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# Food Preservation by Pulsed Electric Fields: An Engineering Perspective

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**Abstract** Along the years, processing technologies for food preservation have been in constant development in order to meet current consumers' claims. In this way, researchers have been continuously working to understand the effects of different novel emerging technologies tested on foods to ensure microbiological stability as well as high quality attributes. Among these novel technologies, pulsed electric fields (PEF) have shown to be a potential non-thermal treatment capable of preserving liquid foods with fresh-like characteristics. As a result, in the last decade, great technological advances have been accomplished regarding to PEF processing, such as improvement on the chamber designs, optimization of the process as well as achievement of high-quality-processed foods. This paper reviews the latest developments related to PEF technology for food preservation, the current designs of PEF treatment chambers, and modeling concepts applied for process optimization. In addition, some scaling-up considerations are included for future industrial implementation of PEF processing.

**Keywords** Pulsed electric fields · Food preservation · Treatment chambers · Modeling · Scale-up

## Introduction

The concept of pulsed electric field (PEF) as a process for food preservation has already more than 80-year history. Pulsed discharges of high voltage across two electrodes for microbial inactivation were first investigated in 1950s [5, 24], resulting in a process called electrohydraulic treatment. Later on, Doevenspeck [23] demonstrated that PEF were able to disrupt cells in food material and were further developed to the inactivation of microorganisms. Based on these studies, numerous research activities have been carried out during the last decades. More information on the history of the application of PEF for food processing can be found elsewhere [89].

Basically, PEF involves the application of high-voltage pulses (20–80 kV/cm) for short periods of time (ms or μs) to a product confined or flowing between two electrodes. A typical PEF unit consists of a pulse generator, treatment chambers, a fluid-handing system, and monitoring and control devices [60]. The PEF treatment chamber, which is one of the key factors for achieving the highest effectiveness of the process, is used to house the electrodes and deliver the high voltage to the food. In addition, treatment parameters such as the electric field strength, treatment time, temperature, pulse frequency, width, and polarity are critical factors that have strong influence on process effectiveness [34]. Hence, the study and evaluation of equipment and chamber design as well as the establishment of optimal treatments to obtain safe products are interesting topics that have been under analysis along the last few years. As a result, various laboratory and pilot-scale PEF treatment chambers have been developed by different research groups [50].

PEF is an effective technology to achieve sufficient reduction of spoilage and pathogenic microorganisms in several foods [29, 35, 61, 74, 86]. Moreover, studies focused on enzyme inactivation have demonstrated that PEF allows the inactivation of deleterious enzymes at different levels, depending on the enzyme itself and the medium in which is suspended [58]. In contrast to a

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conventional thermal treatment, this emerging technology preserves the sensory, nutritional, and functional properties of foods [57, 87, 88], which matches with the increasing consumer demand for "fresh-like" foods. During the last years, numerical simulations and mathematical models have been used for setting treatment conditions and predicting the changes that might occur during PEF processing of liquid foods. Currently, researchers are working on the accurate application of different models in order to optimize treatment conditions and achieve the highest efficiency of the process, as well as developing of suitable equipments [1, 10, 35, 44, 84].

In this way, based on the results obtained at present, PEF could be considered as a good alternative to heat pasteurization for liquid food preservation. Nevertheless, for the sake of comparison and optimization, it has to be demonstrated that PEF is economically interesting regarding to the existing thermal pasteurization methods, in terms of cost of operation and investment as well as product quality and, in particular, consumer acceptance. The present review highlights the current developments of PEF technology regarding to the engineering aspects and gather information related to the most recent advances performed over the last 10 years focused on its potential advantages as food preservation process.

## PEF Treatment System

Analysis of PEF engineering implies such aspects as controlling, measuring, and evaluation of the treatment chambers efficiency and the changes on processed foods. The basic principle of PEF is the application of short pulses of high electric fields during short periods of time. In this way, PEF technology can be applied for fluid foods, in batch or continuous-flow mode, to increase their shelf-life while maintaining its organoleptical and nutritive attributes [64]. While in batch processing, discrete portions of foodstuff are treated as a unit by subjecting all of the fluid to a PEF treatment chamber; in the continuous processing, the treated foodstuff is flowing into in a steady stream by a pump. Batch systems are mostly used to carry out laboratory experiments with small samples. Nevertheless, in order to achieve high-volume capacity and easy integration into industrial food processing lines, PEF should be applied in continuous mode.

Two different fluid handling systems can be used to allow the treatment of liquid foods in continuous flow: 1) the fluid can be repeatedly pumped through the system, as many times as needed, to reach the desired treatment time in what is called the stepwise circulation mode (Fig. 1) and 2) the liquid can also pass through the system and return to the container without interruption in what is called the recirculation mode [31]. According to Abram et al. [1], the use of several treatment chambers in series in continuous processing is a good way of improving the microbial inactivation efficiency. Therefore, PEF systems in continuous mode may have one or more treatment chambers.

