \qquad \text{Eqn [6]}$$

$$\text{gap} = (R_{LV} - R_{HV}) \qquad \text{Eqn [7]}$$

where $L$ is the effective length of the high-voltage electrode, $V_0$ the differential voltage across the gap, $r$ the radial position within the chamber gap, and $R_{LV}$ and $R_{HV}$ the radius of the low- and high-voltage electrodes, respectively. However, Eqn [6] considers a very long $L$ and ignores the true fields that appear at the entry and exit of the treatment gap. If the chamber is properly designed, optimizing the uniformity of electric fields by properly contouring the electrodes to minimize field enhancement at the electrode edges and triple points (Qin *et al.*, 1995, 1997; Bushnell *et al.*, 1996), the assumption in Eqn [6] would be acceptable.

It can be deducted from Eqs [2]–[4] that chambers with high effective areas, and chambers processing highly conductive food materials, will have low resistance. The high resistance of co-field chambers (50–300 Ω) is due to the low effective area in the cross sections of the tubular electrodes. Neither the coaxial nor the co-field chambers can develop a constant electric field across the gap (as with parallel plates), thus yielding nonuniform treatments. However, coaxial chambers can be designed with a uniformly distributed electric field, as indicated in Eqn [6], at a minimum when $r = R_{LV}$ and maximum when $r = R_{HV}$. **Figure 2** shows the electric field limits within a coaxial chamber receiving a slightly underdamped pulse (Góngora-Nieto, 2000). In the coaxial configuration, the higher the radii of the electrodes (at a given gap), the lower the difference in electric fields between them. Another way to increase the uniformity of PEF treatments conducted in chambers with nonuniform electric fields is to increase the number of chambers. This is easier with highly resistive chambers, such as the co-field. A number of PEF chambers (between 6 and 12) are generally reported, but the statistics behind this remain undisclosed (Yin *et al.*, 1997). Furthermore, the scale-up of PEF systems most likely will involve the interconnection of several PEF chambers. Designing coaxial chambers with high resistance is not a trivial task. An electrically parallel combination of two 5 Ω chambers will generate an equivalent resistance of 2.5 Ω, which will increase the current in the system, decrease the effective voltage, and thus reduce the effective electric field. An electrical series combination requires that the peak voltage be nearly double or at least high enough, so that the differential voltage across the two chambers can generate lethal electric fields.

Nonuniformity in the electric field and thus in the treatment should be carefully taken into consideration whenever reliable kinetic data are required, and in conducting the optimization of PEF systems. Based on these premises, some research groups have focused on

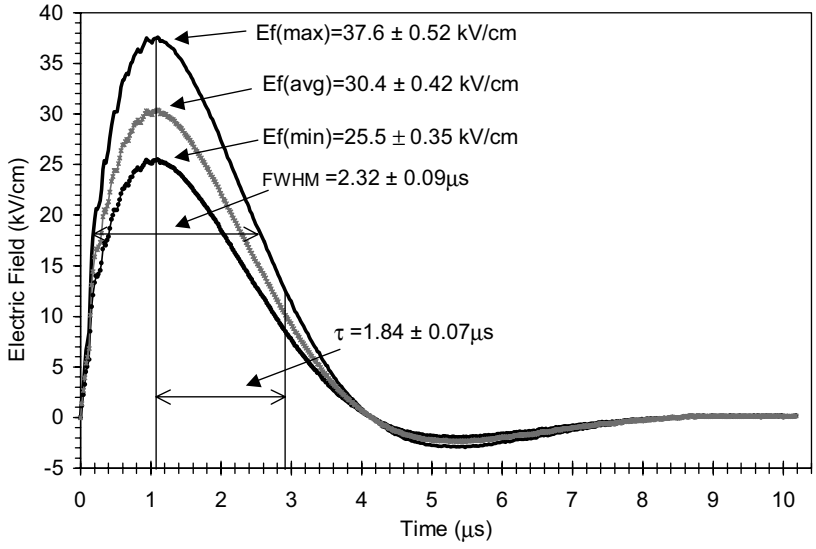


**Fig. 2** Electric field profile across the gap of a coaxial treatment chamber. $E_f$, electric field; $E_f$(min) at low-voltage electrode side; $E_{f(avg)}$ at the middle of the gap; $E_{f(max)}$ at the high-voltage electrode side; $\tau$, time constant; FWHM, full-width of the highest-maxmum; standard deviation values shown after mean values (Góngora-Nieto, 2000)

kinetic studies using very small parallel chambers and well-defined square pulses (Heinz *et al.*, 1999; Raso *et al.*, 2000). As a result, the uncertainty in the accuracy of the values of the two main variables defining the microbial inactivation, treatment time and electric field intensity, is minimized. However, the way in which the voltage transient is measured must also be accurate.

The main limitation of parallel chambers is in the batch nature of the process. However, while co-field and coaxial chambers allow continuous treatment, the electric fields are not as homogeneous as in the case of parallel chambers. Other chamber configurations exist, but in most cases, determining the electrical parameters that define PEF treatment lethality may be very challenging, plus uniformity of treatment is hard to control (Dunn and Pearlman, 1987; Matsumoto *et al.*, 1991; Lubicki and Jayaram, 1997).

In continuous chambers, the number of pulses and thus the treatment time also depend on the food's residence time within the treatment chamber. Therefore, if a minimum treatment is required, processing conditions must assure that the fastest fluid flowing through the chamber receives the required dose. The fluid dynamics of coaxial chambers will most likely reside in the laminar regimen, thus the fastest fluid will flow at double the average speed. This consideration has been seldom reported if addressed at all (McDonald *et al.*, 2000).

Chamber electrodes as well as material used as a physical barrier between the food and the electrode, such as ion permeable membranes must be made of food grade, chemically inert materials to prevent food contamination. Some of the materials recommended are gold, platinum, and metal oxides (iridium and ruthenium) (Bushnell *et al.*, 1996). The use of highly conductive polymer coatings such as polyacetylene, polyacetylene aligned, poly(*p*-phenylene AsFs), poly(*p*-phenylene Na), poly(*p*-phenylene-1,2,4-oxadiozole)–pyrolysed, poly(*p*-phenylene vinilene)–aligned, polysulfur nitride, and polysulfur nitride ($Br_2$) is also recommended (Qin *et al.*, 1997). Regardless the wide variety of available materials, the most commonly used material is stainless steel. However, although sanitary and generally resistant to abrasion or chemical attack, stainless steel has shown some problems when working with products such as milk and eggs, which cause some corrosive effect (Caplot and Cote, 1999; Góngora-Nieto, 2000). Corrosion or a deposit of solids on a high-voltage electrode most likely indicates some electrolysis of the product. This deposit has two major repercussions: the perturbation of electric fields within the chamber and the possibility of transferring some electrode particles to the treated food. Thompon-CSF Detexis Co. (1, Boulevard Jean Moulin, 78852 Elancourt Cedex, France) developed an electrode material that is 200 times more resistant than stainless steel. This material can withstand long pulse widths (20 $\mu$s) and current densities up to 470 A/cm$^2$ before the electrodes are damaged (**Fig. 3**) (Caplot and Cote, 1999). A coaxial chamber with an electrode area of about 28 cm$^2$, in which a highly conductive product is processed, such as liquid egg, can have a current density as high as 200 A/cm$^2$. With such current densities the maximum pulse duration stainless steel can endure is near 0.3 $\mu$s, much less than the generally used pulse widths (**Fig. 2**) (Góngora-Nieto, 2000). In this particular case, a graphite electrode would be suitable, since it can withstand current densities of about 250 A/cm$^2$ at a maximum pulse width of 2–2.5 $\mu$s. Since controlling the movement of particles within the chamber can minimize build up in the electrodes, the use of bipolar pulses (Qin *et al.*, 1994), instant reverse pulses (Mittal *et al.*, 2000), and low energy pulses (Mittal *et al.*, 2000; Robbins, 2001), are good alternatives to significantly minimize electrode damage. Strategies such as increasing flow rate, close temperature control and reduction of the build up area (as in the case of co-field chambers), have as well a potential to reduce electrode damage and deposition.