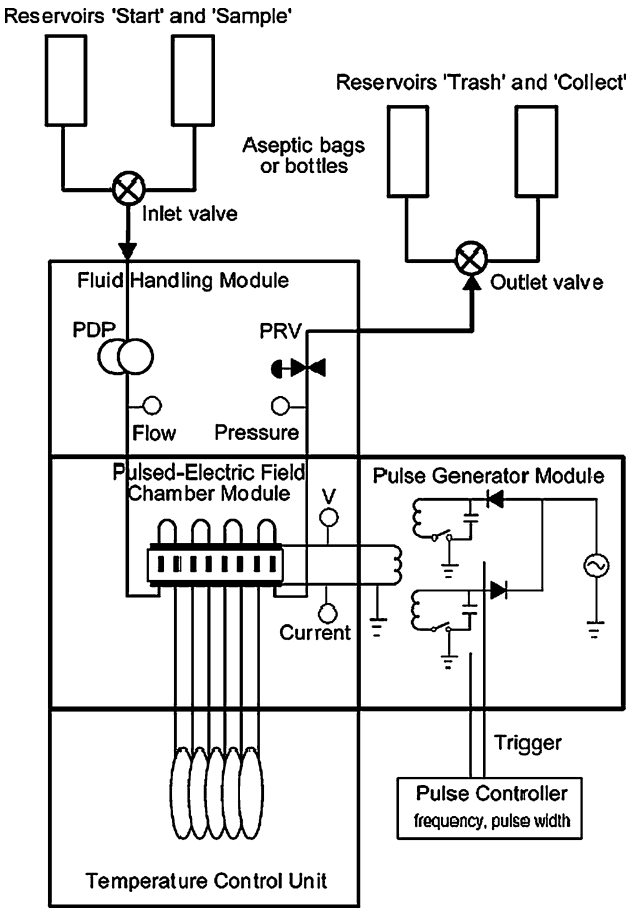


**Fig. 1** Configuration of a stepwise circulation mode (OSU-4F laboratory scale PEF unit, Ohio State University, Colombus, Ohio USA). PDP: precision dispense pump, PRV: pump pressure relief valve. From Sobrino-López and Martín-Belloso [84]

### PEF Treatment Chambers

The treatment chamber is a key component of the PEF unit since it is the part where direct application of the electric field to the products occurs [36, 52]. Its design is of high relevance for the appropriate application of a spatially uniform field distribution [32]. Basically, a PEF treatment chamber is composed of two electrodes held in position by an insulating material forming an enclosure where the food will be placed or pumped through. Different treatment chambers have been designed for batch or continuous PEF systems along years; nevertheless, continuous-flow treatment chambers have been mostly evaluated. Huang and Wang [50] published an interesting review related to the latest PEF chambers designs.

Up to now, the parallel, co-axial, and co-linear configurations are the most known treatment chambers (Fig. 2); they present some advantages and drawbacks during PEF processing. According to Toepfl et al. [86], a linear and uniform electric field distribution can be achieved using the parallel configuration; nevertheless, treatment intensity is reduced in boundary regions depending on the product and processing parameters. Its low resistance results in unwanted high current flow and consequently in higher energy requirements to achieve specific electrical field [53]. On the other hand, although co-axial and co-linear treatment chambers have a less uniform electric field distribution, which depends on constructional properties, they are easy to clean and have a high load resistance [29, 50, 86–88]. In this way, some researchers are currently working to improve the electric field distribution, enhancing the homogeneity of the flow velocity or avoid the temperature peaks inside the chambers.

In a recent study, Li et al. [55] evaluated the use of co-axial and tube-plate treatment chambers for bacteria inactivation in order to provide technical basis for industrial application. Their results showed that, in co-axial configuration, only part of the fluid passes through the high electric field intensity region, while in the tube-plate chamber, the entire fluid passes through the region resulting in major bacteria inactivation levels. Jaeger et al. [52] affirmed that the homogeneity of the PEF treatment in a continuous chamber is linked to the flow velocity profile. They modified a co-linear treatment chamber configuration in order to alter the field strength distribution and improve the flow rate profile. Stainless steel and polypropylene grids were inserted in the chamber (Fig. 3). Their results showed that the insertion of grids in the electric field zone produces homogeneous and more intense electric fields. The modification of the treatment chamber resulted in an increase microbial inactivation and retention of heat sensitivity compounds.

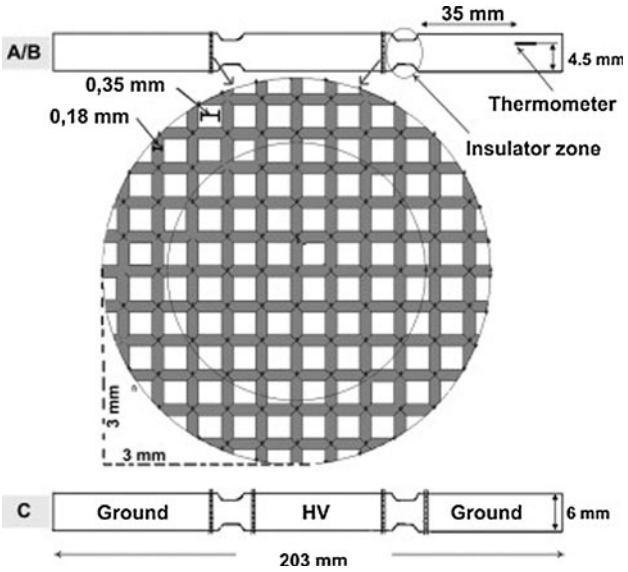


**Fig. 3** Grid dimensions and location for models A, B, and C as well as position of the fiber optic temperature sensor in the treatment chamber. From Jaeger et al. [52]

Modifying chamber configuration let avoid electrical breakdown, sparking, electrolysis reactions, bubble creation within the fluid food, and microbial stagnant zones formation. A chamber with a smooth inner surface which consisted of a circular tube and a group of electrodes insulated from each other and extended parallel to the

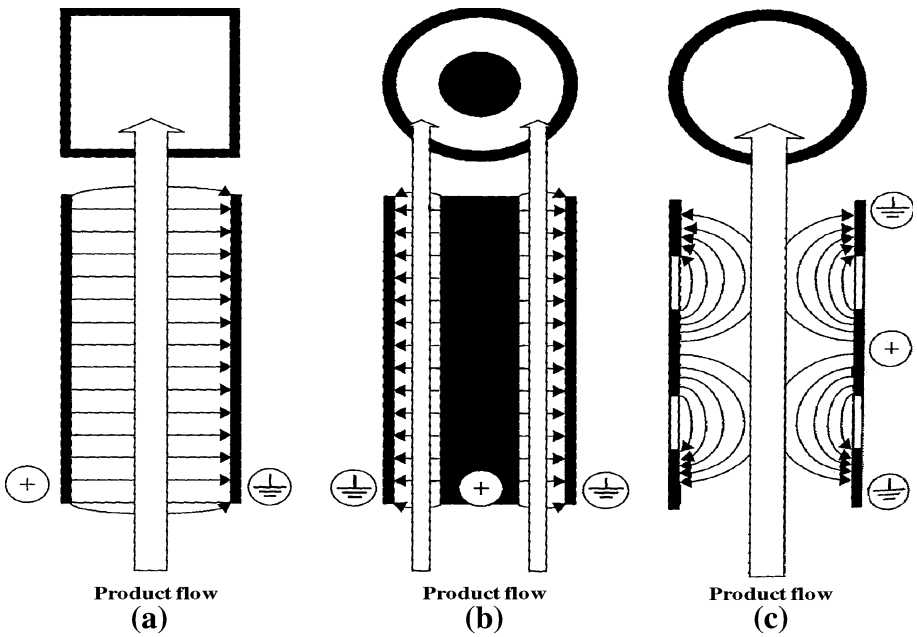


**Fig. 2** Configurations of treatment chambers for continuous PEF treatment: **a** parallel plate, **b** co-axial and **c** co-linear configuration. From Toepfl et al. [86]

longitudinal center line of the chamber was proposed by Morshuis et al. [63]. They achieved a uniform electric field during the processing without perturbing the product flow. Alkhafaji and Farid [4] proposed an interesting chamber design to concentrate the electric field in a small region. The chamber included two stainless steel mesh electrodes isolated from each other by an insulator element. This design enables high electric field intensities with only low increase in liquid temperature and limited fouling of electrodes, avoiding stagnant zones where the microorganisms can accumulate and multiply.

During PEF process, temperature increases in the interior of the treatment chambers as a result of ohmic heating [56]. This effect can contribute to the inactivation of microorganisms and enzymes but may also lead to undesired destruction of heat-sensitive components of the food. The maximum temperature increase ($\Delta T$) can be calculated using a basic thermodynamic equation (Eq. 1).

$$\Delta T = \frac{Q}{\rho_f C_p} \tag{1}$$

where $Q$ is the energy density, $\rho_f$ and $C_p$ are the density and specific heat of fluid food inside the treatment chamber, respectively.