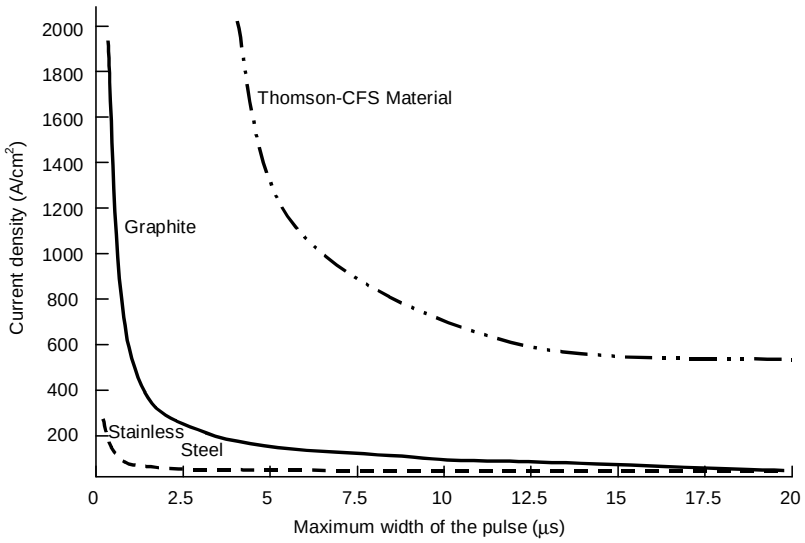


**Fig. 3** Comparison of different electrode materials on their resistance to corrosion [High-energy monopolar square pulses ~100 J/pulse] (Courtesy of Thomson-CSF Detrexis, France)

*PEF switching devices.* The switch on the PFN plays a critical role in the efficiency of the system. This component must hold the high voltage stored in the PFN and control the current flow reliably when fired. The type of switch used will determine how fast it can perform and how much current and voltage it can withstand. In PEF technology, the charging voltages of power supplies are 10–40 kV, while current depends on the resistance of the chambers. Low equivalent resistance generates driving currents on the order of 3–7 kA. Highly resistive loads near 100 Ω drive significantly lower currents ($\ll$1000 A). In general, the firing rate and service life have an inverse relationship with the current that the switches can handle. In increasing order of service life, suitable switches for PEF systems include: ignitrons, spark gaps, trigatrons, thyratrons, and semiconductors. The ignitron's performance can be very limited due to improper handling and triggering, so it is not likely to be implemented in industrial PEF systems. Spark gap switches can hold $\sim$100 kV and withstand very high currents in the order of MA, but can be fired at rates of $<$1–100 Hz. Spark gap switches are basically a two-electrode configuration, which can arc-over when the voltage supplied exceeds the dielectric breakdown voltage of the gas surrounding the electrodes. A controlled spark gap switch is the trigatron, which includes a trigger element that produces a controlled spark, promoting a plasma reaction and the arc-over of the main gap (Bhasavanich *et al*., 1991). The thyratron is a gas-filled discharge chamber with a cathode, one or several grids, and an anode. Hydrogen or deuterium are the most common gases used, with deuterium thyratron voltages up to $\sim$100 kV at $\sim$10 kA and 5–10 kHz. This switch is widely used in PEF. However, because they are 'on' switches, they often require a large box full of driver/control circuitry, making it also one of the most expensive devices (i.e. Thyratron handling 50 kV, 10 kA $\sim$ $12,000). Solid-state semiconductor switches are considered by the experts as the future of high power switching (Bartos, 2000). Solid-state switches present better performance and are easier to handle, require fewer components, allow faster switching times compared to older power switches, and are more economically sound. One of the first switches in this group is the gate turn-off (GTO) thyristor. This switch is the least efficient of all the current semiconductor switches. New generations, such as the insulated-gate bipolar transistor (IGBT), have combined the best features of a metal-oxide semiconductor field-effect transistor (MOSFET) input and a bipolar transistor output into a newer power-switching device. Its advantages are very rapid switching and small power consumption. The only disadvantage of this switches is a voltage switching limit $\sim$3 kV, thus requiring a series connection for high voltages. The latest solid-state switch is the symmetrical gate-commutated thyristor (SGCT), a modified version of a thyristor that combines optimum characteristics of intermediate generations. The advantages of the SGCT are high-switching frequency, low-switching losses, high voltage handling capability, and low on-state (conduction) losses, allowing only one-directional current to flow through the device, all with the lowest possible component count (Bartos, 2000).

*Process monitoring, control, and data gathering systems.* Optimum PFN performance should be controlled by a central computer through GPIB or fiber optic interfaces. The computer controls triggering and charging periods of the capacitor units. The electric field in the treatment chamber can be calculated from the measured voltage across the chamber considering the corresponding geometry (Eqn [6] for coaxial geometry). Therefore, it is very important that the voltage sensors have a high bandwidth and fast response to accurately measure the applied voltage (Kreuger, 1989). For this purpose, a Tectronix-P6015A (Wilsonvile, OR, U.S.A.) high-voltage probe with 75 MHz bandwidth and maximum voltage limit of 40 kV, or a high voltage probe Northstar–PVM5 (Albuquerque, NM, U.S.A.) with 90 MHz bandwidth and maximum voltage limit of 60 kV are suitable options. Since a voltage measurement is relative (a single point measure is relative to ground), ideal metering requires differential voltage measurement across the chamber. The A/D conversion of PEF voltage signals is conducted by fast sampling (100 MHz sampling rates) digital oscilloscopes, which can also be controlled through GPIB cards by computerized systems. (Heinz *et al*., 1999; Raso *et al*., 2000). However, the use of A/D cards instead of GPIB interfaces may be better because of the higher and more efficient transmission rate of the former (Góngora-Nieto, 2000).

*Available systems*

By early 1999 (Barbosa-Cánovas *et al*., 2001) there were at least 35 research groups working on PEF technology, doubling those working in PEF in 1996 (Barbosa-Cánovas *et al*., 1999). Such research is mostly conducted at laboratory and pilot plant levels. The number of commercially available units is still limited to one industrial plant size and a few pilot plants and laboratory scale systems. Leading institutions like Pure Pulse Technologies, USA, Thomson CSF, France, Ohio State University, U.S.A., Diversified Technologies Inc., U.S.A., and CENTRALP, France produce these units. The major concern of industrial consortiums interested in PEF application is in the initial investment (Barbosa-Cánovas *et al*., 1999). The price of such devices ranges from $40,000 to $500,000 usd. Some systems have estimated operating costs of $0.2/Lt (EPRI, 1998). Since strong cooperative efforts between academia and industry are now evident, this limited availability will certainly change in the years to come. Current laboratory research is carefully generating kinetic data for microorganisms of interest, while pilot plant research is defining scale up criteria.