Temperature can also modify density, viscosity, as well as electrical and thermal conductivity of different foods; as a consequence, electric field distribution and product flow are intrinsically coupled to temperature variations [84]. Therefore, to maintain a non-thermal operation, the energy input to the food being treated must be controlled. Different authors have conclude that a cooling system is desirable for controlling the temperature range during the processing, located either in the treatment chamber itself or between chambers in the case of systems with more than one [18, 31, 82, 90]. The possibility of two or more chambers in series may facilitate heat dissipation by placing in a cooling system after a determined number of chambers [1]. According to Jaeger et al. [52], the distribution of temperature inside the treatment chamber can vary at different points as a result of the dissipation of electrical energy depending on field intensity, flow behavior, and residence time. Insertion of grids inside the treatment chamber promoted an improved homogeneity of the flow velocity profile and produces higher turbulence intensity [17]. In the study carried out by Jaeger et al. [52], two different kinds of grids were inserted into a co-linear treatment chamber in order to improve temperature homogeneity. The authors calculated temperature distribution inside the chamber during the processing by applying mathematical equations (Eqs. 2 and 3).

$$pC_p\frac{\partial T}{\partial t} + \nabla \cdot (-(k + k_{T,}C) \cdot \nabla T + pC_pTu) = W \tag{2}$$

$$k_T = C_p\eta_T \tag{3}$$

where $C_p$ denotes the specific heat capacity, $T$ is the temperature, $k$ is the thermal conductivity, $k_T$ is the turbulent heat conductivity, $\eta_T$ denotes turbulent kinematic viscosity, $\rho$ is the density, $u$ is the velocity vector, and $W$ is a sink of source term.

Their results demonstrated that the insertion of grids modified the flow velocity and avoided the formation of temperature peaks. Hence, minor or major modifications on electrode configuration and chamber geometry could result in more efficient PEF treatments.

The resistance of the treatment chamber is strongly related to the electrical conductivity of the food being treated, which is an intrinsic property of the product that influences the crucial process parameters [48, 92]. According to Heinz et al. [48], a raise in the food conductivity, produced by an increase in temperature due to the dissipated pulse energy, can reduce the chamber resistance. Góngora-Nieto et al. [45] reported that the resistance of the chamber ($R$) is defined by its dimensions and geometry, as well as the conductivity of the food product being treated. Ohm's law (Eq. 4) describes $R$ as the voltage ($V$) across it divided by the current ($I$) (Eq. 5) through it.

$$R = \frac{V}{I} \tag{4}$$

$$I = (|J_c| \cdot 1 \cdot A) \tag{5}$$

where $A$ is the effective area, and $J_c$ is the conduction current density (Eq. 6).

$$J_c = \sigma E_f \tag{6}$$

where $\sigma$ is the conductivity of the food, and $E_f$ is the electric field developed in the gap.

It can be deduced from Eqs. 4, 5, and 6 that chambers with high effective areas and processing food with high-conductivity values could have low $R$. Therefore, foods with high ionic concentration seem to be less suitable for PEF treatments than food with low conductivity values. In addition, there are more aspects related to the food properties to be considered before applying a PEF treatment. For example, air bubbles must be removed because they cannot withstand high electric fields and can influence the efficacy of the treatment [86]. Additionally, the maximum particle in the liquid must be smaller than the gap of the treatment region in the chamber in order to maintain proper processing operation [70].

Additionally, energy density ($Q$) is an important aspect for being considered to achieve effective treatments without over- or under-processing. Overall, depending on the product, experimental setup, treatment chamber geometry, and processing parameters such as pulse shape and temperature, the specific energy input requirements vary in a

broad range from 50 up to several hundreds of kJ/kg [87]. At present, $Q$ is rarely reported in the studies. It is only provided to be correlated with microbial and enzyme inactivation [30, 38, 48]. In fact, as reported by Góngora-Nieto et al. [46], the low energy consumption of PEF treatment is very attractive to the food industry because it allows a reduction of costs in comparison with other preservation technologies.

## Implementing and Monitoring a PEF Treatment

The application of PEF process requires suitable and appropriate control throughout the entire treatment time. Today, numerical simulations of fluid dynamics coupled with the electric and thermal fields inside the treatment chambers have been developed to provide such information with high spatial and temporal resolution. They can be employed to study crucial parts of the process in detail and avoid the over- or under-processing of food as well as the electrical breakdown, resulting in an efficient application of PEF. This approach can be considered as a complementary method in such cases, when the experiments are difficult or impossible to be performed. Detailed information of this topic is given by Gerlach et al. [36] in which authors describe the theoretical concepts of numerical simulations with some examples. Most simulations carried out up to now have been completed using commercial software based on fluid dynamic solvers, which let use equations for describe temperature profile into the chamber and other treatment variables.