PEF technology should also be considered for incorporation into existing processes, for example, replacing a thermal operation with a PEF pasteurization unit, or a combination of both. This could be a sound approach to introducing PEF at industrial levels. The processing

capacity of the available PEF equipment is still limited to around 1800 L/h (Mittal *et al.*, 2000), while the industry demands at least 10,000 L/h. Capital investment and operating costs have an important impact on the decision-making process to implement PEF. Processing of high-acidity food products or combining PEF with mild thermal processes in a hurdle approach are a couple of potential short-term options for commercialization of PEF.

*Energy evaluation*

As in other preservation techniques, in PEF we can identify two energy expenditures of relevance, that delivered to the product and that used to run the system. In PEF, the system energy depends on the requirements of the power supply, switch trigger circuitry, computer(s), cooling devices, and pumping system. The energy delivered to the product must be defined in terms of voltage and current transients experienced within the food product gap, especially if its value is to be correlated with the lethality of the process. Eqn [8] should be used to define the delivered energy (En) by each PEF pulse:

$$\mathrm{En}(t_1) = \int_0^{t_1} (V_D(t) I(t)) \mathrm{d}t \qquad \text{Eqn [8]}$$

where, $V_D(t)$ is the voltage across the chamber gap as a function of time, $I(t)$ the current flowing through the gap as a function of time, and $t_1$ is the pulse duration. However, this energy must be normalized by the processing conditions and reported as energy density ($E_d$):

$$E_d = \frac{\mathrm{En}(t_1) n}{\mathrm{Vol}} \qquad \text{Eqn [9]}$$

where Vol is the effective volume of the treatment chamber and $n$ the number of pulses applied to the sample. Because food is a conductive element, some ohmic heating takes place during PEF processing. If nonthermal conditions need to be guaranteed, a cooling energy equal to the energy density of the treatment must be provided. The temperature rise, $\Delta T$, of the product during treatment can be estimated by

$$\Delta T = \frac{E_d}{\rho C_p} \qquad \text{Eqn [10]}$$

where $\rho$, is the density of the product, $E_d$ the energy density, and $C_p$ the specific heat of the product.

There is very little published data regarding energy consumption from both the system and the product during PEF processing. There is also scarce information that can quantitatively correlate the lethality of a treatment to the energy received by the food (Grahl and Markl, 1996; Heinz *et al.*, 1999). Even if the energy delivered to the treatment chamber is accurately measured, depending on the pulse shape, the energy received by a food may not be entirely used to inactivate microbial flora. As it can be expected, not all electric field intensities have a lethal effect on bacteria and there is minimum electric field intensity required to cause bacterial inactivation. In a close to ideal square pulse, most of the energy will be effective for inactivation purposes, however, in exponential and over/under-damped pulses, nearly 35% of the energy corresponds to electric fields below the threshold level (Qin *et al.*, 1994) and is noneffective for inactivation purposes, providing only heating (**Fig. 4**).

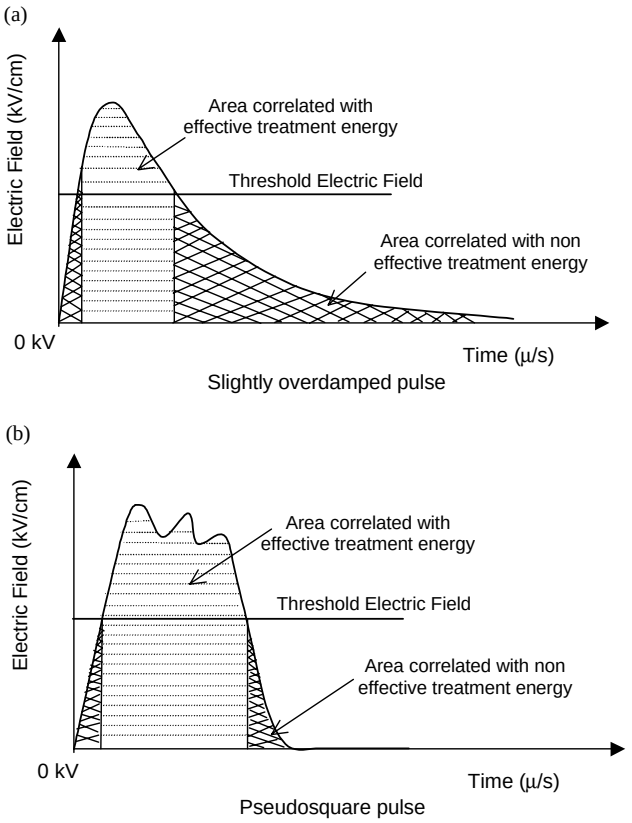


**Fig. 4** Effective energy of pseudosquare and decaying pulses, useful for food preservation/processing purposes

## Inactivation Studies

*Process variables*

The main lethality factors contributing to the effectiveness of PEF can be classified as those depending on (a) the PEF system set up and processing conditions, (b) the attributes of the product being treated, and (c) the microorganism characteristics. Electric field intensity and total treatment time are the most important PEF processing factors. An increase of these factors generally tends to increase the inactivation of microorganisms. In addition, pulse wave-shape and frequency, chamber design, and treatment temperature are also factors influencing PEF lethality. The effectiveness of the process also depends on the physical characteristics of the product, primarily its conductivity but also pH, and ionic strength, the latter being closely related to the conductivity. The pH will act as an additional stressing factor along with the PEF action. On the other hand, factors such as the presence of bivalent cations seems to have a strong effect on reducing PEF lethality. The type, growth stage, and size and shape of the microorganism also influence the lethality of the process. In general,

spores that are smaller and rounder and have a stronger shield protection are harder to inactivate (Marquez *et al*., 1997; Yin *et al*., 1997; Pagan *et al*., 1998) than bacteria, yeast (Wouters and Smelt, 1997), and molds (Raso *et al*., 1998) while microorganisms in the logarithmic growth stage are the least resistant to PEF. For a given input power, chamber configuration and treatment time, the electrical conductivity of the media will determine the maximum achievable electric field and the maximum temperature rise during processing. Low conductivity allows lower power input and will result in lower temperature rises, especially in low resistance chambers. A brief list displaying the conductivity of some of the most studied food products at temperatures below 60 °C includes: beer (0.1–0.25 S/m), coffee (0.1–0.3 S/m), fruit juices (0.1–0.7 S/m), milk products (0.4–1 S/m), and liquid egg (0.5–1 S/m) (Fernández-Martín and Sanz, 1985; Kent, 1987; Barbosa-Cánovas *et al*., 1999; Ruhlman *et al*., 2001).

*Inactivation mechanism*

Application of high-intensity pulsed electric fields generates stress and destabilizes microbial cell membranes, and in some cases may lead to irreversible cell membrane breakdown (Zimmermann, 1986; Pothakamury *et al*., 1997; Jin *et al*., 1998; Calderón-Miranda *et al*., 1999*c*), alterations in ion transport processes (Vega-Mercado *et al*., 1996*b*; Kim *et al*., 2001), and changes in the structural conformation of enzymes (Vega-Mercado *et al*., 1995; Fernandez-Diaz *et al*., 2000). The membrane dielectric breakdown theory explains microbial inactivation in terms of a large increase in the permeability of the microbial membrane as a result of pores induced by a critical potential between 0.7 and 2.2 V (Angersbach *et al*., 2000), which is larger than the natural cell potential. This is analogous to the electrical breakdown of gases caused by a sudden rise in current across two points with high potential. When the critical value is reached some reversible micropores are formed, followed by increase in the permeability of the cell membrane, which leads to ion transport distortions, cell swelling, and eventually permanent damage (Tsong, 1990).

Electromechanical compression and instability theories explain how the electric field creates an accumulation of opposite charges on opposite sides of the microbial cell wall, causing compression and leading to an unstable thin membrane (Coster and Zimmermann, 1975; Ho and Mittal, 1996). In the osmotic imbalance theory, the electric field induces pores into the membrane through which ions and small molecules leak out of the cell, leading to an osmotic imbalance and swelling of the cell until lysis occurs (Kinosita and Tsong, 1977). The viscoelastic model takes into account the rheological behavior of the cell to evaluate the breakdown potential (Dimitrov, 1984). In the hydrophobic to hydrophilic pore transition theory, reversible pores tend to become irreversible (or require longer time to reseal), depending on their radii and energy (Dimitrov, 1984). In the conformational change theory, the electric field may induce phase transitions and conformational transitions of lipids and proteins that could lead to transient pore formations (Newman and Rosenheck, 1972). Experimental data and mathematical simulations (Bruhn *et al*., 1997; Angersbach *et al*., 2000) support these theories. Barbosa-Cánovas *et al*. (1999), presented a comprehensive description of the above-mentioned theories on bacteria inactivation by PEF.

Although electroporation is believed to be the principle of action in PEF, pore formation has not always been confirmed by microstructure studies. However, damage to cell membrane, leakage of intracellular material, and alterations in cell protein have been confirmed (Calderon-Miranda *et al*., 1999*c*; Kim *et al*., 2001).

*Microbial and enzyme inactivation*

Since the early 1950s, pulsed electric discharges at different energy levels have shown successful inactivation of *Escherichia coli*, *Streptococcus faecalis*, *Bacillus subtilis*, *Streptococcus cremoris*, and *Micrococcus radiodurans*, as well as trypsin and a protease from *Bacillus subtilis* (Dovenspeck, 1960). Also, the nonthermal lethal effect of homogeneous electric fields on bacteria like *E. coli*, *Staphylococcus aureus*, *Micrococcus lysodeikticus*, *Sarcina lutea*, *Bacillus subtilis*, *B. cereus*, *B. megaterium*, *Clostridium welchii*, *and yeasts* (i.e. *Saccharomyces cerevisiae* and *Candida utilis*) was demonstrated (Sale and Hamilton, 1967; Hamilton and Sale, 1967). Today extensive research is being conducted on the inactivation of important spoilage microorganisms (i.e. yeast, molds, *Pseudomonas* spp.) and pathogenic flora (i.e. *E. coli* O157:H7, *Listeria* spp., and *Salmonella* spp.) suspended in real food products, such as juices, salsas, milk, and liquid eggs. In some cases, more than 7 log cycles of inactivation have been achieved, and even total inactivation (negative detection) of important pathogens following treatment has been verified during storage (Barbosa-Cánovas *et al*., 1999). Different inactivation percentages (30–99%) of important enzymes, such as trypsin, lactic dehydrogenase, galactosidases, plasmin, proteases, alcaline phosphatase, lipases, glucose-oxidase, α-amylase, peroxidase, phenol oxidase, pectin methylesterase, and papain, have also been achieved (Vega-Mercado *et al*., 1995, 2001*a*,*b*; Ho *et al*., 1997; Yeom *et al*., 1999; Giner *et al*., 2000; Castro *et al*., 2001*a*,*b*; Palomeque *et al*., 2001; Van Loey *et al*., 2002). Such enzyme inactivation levels are considered the result of changes in secondary and tertiary structures that modify certain molecular linkages in the active centers and entire globular configuration. Spore inactivation is still under research, but between a 90 and 99% inactivation of *Bacillus* spores suspended in water has been reported, which may be correlated with the loss of dipicolinic acid (Marquez *et al*., 1997). Important inactivation levels of mold conidiospores suspended in fruit juices, have been achieved with minimum treatment (36 kV/cm, 7 μs treatment time). However, inactivation of mold ascospores under the same treatment conditions does not seem to be as efficient (~90%) (Barbosa-Cánovas *et al*., 1999).

*Hurdle approach*

The combination of several preservative factors to control microbial spoilage and food poisoning is not a new concept. Introduced to the food industry by Leistner (1978), this intelligent concept has also proven to be a sound approach to implementing PEF technology.

The use of antimicrobials, low water activities and pHs, and mild temperatures in combination with PEF has been more effective in inactivating microbial flora and extending the shelf-life of refrigerated products than PEF treatment alone.

Vega-Mercado *et al*. (1996*b*) reported a slightly greater inactivation of *E. coli* in SMUF at low pH (5.69) than at neutral pH (6.82). Media pH also plays an important role in inactivation kinetics when PEF treatment is combined with organic acids. Treatment of *E. coli* with 12.5 kV/cm in a solution containing 1,000 ppm of benzoic acid (pH of 2.4) achieved a 4-log cycle reduction. The strong synergistic killing effect achieved by combining organic acids and PEF treatment at low pH indicates that the entry of undissociated acids into bacterial cells is enhanced by PEF (Liu *et al*., 1997).

The effect of nisin on the cytoplasmic membrane of Gram-positive cells is enhanced by its combination with PEF. The inactivation of *listeria inocua* suspended in skim milk or liquid whole egg, by less than 60 $\mu$s with 50 kV/cm and as much as 100 IU/mL of nisin, shows a significant synergistic effect, achieving 3.8 and 5.5 log unit reductions in skim milk and liquid egg, respectively (Calderón-Miranda *et al*., 1999*a*,*b*). However, the synergistic effect of nisin and PEF is not as marked in the inactivation of Gram-negative bacteria, due to the lipopolysaccharides in outer cell membrane. (Kalchayanand *et al*., 1994; Terebiznik *et al*., 2000; Góngora-Nieto *et al*., 2001).

Electric field treatments at moderate temperatures ($\sim$50–60 °C) exhibit a synergistic effect on the inactivation of microorganisms, where at constant electric field strength, inactivation increases with a rise in temperature. Sensoy *et al*. (1997) achieved nearly a 2-log cycle increase in the inactivation of *Salmonella dublin*, by increasing the process temperature from 10 to 50 °C with 100 µs PEF treatment at 25 kV/cm. A similar result was found with *Listeria monocytogenes* inoculated in whole milk and treated with 600 µs at 30 kV/cm, where 3.5 and 4 log reductions were achieved at 10 and 50 °C, respectively (Reina *et al*., 1998). Other microorganisms such as *Salmonella enteritidis*, *E. coli*, and *Lactobacillus brevis* have shown similar effects. It seems that slightly higher temperatures weaken the cell membrane, thus favoring destabilization and cell death (Pothakamury *et al*., 1996; Vega-Mercado *et al*., 1996*a*; Romain *et al*., 1999). However, it should be noted that if nonthermal inactivation is claimed, the temperatures used in combination with PEF must be held far below those used in pasteurization. Since application of electric fields causes some increase in the food temperature, proper cooling should be provided to maintain food temperatures far below those generated by pasteurization. Sufficient time between pulses, high flow rates, and the use of cooling devices will help to ensure proper food temperatures.