Fiala et al. [33] proposed a computerized modeling of a co-field continuous flow through chamber based on the mutual influence of the electric field induced and the product flow. They observed that changing the diameter and position of the probes fitted in the pipe, both the degree of uniformity in food processing and the resident time could be regulated. Later, Lindgren et al. [56] added the effect of temperature to the model developed by the previously cited authors. Analyzing the geometry of different continuous-flow chambers, Lindgren et al. [56] concluded that the electrical field could be more homogeneous if the insulator and the electrodes intersected at angles close to 90° and if a cooling system was incorporated in order to minimize heating of the product close to the pipe wall, where the flow velocity is low.

Recently, a static chamber was evaluated by Saldaña et al. [76] to obtain kinetic data on microbial inactivation. The authors estimated the distribution of the electric field strength and the temperature within a parallel treatment chamber with tempered electrodes by a finite element method. They conclude that the treatment chamber designed in their investigation permits to apply uniform electric field strengths at different temperatures and at quasi-isothermal conditions achieving high-microbial inactivation levels. In another interesting work, Jaeger et al. [53] applied numerical simulation to calculate the temperature–time profile of milk while passing the PEF system. Using FLUENT software and identifying the characteristics for heat transfer phenomena during the process, the authors obtained Eq. 7 that allowed the estimation of temperature increase within the treatment chamber between the inlet ($T_{\text{inTC}}$) and the outlet ($T_{\text{outTC}}$) temperature due to the dissipation of the electrical energy, considering the energy delivered per pulse ($W_{\text{pulse}}$), pulse frequency ($f$), mass flow rate ($m$), and specific heat capacity ($c_p$).

$$W_{\text{pulse}} \cdot f = m \cdot c_p \cdot (T_{\text{outTC}} - T_{\text{inTC}}) \tag{7}$$

In addition, the temperature at certain time ($t$) after the milk entered the treatment chamber was calculated according to Eq. 8.

$$T_{\text{outTC}} = T_{\text{inTC}} + \frac{W_{\text{pulse}}}{m \cdot c_p} \cdot f \cdot t_{\text{res}} \tag{8}$$

where $t_{\text{res}}$ was the total residence time of the product in the treatment chamber.

Based on Eq. 8, the authors observed that temperature increase during PEF treatment due to the dissipation of electrical energy depended on the total specific energy input (which depends on $W_{\text{pulse}}$, $f$ and $m$ in turn). The application of numerical simulations enables these authors to confirm that there was a synergistic effect between temperature and pulsed electric fields which enhance microbial inactivation. They conclude that an optimal combination of the application of thermal and electrical energy can be determined to allow a maximum of microbial inactivation with a minimum of required energy and degradation of heat-sensitive compounds.

Hence, the advantage of applying numerical simulations to investigate PEF processes lies in the capability to provide detailed information about electric and thermal fields inside the treatment chambers, which cannot be provided otherwise. Moreover, information obtained from simulation could be useful to obtain kinetics data on microorganism and enzymes destruction by PEF or the changes caused in other component of interest, as a result processing optimization can be easily attained. Therefore, numerical simulations can be a prospective tool to study crucial parts of the process in detail and its behavior in specified parameter ranges for optimization [36].

On the other hand, response surface methodology (RSM) design has been used to evaluate the effect of PEF process parameter (as electric field strength ($E$), treatment time ($t$), pulse width ($\tau$), frequency ($f$), and polarity) over a specific response (microbial or enzyme inactivation). Consequently, the development of mathematical relationships between the response and the independent parameters

are achieved [66]. These relationships allow estimation of the optimum response and the values of the PEF parameters needed to achieve such a response [59].

Different authors have applied RSM design to optimize the effects of PEF treatment over microbial and enzyme inactivation in different foods such as milk and fruit and vegetable juices [2, 3, 59, 83]. Marsellés-Fontanet et al. [59] evaluated the effect of $E$, $f$, and $t$ on a mixture of microorganisms typically present in grape juice by the application of an experimental design based on RSM. They founded that the optimal inactivation of a mixture of microorganisms could be obtained by applying a PEF treatment of 35 kV/cm during 1,000 μs with 303 Hz of frequency. Moreover, the RSM also indicated that inactivation levels were greater for yeast than for bacteria. In a recent study, Aguiló-Aguayo et al. [2] found, by the RSM, that longer PEF treatments resulted in the reduction of polyphenoloxidase (PPO) activity in strawberry juice. Moreover, as reported by the authors, it was feasible to minimize the residual PPO activity (down to 2.5%) by selecting bipolar pulses at $f$ higher than 229 Hz and pulse widths between 3.23 and 4.23 μs for a constant treatment time of 2,000 μs. Additionally, this approach could be applied to optimize PEF treatment based on the retention of bioactive compounds and antioxidant properties of foods. For example, Odriozola-Serrano et al. [68] used an RSM to determine the combined effect of PEF critical parameters on vitamin C, anthocyanins, and antioxidant activity of strawberry juices. The RSM allowed the authors conclude that the strawberry juice antioxidant potential was affected linearly by $f$, pulse width, and polarity. They established that a PEF treatment of 35 kV/cm for 1,000 μs conducted at 232 Hz with bipolar pulses of 1 μs led to strawberry juices with the greatest presence of health-related compounds.