*Microbial inactivation kinetics*

Most of the research conducted on PEF is related in one way or another to inactivation kinetics. However, a recent document put together by experts in the area indicates a need for further development and evaluation of mathematical models to express the inactivation kinetics of PEF (CFSAN-FDA, 2000). Many studies have used first-order kinetics to describe the relationship between inactivation and electric field strength or treatment time, despite a significant reduction in the inactivation rate during long treatment (at a given electric field), the so-called tailing behavior. With pressure from some scientists (Peleg, 1995; Cole, 1999), the scientific community is now more aware that not all cells in a given population have identical sensitivities; instead, there is a spectrum of resistances. Thus, under highly stressful situations the microbes die close together, showing a tight inactivation distribution, while in less stressful situations they die further apart.

Identification of certain tailing when a first-order model is assumed has led researchers (Peleg, 1995; Sensoy *et al*., 1997) to describe microbial kinetics as follows:

$$S = 100/[1 + \exp((E - E_c)/\alpha)] \qquad \text{Eqn [11]}$$

where $S$ is the percent of surviving microorganisms, $E$ the field strength in kV/cm, $E_c$ the critical electric field where the survival level is 50% (inflection point), and $\alpha$ is a parameter that indicates the steepness of the survival curve around $E_c$. A large $\alpha$ value means a wide span, while a small value indicates a very steep decline, high PEF sensitivity, or faster inactivation. Furthermore, $E_c$ and $\alpha$ depend on the number of pulses or treatment time and such dependence can be described as

$$E_c = E_{c0}\exp(-K_1 n) \qquad \text{Eqn [12]}$$

$$\alpha = \alpha_0 exp(-K_2 n) \qquad \text{Eqn [13]}$$

where $K_1$ and $K_2$ are dimensionless constants, and $n$ the number of pulses.

A relatively new model to accurately describe the inactivation kinetics of microorganisms by PEF is the log-logistic model (Cole *et al*., 1993; Anderson *et al*., 1996; Raso *et al*., 2000). It has proven to be a good alternative for fitting survivor curves when a distribution of resistance within the bacterial population occurs. This model is an alternative for describing microbial inactivation kinetics when the logarithm of the survival fraction is not a linear function of treatment time. This log-logistic model is defined by

$$\mathrm{Log}S = \mathrm{Log}S_0 + \frac{\mathrm{Log}S_n - \mathrm{Log}S_0}{1 - \exp(4\sigma(\tau - \mathrm{Log}t)/(\mathrm{Log}S_n - \mathrm{Log}S_0)} \qquad \text{Eqn [14]}$$

where Log $S$ is the log of the survival fraction as a function of time; Log $S_0$ is the log of the initial survival fraction at time zero; Log $S_n$ is the log of the final survival fraction; $\sigma$ is the maximum slope of the log of

survival fraction curve; $\tau$ is the log time when the maximum slope is reached; and $t$ is the processing time. The use of different models indicates the lack of agreement among researchers as to the best way to define the microbial inactivation kinetics of PEF processing.

Other technical issues linked to inactivation kinetics and PEF processing that still need attention from the scientific community include:

(a) The use of specific target microorganisms for products of interest. (EPRI/Army, 1997).
(b) The use of cocktails (a mixture of more than two strains) for boundary models. (EPRI, 1998).
(c) Avoidance of first-order kinetics assumptions (Cole, 1999).
(d) Use of high enough inoculate levels, so there is no need of extrapolation (Cole, 1999).
(e) Gathering enough data points (5–6 minimum) (Cole, 1999).
(f) Evaluating the recovery of injured cells (EPRI, 1998).
(g) Identifying nonpathogenic surrogates (Barbosa-Cánovas *et al.*, 2000*b*).
(h) Developing an approach to establishing equivalencies (Cole, 1999).

Addressing these issues will certainly enhance the validity of conclusions drawn on the effectiveness of PEF, setting a strong foundation for the commercialization and adoption of this technology.

## Regulatory Aspects

PEF is a physical process used to preserve food, and thus in the U.S.A. it falls under the U.S. Food and Drug Administration (FDA) regulations. The difficulty experienced demonstrating the equivalency of nonthermal methods to existing thermal processes is one of the main reasons for slow commercialization of new preservation technologies (Cole, 1997).

Before the commercialization of processed foods by a nonthermal technology can take place, each process must comply with appropriate safety regulations set forth by the FDA according to the type of product. FDA regulations have been established to assure the mission of protecting public health. However, the FDA does not approve processes *per se*, but rather the use of substances or components used in the process.

Besides complying with current regulations that might apply, PEF processing may also require premarket approval. This is due to the use of food contact surfaces under different conditions (with respect to current preservation techniques), such as those present inside the PEF treatment chamber, where electrode material could possibly migrate to the food. To determine if these or other aspects are safety concerns for a particular PEF process, a petition must be submitted to FDA for evaluation. After the evaluation a ruling will be made and issued and then included in the Codex of Federal Regulations (CFR).

In the premarket approval process, PEF processors are asked to submit a scientific review with necessary data to clearly characterize the process and the product. The information package submitted to the FDA should address the following: (a) type of product or process being evaluated, (b) how the product will be used, and (c) what the product will accomplish. The environmental impact of the product and process are also important (Hansen, 1999). However, similar dialog can be established to demonstrate that a petition is not needed for premarket approval, as was the case in 1997, when Pure Pulse received a letter of no objection, and therefore no rule-making was required. Each processor must follow the same procedure through intense communication with the FDA.

When a new process is filed, it is necessary to: (Larkin and Spinak, 1997) (a) establish an active and continuous dialog with the FDA during process development, (b) meet with the FDA to describe the process, (c) invite the FDA for an on-site visit (pilot and production facility), (d) draft and provide the FDA with an outline of the proposed filing, and (e) identify the most resistant organism of public health concern, the most resistant organism to commercial viability, and the least lethal treatment zone in the system.

Regarding the novelty of the process, the FDA is interested in reviewing equipment design, product specification, process design, and process validation (Larkin, 1999).

(a) *Equipment design*: a description of the system, control mechanisms used, and fail-safe procedures.
(b) *Product specifications*: a full description of the product, including physical/chemical aspects, critical factors, and influence of processing on the critical factors.
(c) *Process design*: a complete description of the critical/processing conditions used in manufacturing the product. Included process records: pulse shape, frequency and width, electric field, and treatment time. Including monitoring frequency and repetitions. Records must be easy to access, so the FDA can verify if the required electric field and time are delivered according to operating procedures.
(d) *Validation*: a physical demonstration of the accuracy, reliability, and safety of the process. Since PEF processes vary considerably, reproducibility must be assured. Evidence of injury recovery studies, as well as resistance of certain pathogens concerning a given product must also be included.

These four aspects should address concerns in areas of toxicology, nutrition, and microbiology. Information submitted must address the following questions: (a) does the process generate chemical changes producing compounds that may be toxic, (b) are there nutrient losses, and (c) if the final product is not sterile, how will the product or process control *Clostridium botulinum* and production of toxin? (assessing the risk under

normal use and abusive conditions) Some of these questions were addressed in the past, but at the time the research priority was to demonstrate the potential of PEF in the area of food preservation. Today, the basis for implementing this technology exists and is well supported by several decades of research. Now is the time for systematic research that addresses specific FDA questions and other issues regarding implementation of PEF in the production line.

Furthermore, FDA regulatory control may be specific to the U.S., but the relevance of delivering safe foods to consumers goes beyond U.S. borders. Similar safety concerns may soon be raised by regulatory organizations in other countries as well.

## Conclusion

In the past decade research groups working on PEF technology have made tremendous progress toward understanding its principles and identifying key aspects of the industrialization process. In terms of PEF equipment components, since progress in switching often promotes progress in pulsed power applications, PEF will directly benefit from the availability of forthcoming solid-state switches, which are reliable, have high performance, are easier to work with and reasonably priced, and can also deliver accurately the required dose. Along this line, close attention must be paid to the way in which treatment delivery is defined. Each chamber configuration (single or multiple units) should guarantee that the entire product receive at least the minimum required dose. However, minimum required dosage has yet to be determined. Once a lethal dose is determined for a particular product, each PEF processor will need to adjust the processing conditions to make sure the system is delivering the correct dose, regardless of pulse shape and treatment chamber configuration.

The flexibility of laboratory-scale PEF units may not be practical or economically sound for industrial applications. However, if equivalency could be established between the kinetic data obtained in laboratory units and the processing conditions of scaled up systems, product safety and system performance would be easily assured and controlled, respectively. Accurate definition and measurement of process variables, and complete kinetic studies are now the goal.

Much work remains before PEF industrial implementation can be a reality: (a) development of processing protocols for each food product and PEF system, (b) proof that commercial scale processing is feasible and economically sound, (c) design of suitable, more user-friendly pulse generators, (d) gathering more data on the inactivation kinetics of microbial pathogens in various food media, (e) finding new ways to define the inactivation kinetics of this method, and (f) implementation of PEF in combination with other hurdles. Preservation of foods with PEF has provided an open window to imagination, science, and innovation.

## References


Anderson, W. A., McClure, P. J., Bird-Parker, A. C. and Cole, M. B. The application of a log-logistic model to describe the thermal inactivation of *Clostridium botulinum* 213B at temperatures below 121.1 °C. *Journal of Applied Bacteriology*, **80**, 283–290 (1996)

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

Barbosa-Cánovas, G. V., Góngora-Nieto, M. M., Pothakamury, U. R. and Swanson, B. G. *Preservation of Foods with Pulsed Electric Fields*. New York: Academic Press, pp. 1–9, 76–100, 104, 108–155 (1999)

Barbosa-Cánovas, G. V., Góngora-Nieto, M. M. and Swanson, B. G. Processing fruit and vegetables by pulsed electric field technology. In: Alzamora, S., Tapia, M. S. and Lopez-Malo, A. (Eds), *Minimal Processing Technologies for Fruits and Vegetables*. Aspen, Inc., pp. 223–235 (2000*a*)

Barbosa-Cánovas, G. V., Góngora-Nieto, M. M. and Swanson B. G. Pulsed electric power in food preservation. *International Food Information System* (2001). http://www.ifis.org/hottopics/pulsedpower.html

Barbosa-Cánovas, G. V., Pierson, M. D., Zhang, Q. H. and Schaffner, D. W. Pulsed electric fields. In: Kinetics of microbial inactivation for alternative food processing technologies: Special Supplement. *Journal of Food Science*, 65–79 (2000*b*)

Barsotti, L., Merle, P. and Cheftel, J. C. Food processing by pulsed electric fields. 1. Physical aspects. *Food Reviews International*, **15**, 163–213 (1999)

Bartos, F. J. Medium voltage AC drives. *Control Engineering Online*, No. **20**, Feature Article http://www.controleng.com/archives/2000/ctl0201.00/000203.htm (2000)

Bhasavanich, D., Hitchcock, S. S., Creely, P. M., Shaw, R. S., Hammon, H. G. and Naff, J. T. *International Pulsed Power Conference*, San Diego, CA (1991)

Bruhn, R. E., Pedrow, P. D., Olsen, R. G., Barbosa-Cánovas, G. V. and Swanson, B. G. Electrical environment surrounding microbes exposed to pulsed electric fields. *IEEE Transactions on Dielectrics and Electrical Insulation*, **4**, 806–812 (1997)

Bushnell, A. H., Clark, R. W., Dunn, J. E. and Lloyd, S. W. Process for reducing levels of microorganisms in pumpable food products using a high pulsed voltage system. *U.S. Patent 5,514,391* (1996)

Bushnell, A. H., Dunn, J. E., Wayne, R., Clark, R. W. and Pearlman, J. S. High pulsed voltage systems for extending the shelf life of pumpable food products. *U.S. Patent 5,235,905* (1993)

Calderón-Miranda, M. L., Barbosa-Cánovas, G. V. and Swanson, B. G. Inactivation of *Listeria innocua* in skim milk by pulsed electric fields and nisin. *International Journal of Food Microbiology* **51**, 19–30 (1999*a*)

Calderón-Miranda, M. L., Barbosa-Cánovas, G. V. and Swanson, B. G. Inactivation of *Listeria innocua* in liquid whole egg by pulsed electric fields and nisin. *International Journal of Food Microbiology*, **51**, 7–17 (1999*b*)

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

Caplot, M. and Cote, G. Les technologies nécessaires aux machines de traitment par Champs Electriques Pulsés. In: Proceedings: Formation Technologique en Agro-Alimentaire: La conservation de demain, *Action-Rdta*, *Agir*, *Agrotec*, *Novelect*. Bordeaux Pessac France (1999)

Castro, A. J., Swanson, B. G., Barbosa-Canovas, G. V. and Zhang, Q. H. Pulsed electric field modification of milk

alkaline phosphatase activity. In: Barbosa-Cánovas G. V. and Zhang, H. (Eds), *Electric Fields in Food Processing*. Lancaster, PA: Technomic, pp. 65–82 (2001*a*)

Castro, A. J., Swanson, B. G., Barbosa-Canovas, G. V. and Dunker, A. K. Pulsed electric field denaturation of alkaline phosphatase. In: Barbosa-Cánovas G. V. and Zhang H. (Eds), *Electric Fields in Food Processing*. Lancaster, PA: Technomic, pp. 83–104 (2001*b*)

CFSAN-FDA (Center for Food Safety and Applied Nutrition) Kinetics of Microbial Inactivation for Alternative Food Processing Technologies. *U.S. Food and Drug Administration*, URL: http://vm.cfsan.fda.gov/~comm/ift-toc.html and http://vm.cfsan.fda.gov/~comm/ift-pef.html (2000)

Cogdell, J. R. *Foundations of Electric Circuits*. NY: Prentice-Hall, p. 333 (1999)

Cole, M. B. The outlook for novel preservation technologies: a food industry perspective. Presented at the *Fifth Conference of Food Engineering*, Los Angeles (1997)

Cole, M. B. A critical review on microbiology issues in nonthermal food processing. *Institute of Food Technologists, Annual Meeting*, Chicago, IL [Session 30-4] (1999)

Cole, M. B., Davies, K. W., Munro, G., Holyoak, C. D. and Kilsby, D. C. A vitalistic model to describe the thermal inactivation of *Listeria monocytogenes*. *Journal of Industrial Microbiology*, **12**, 232–239 (1993)

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

De Jong, P. and Van Heesh, E. J. M. Review: Effect of pulsed eletric fields on the quality of food products. *Milchwissenschaft*, **53**, 4–8 (1998)

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

Dovenspeck, H. Verfahren und vorrichtung zur gewinnung der einzelnen phasen nus dispersen systemen. *German Patent 1,237,541* (1960)

Dunn, E. and Pearlman, J. S. Methods and apparatus for extending the shelf life of fluid food products. *U.S. Patent 4,695,472* (1987)