Given the large number of variables involved in a PEF treatment, RSM seems to be a potential mathematical and statistical tool that could be used for accurate prediction of microbial and enzyme inactivation and also to achieve optimal processing conditions for obtaining foods with high nutritional values. Moreover, these types of statistical design allow researchers to reduce the number of assays required for a full factorial design, thereby omitting information that may be unnecessary for statistical conclusions [84]. Food industry could apply this approach in order to optimize PEF process for food preservation.

## Modeling the PEF Process

Modeling and prediction of microbial and enzyme inactivation, as well as changes on the content of bioactive compounds in foods treated by PEF, are interesting concepts that has been under evaluation during the last years. Mathematical models describing the effects caused by PEF are important tools that can be applied in order to: 1) optimize the treatment conditions and efficiency of the process, 2) develop suitable equipments, and 3) obtain microbiologically and enzymatically stable products without over-processing [25, 84]. According to Martín-Belloso and Elez-Martínez [58], models should be as simple as possible and based on understanding the changes caused by PEF. In addition, they should include, or at least reflect, the mechanisms of the changes that take place during the processing in order to facilitate the comparison of such factors as differences in the resistance of target microorganisms or enzymes to PEF and in the efficacy of the PEF equipment [84].

### Modeling Microbial Inactivation by PEF

Based on the results reported during the last years, it has been pointed out that PEF is as effective as thermal treatment for microorganism inactivation in liquid foods such as fruit and vegetable juices, milk, liquid eggs, beer, mixed beverages, among others [11–13, 29, 35, 61, 62, 67–69, 74, 78, 86]. Different authors have mentioned that cell membrane is the main place where the energy of the electric fields is concentrated during PEF processing and its rupture (electroporation) is the principal cause of microbial death [7, 47, 72, 94]. This effect could be reversible or irreversible depending on the treatment intensity, the microorganism characteristics, and the physical–chemical parameters of the media [34].

Along the years, modeling microbial inactivation by PEF has enabled scientist to improve PEF process efficiency for food preservation. The first studies on microbial inactivation by PEF suggested that it followed a first-order kinetics model (Eq. 9), which is one of the simplest approaches [80].

$$S = e^{-kt} \tag{9}$$

where $S$ is the survival fraction, defined as the ratio between the number of survivors and the number of initial microorganisms ($N/N_0$), $t$ is the treatment time, and $k$ is the kinetic constant that depends on the intensity of electric field strength ($E$). This model describes, basically, the log curves of microbial survival fraction and it has been used in defining decimal reduction time values ($D$) for thermal and non-thermal treatments [65]. Nevertheless, the presence of tails and shoulders on inactivation curves makes difficult to establish a fit of this model. Therefore, in order to overcome the limitations of assuming typical first-order kinetics, other models such as the Hülsheger (Eq. 10), the Peleg (Eq. 11), and the Weibull distribution function (Eq. 12), have been also evaluated during the last decades [34].

$$S = \left(\frac{t}{t_c}\right)^{\frac{-(E-E_c)}{k}} \tag{10}$$

$$S = \frac{1}{1 + e^{\frac{E-E_{50}}{k}}} \tag{11}$$

$$S = \exp\left(-\frac{t}{a}\right)^{b} \tag{12}$$

where $t_c$ is the critical treatment time, $E$ is the electric field strength, $E_c$ is the critical electric field strength needed for electroporation, $E_{50}$ is the 50% microorganism survival point, $a$ is a time parameter, and $b$ is a shape parameter defining the concavity of the inactivation curve.