EPRI and Army Workshop II. Pulsed electric field Workshop. Minutes: Appendix 13: Food Microbiology Goals. *Industrial and Agricultural Technologies and Services*, Palo Alto, CA (1997)

EPRI. Pulsed electric field processing in the food industry: a status report on PEF. Report CR-109742. *Industrial and Agricultural Technologies and Services*, Palo Alto, CA (1998)

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

Fernandez-Díaz, M. D., Barsotti, L., Dumay, E. and Cheftel, J. C. Effects of pulsed electric fields on ovalbumin solutions and dialyzed egg white. *Journal of Agricultural Food Chemistry*, **48**, 2332–2339 (2000)

Fernandez-Martin, F. and Sanz, P. D. Influence of temperature and composition on some physical properties of milk and milk concentrates: V,*electrical conductivity. *International Agrophysics*, **1**, 41–54 (1985)

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

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

Góngora-Nieto, M. M. *Food preservation by pulsed electric fields: evaluation of critical processing parameters and process optimization*. Ph.D. Dissertation. Washington State University, Pullman, WA, pp. 159–176 (2000)

Góngora-Nieto, M. M., Seignour, L., Riquet, P., Davidson, P. M., Barbosa-Cánovas, G. V. and Swanson, B. G. Nonthermal inactivation of *Pseudomonas fluorescens* in liquid whole egg. In: Barbosa-Cánovas, G. V. and Zhang, H. (Eds), *Pulsed Electric Fields in Food Processing: Fundamental Aspects and Applications*. Lancaster, PA: Technomic Publishers pp. 193–210 (2001)

Hansen, P. A. Novel food processing technologies: regulatory perspectives. *Institute of Food Technologists, Annual Meeting*, Chicago, IL [Session 59-3] (1999)

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

Heinz, V., Alvarez, I., Angersbach, A. and Knorr, D. Preservation of liquid foods by high intensity pulsed electric fields—basic concepts for process design. *Trends in Food Science and Technology*, **12**, 103–111 (2002)

Heinz, V., Phillips, S. T., Zenker, M. and Knorr, D. Inactivation of *Bacillus subtilis* by high intensity pulsed electric fields under close to isothermal conditions. *Food Biotechnology*, **13**, 155–168 (1999)

Ho, S. Y. and Mittal, G. S. Electroporation of cell membranes: a review. *Critical Reviews in Biotechnology*, **16**, 349–362 (1996)

Ho, S. Y., Mittal, G. S. and Cross, J.D. Effect of high field electric pulses on the activity of selected enzymes. *Journal of Food Engineering*, **31**, 69–84 (1997)

Ho, S. Y., Mittal, G. S., Cross, J. D. and Griffith, M. W. Inactivation of *P. fluorescens* by high voltage electric pulses. *Journal of Food Science*, **60**, 1337–1340 (1995)

Jin, Z. T., Qiu, X. and Zhang, Q. H. Application of high speed microscopy imaging technology in evaluation of inactivation of microorganisms by pulsed electric fields. *Institute of Food Technologies, Annual Meeting*, Paper 59C-16 (1998)

Kalchayanand, N., Sikes, T., Dunne, C. P. and Ray, B. Hydrostatic pressure and electroporation have increased bactericidal efficiency in combination with bacteriocins. *Applied and Environmental Microbiology*, **60**, 4174–4177 (1994)

Keith, W. D., Harris, L. J. and Griffiths, M. W. Reduction of bacterial levels in flour by pulsed electric fields. *Journal of Food Process Engineering*, **21**, 263–269 (1998)

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

Kempkes, M. A., Zhang, Q. H., Gaudreau, M. P. J. and Hawkey, T. J. Scalable, solid state pulsed power systems for pulsed electric field (PEF) processing of pumpable foods. *Institute of Food Technologists, Annual Meeting*, Paper 59H-26 (2001)

Kent, M. *Electrical and Dielectric Properties of Food Materials*. Science and Technology Publishers, pp. 72–73, 76–77,86–87 (1987)

Kim, H., Ye, J. and Li, Y. Inactivation of *Listeria monocytogenes* in chilling brine using a flow through electrochemical treatment. *Institute of Food Technologists, Annual Meeting*, Paper 59H-22 (2001)

Kinosita, K. Jr. and Tsong, T. Y. Hemolysis of erythrocytes by a transient electric field. *Proceedings of the National Academy of Science U.S.A.* **74**, 1923–1927 (1977)

Knorr, D. and Angersbach, A. Impact of high intensity electric fields on plant membrane permeabilization. *Trends in Food Science and Technology*, **9**, 185–191 (1998)

Kreuger, F. H. *Partial Discharge Detection in High-voltage Equipment*. London: Butterworths (1989)

Larkin, J. W. Regulatory issues and consumer acceptance in nonthermal food preservation. *Institute of Food* Technologies, *Annual Meeting*, Paper 30-6 (1999)

Larkin, J. W. and Spinak, S. H. Aspects of new technology implementation in the U. S. Presented at the *New Technologies Symposium NFPA Convention*. 29 October, Chicago, IL (1997)

Leistner, L. Hurdle effect and energy savings. In: Downey, W. K. (Ed.), *Food Quality and Nutrition*. Barking: Applied Science Publishers, p. 553 (1978)

Liu, X., Yousef, A. E. and Chism, G. W. Inactivation of *Escherichia coli* 0157:H7 by the combination of organic acids and pulsed electric fields. *Journal of Food Safety*, **16**, 287–299 (1997)

Lubicki, P. and Jayaram, S. High voltage pulse application for the destruction of the Gram negative bacterium *Yersinia enterocolitica*. *Bioelectrochemistry and Bioenergetics*, **43**, 135–141 (1997)

MacGregor, S. J., Farish, O., Fouracre, R., Rowan, N. J. and Anderson, J. G. Inactivation of pathogenic and spoilage microorganisms in a test liquid using pulsed electric fields. *IEEE Transactions on Plasma Science*, **28**, 144–149 (2000)

Márquez, V. O., Mittal, G. S. and Griffiths, M. W. Destruction and inhibition of bacterial spores by high voltage pulsed electric fields. *Journal of Food Science*, **62**, 399–409 (1997)

Matsumoto, Y., Satake, T., Shioji, N. and Sakuma, A. Inactivation of microorganisms by pulsed high voltage applications. Conference Record of *IEEE Industrial Applications Society* Annual Meeting, pp. 652–659 (1991)

McDonald, C. J., Lloyd, S. W., Vitale, M. A., Petersson, K. and Innings, E. Effects of pulsed electric fields on microorganisms in orange juice using electric field strengths of 30 and 50 kV/cm. *Journal of Food Science*, **65**, 984–989 (2000)

Mittal, G. S., Ho, S. Y., Cross, J. D. and Griffiths, M. W. Method and apparatus for electrically treating foodstuffs for preservation. *U.S. Patent 6,093,432* (2000)

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

Palomeque, L. A., Gongora-Nieto, M. M., Bermudez, A. S., Barbosa-Cánovas, G. V. and Swanson, B. G. Nonthermal inactivation of endoproteases by pulsed electric field technology. In: Barbosa-Cánovas, G. V. and Zhang, H. (Eds), *Electric Fields in Food Processing*. Lancaster, PA: Technomic, pp. 135–148 (2001)

Pagán, R., Esplugas, S., Góngora-Nieto, M. M., Barbosa-Cánovas, G. V. and Swanson, B. G. Inactivation of *Bacillus subtilis* spores using high intensity pulsed electric fields in combination with other food conservation technologies. *Food Science and Technology International*, **4**, 33–44 (1998)