The mathematical model proposed by Hülsheger et al. [51] (Eq. 10) describes the survival curves of different microorganisms supposing a logarithmic dependency between $S$ and $E$ as well a double-logarithmic relation between the $S$ and $t$. Generally, this model indicates that an increase in $E$ could be much more profitable than an increase in $t$. On the other hand, it has been considered that there is a natural variability in microbial populations and each microorganism has specific resistance level to PEF process; hence based on vitalistic conception that individuals in a population are not identical, Peleg [71] proposed a model based on Fermi's equation (Eq. 11). This model includes the characteristic parameters of the microorganisms in order to achieve a better correlation of survival fraction around the critical values. In recent years, the Weibull distribution function (Eq. 12) has gained attention as an alternative model for the description of microbial kinetics. According to van Boekel [91], Weibull distribution is a flexible, yet simple model to describe microbial inactivation. Hence, the main advantages of this model are its simplicity and capability of modeling survival curves that are linear and those that contain shoulder or tailing regions.

Along the years, different authors have applied and compare these models for prediction of microbial inactivation by PEF in different buffer solutions and liquid foods [6, 44, 78]. Generally, their results show that, based on the specific microbial characteristics as well as media properties and treatment conditions, some models fit better than others. Rodrigo et al. [75] evaluated the inactivation of *L. plantarum* in a 0.6% peptone water solution treated by PEF (20–28 kV/m, 30–240 μs). The inactivation data were adjusted to the Hülsheger model and Weibull distribution. The authors observed that Weibull gave better fittings for the inactivation than the Hülsheger model. Likewise, inactivation kinetic of *L. plantarum* inoculated in an orange juice–milk beverage treated by PEF was studied by Sampedro et al. [78]. The authors also evaluated Hülsheger model and Weibull distribution function. Their results indicated that both models fit well the experimental data. Gómez et al. [44] observed that the mathematical model based on Weibull distribution function accurately described the survival curves of *L. monocytogenes* at different pH. The model indicated that, at all electric fields investigated (15, 22 and 28 kV/cm), the microorganism was more sensitive to PEF in media of low pH. Similarly, in a recent study, Saldaña et al. [77] developed mathematical models (Eqs. 13 and 14) based on Weibull distribution to describe the influence of $E$, $t$, and pH on the lethality of *L. monocytogenes* STCC 5672 and *S. aureus* SSTC 4459 after PEF treatments.

$$\log_{10}\frac{N_t}{N_0} = -\left(\frac{t}{10^{(6.94-0.25E-0.07\mathrm{pH}^2+0.03E\mathrm{pH})}}\right)^{0.408+0.302e^{-e^{(-4.573\mathrm{pH}+23.153)}}} \tag{13}$$

$$\log_{10}\frac{N_t}{N_0} = -\left(\frac{t}{10^{(-4.33+4.86\mathrm{pH}-0.48\mathrm{pH}^2-0.15E^2\mathrm{pH}+0.0092E\mathrm{pH}^2)}}\right)^{0.959+1.267e^{-e^{(-41.183\mathrm{pH}+1.389)}}} \tag{14}$$

where, $N_t$ is the number of microorganisms that have survived to the treatment, $N_0$ is the initial number of the microbial population, $t$ is the treatment time (in μs), pH is the pH of the medium, and $E$ is the electric field strength.

As well, their results demonstrate that both microorganisms were more sensitive to media with low pH, although the influence of the pH on the PEF resistance was more significant in *S. aureus*. Another interesting model to accurately describe the inactivation kinetics of microorganisms by PEF is the log-logistic model (Eq. 15) proposed by Cole et al. [19]. Raso et al. [73] affirmed that survivor curves of *Salmonella senftenberg* at different $E$ did not follow first-order kinetics; instead the log-logistic model accurately described the inactivation of *S. senftenberg* by PEF in the range of 12–28 kV/cm. This model is a good alternative for describing microbial inactivation kinetics when logarithm of the survival fraction is not a linear function of $t$ and when a distribution of resistance within the bacterial population occurs.

$$\log S = \alpha + (\omega - \alpha)/1 + \exp[4\sigma(\tau - \log t)/(\omega - \alpha)] \tag{15}$$

Eq. 15 represents a symmetric curve where $\alpha$ is the upper asymptote (log CFU/mL), $\omega$ is the lower asymptote (log

CFU/mL), $\sigma$ is the maximum slope of the log of the survival fraction curve, $\tau$ is the log time when the maximum slope is reached (position of the curve), and $t$ is the treatment time.

In general, developed mathematical models successfully describe the microbial behavior as a function of the changes in process parameters and environmental factors. Throughout the years, scientists have improved the existing mathematical equations and proposed new ones for the establishment of the optimal PEF process conditions to obtain safe and innocuous products. However, most of the models used at present are purely empirical and based on a simple approximation of the experimentally obtained dependences by some of the well-known mathematical equations, without much attention on the actual mechanism that govern microbial inactivation [81]. Therefore, further studies to develop new models for the description of microbial kinetic inactivation by PEF in real foods are needed to assure the best possible yield of microbial inactivation.