Peleg, M. A model of microbial survival after exposure to pulsed electric fields. *Journal of Science of Food and Agriculture*, **67**, 93–99 (1995)

Pothakamury, U. R., Vega-Mercado, H., Zhang, Q., Barbosa-Cánovas, G. V. and Swanson, B. G. Effect of growth stage and temperature on inactivation of *E. coli* by pulsed electric fields. *Journal of Food Protection*, **59**, 1167–1171 (1996)

Pothakamury, U. R., Barbosa-Cánovas, G. V., Swanson, B. G. and Spence, K. D. Ultrastructural changes in *Staphylococcus aureus* treated with pulsed electric fields. *Food Science and Technology International*. **3**, 113–121 (1997)

Qin, B. L., Barbosa-Cánovas, G. V., Pedrow, P. D., Olsen, R. G., Swanson, B. G. and Zhang, Q. Continuous flow electrical treatment of flowable food products. *U.S. Patent 5,662,03* (1997)

Qin, B. L., Zhang, Q., Barbosa-Cánovas, G. V., Swanson, B. G. and Pedrow, P. D. Inactivation of microorganisms by pulsed electric fields with different waveforms. *IEEE Transactions of Dielectrics and Electrical Insulators*, **1**, 1047–1057 (1994)

Qin, B. L., Zhang, Q., Barbosa-Cánovas, G. V., Swanson, B. G. and Pedrow, P. D. Pulsed electric field treatment chamber design for liquid food pasteurization using finite element method. *Transactions of ASAE*, **38**, 557–565 (1995)

Raso, J., Alvarez, I., Condón, S. and Sala, F. J. Predicting inactivation of *Salmonella seftenberg* by pulsed electric fields. *Innovative Food Science and Technology*, **1**, 21–29 (2000)

Raso, J., Calderon, M., Gongora-Nieto, M. M., Barbosa-Canovas, G. V. and Swanson, B. G. Inactivation of mold ascospores and conidiospores suspended in fruit juices by pulsed electric fields. *Lebensmittel-Wissenschaft und-Technologie*, **31**, 668–672 (1998)

Reina, L. D., Jin, Z. T., Zhang, Q. H. and Yousef, A. E. Inactivation of *Listeria monocytogenes* in milk by pulsed electric field. *Journal of Food Protection*, **61**, 1203–1206 (1998)

Robbins, J. A. Process and apparatus for reduction of microorganisms in a conductive medium using low voltage pulsed electrical energy. *U.S. Patent 6,331,321* (2001)

Romain, J., Florence, B., Francoise, N., Roignant, M. and Brule, G. High intensity pulsed electric fields applied to egg white: effect on *Salmonella enteritidis* inactivation and protein denaturation. *Journal of Food Protection*, **62**, 1381–1387 (1999)

Ruhlman, K. T., Jin, Z. T. and Zhang, Q. H. Physical properties of liquid foods for pulsed electric fields treatment. In: Barbosa-Cánovas, G. V. and Zhang H. (Eds), *Electric Fields in Food Processing*. Lancaster, PA: Technomic, pp. 45–56 (2001)

Sale, A. J. H. and Hamilton, W. A. Effects of high electric fields on microorganisms. I. Killing of bacteria and yeast. *Biochimica Biophysica Acta* **148**, 781–788 (1967)

Sensoy, I., Zhang, Q. H. and Sastry, S. K. Inactivation kinetics of *Salmonella dublin* by pulsed electric fields. *Journal of Food Processing Engineering*, **20**, 367–381 (1997)

Terebiznik, M. R., Jagus, R. J., Cerrutti, P., Huergo, M. S. and Pilosof, A. M. R. Combined effect of nisin and pulsed electric fields on the inactivation of *Escherichia coli*. *Journal of Food Protection*, **63**, 741–747 (2000)

Tsong, T. Y. Electrical modulation of membrane proteins: enforced conformational oscillations and biological energy and signal transductions. *Annual Review of Biophysics and Chemistry* **19**, 83–106 (1990)

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

Vega-Mercado, H., Martin-Belloso, O., Chang, F. J., Barbosa-Cánovas, G. V. and Swanson, B. G. Inactivation of *Escherichia coli* and *Bacillus subtilis* suspended in pea soup using pulsed electric fields. *Journal of Food Process and Preservation*, **20**, 501–510 (1996*a*)

Vega-Mercado, H., Pothakamury, U. R., Chang, F. J., Barbosa-Cánovas, G. V. and Swanson, B. G. Inactivation of *E. coli* by combining pH, ionic strength and pulsed electric field hurdles. *Food Research International*, **29**, 117–121 (1996*b*)

Vega-Mercado, H., Powers, J. R., Barbosa-Cánovas, G. V. and Swanson, B. G. Plasmin inactivation with pulsed electric fields. *Journal of Food Science*, **60**, 1132–1136 (1995)

Vega-Mercado, H., Powers, J. R., Barbosa-Cánovas, G. V. and Swanson, B. G. Effect of added calcium and EDTA on the inactivation of a protease from *Pseudomonas fluorescens* M3/6 when exposed to pulsed electric fields. In: Barbosa-Cánovas, G. V. and Zhang, H. (Eds), *Electric Fields in Food Processing*. Lancaster, PA: Technomic, pp. 121–134 (2001*a*)

Vega-Mercado, H., Powers, J. R., Martin-Bellosos, O., Luedecke, L., Barbosa-Cánovas, G. V. and Swanson, B. G. Change in susceptibility of proteins to proteolysis and the inactivation of an extracellular protease from *Pseudomonas fluorescens* M3/6 when exposed to pulsed electric fields. In: Barbosa-Cánovas, G. V. and Zhang, H. (Eds), *Electric*

*Fields in Food Processing*. Lancaster, PA: Technomic, pp. 105–120 (2001*b*)

Wouters, P. C. and Smelt, J. P. P. Inactivation of microorganisms with pulsed electric fields: potential for food preservation. *Food Biotechnology*, **11**, 193–229 (1997)

Yeom, H. W., Zhang, Q. H. and Dunne, C. P. Inactivation of enzyme papain by pulsed electric field in a continuous system. *Food Chemistry*, **67**, 53–59 (1999)

Yin, Y., Zhang, Q. H. and Sastry, S. H. High voltage pulsed electric field treatment chambers for the preservation of liquid food products. *U.S. Patent 5,690,978* (1997)

Zhang, Q., Monsalve-González, A., Qin, B. L., Barbosa-Cánovas, G. V. and Swanson, B. G. Inactivation of *Saccharomyces cerevisae* by square wave and exponential-decay pulsed electric fields. *Journal of Food Process Engineering*, **17**, 469–478 (1994*a*)

Zhang, Q., Barbosa-Cánovas, G.V. and Swanson, B. G. Engineering aspects of pulsed electric field pasteurization. *Journal of Food Engineering*, **25**, 268–281 (1995)

Zhang, Q. H., Chang, F. J. and Barbosa-Cánovas, G. V. and Swanson, B. G. Inactivation of microorganisms in a semisolid model food using high voltage pulsed electric fields. *Lebensmittel-Wissenschaft und-Technologie*, **27**, 538–543 (1994*b*)

Zimmermann, U. Electrical breakdown, electropermeabilization and electrofusion. *Reviews on Physiological Biochemical Pharmacology*, **105**, 175–256 (1986)