## Modeling Enzymatic Activity During PEF Treatment

Food stability not only depends on microbiological control but enzymatic activity has also to be under consideration in order to avoid negative effects on food quality, such as color deterioration, off-odor, off-flavor, changes on viscosity, among others. In general, it has been observed that PEF treatment conditions required for enzyme inactivation are more severe than those required for inactivation of microorganisms. According to Martín-Belloso and Elez-Martínez [58] and Elez-Martínez and Martín-Belloso [28] depending on the enzyme itself, the food characteristics and the treatment conditions and some enzymes are almost completely inactivated by PEF processing, while others show no inactivation or their initial activity is even enhanced. Since enzymes are proteins, their structural stability and catalytic functions are maintained for a complex network of non-covalent and covalent interactions [54]. Therefore, if interactions cause a shift, changes in enzyme activity may occur due to the effects on its three-dimensional molecular structure [93]. Hence, it is believed that the main reason of enzyme inactivation is the denaturation caused by the changes occurred on its conformational state [95, 97]. According to Elez-Martínez and Martín-Belloso [28], owing to the alteration of the enzyme structure, the substrate cannot fit the active site, preventing its conversion into products and resulting in a reduction of the enzyme activity.

Modeling enzyme inactivation by PEF has been carried out by several authors evaluating different empirical models, such as exponential decay, fractional conversion, Fermi's, Hülsheger's, and Weibull's equations to predict well enough the possible enzymatic changes caused by PEF. Hülsheger's (Eq. 16) and Fermi's (Eq. 17) models were used for predicting and describing the evolution of enzyme activity under PEF at different treatment conditions. On one hand, Hülsheger's model describes the decrease in enzyme activity as a function of both $E$ and $t$, whereas Fermi's model describes the level of residual relative enzyme activity only as a function of the $E$ [38].

$$\mathrm{RA} = \mathrm{RA_o} \cdot \left(\frac{t}{t_c}\right)^{-\left(\frac{(E-E_c)}{k_H}\right)} \tag{16}$$

$$\mathrm{RA} = \frac{\mathrm{RA_o}}{1 + \exp\left(\frac{E-E_h}{k_F}\right)} \tag{17}$$

where RA is the residual enzyme activity, $\mathrm{RA_o}$ is the initial residual activity (100%), $E$ is the electric field strength (kV/cm), $E_h$ is a critical level of $E$ where RA is 50%, $k_F$ (kV/cm) indicates the steepness of the curve around $E_h$, $k_H$ is an independent constant factor (kV/cm), and $t_c$ and $E_c$ are the extrapolated critical $t$ and $E$ values for RA equal to 100%.

Other authors have observed that enzyme inactivation by PEF could be also described as an exponential function of $E$ and $t$ (Eqs. 18 and 19).

$$\mathrm{RA} = \mathrm{RA_0} \cdot \exp(-k_E \cdot t) \tag{18}$$

$$\mathrm{RA} = \mathrm{RA_0} \cdot \exp(-k_t \cdot E) \tag{19}$$

where $k_E$ and $k_t$ are the inactivation rate constants.

These models have been applied to describe enzyme inactivation on vegetable [37–40] as well microbial dairy enzymes [11, 14, 15] among others. Giner et al. [38] reported that the classical exponentially decay model as well as Hülsheger's and Fermi's equations adequately described tomato pectin methyl esterase (PME) inactivation by PEF with 400 pulses of 20 μs pulse width at 24 kV/cm. They observed that tomato PME inactivation begins at lower $E$ than microorganism destruction but requires a longer $t$ to start less pronounced depletion of enzymatic activity. In addition, they conclude that Hülsheger's and Fermi's equations provide different information to describe the enzymatic inactivation. Whereas Hülsheger model express minimal values of working conditions ($E$ and $t$) to start the enzyme inhibition, Fermi's parameters correspond to the way tomato PME inactivation occurs. Min et al. [3] evaluated different inactivation models to describe the changes on tomato juice lipoxygenase (LOX) treated by PEF with the combination of $E$ (0–35 kV/cm), $t$ (20–70 μs) and treatment temperature (10–50 °C). Their results showed that the first-order kinetic (Eqs. 20 and 21), Hülsheger's (Eq. 16), Fermi's (Eq. 17), and second-order polynomial equation (Eq. 22) models adequately described the LOX inactivation by PEF.

$$\ln(\mathrm{RA}) = -k_E t \tag{20}$$

$$\ln(\mathrm{RA}) = -k_N E \tag{21}$$

$$\mathrm{RA} = \beta_0 + \beta_1(E) + \beta_2(T) + \beta_3(t) + \beta_{11}(E^2) + \beta_{22}(T^2) + \beta_{33}(t^2) + \beta_{12}(E * T) + \beta_{13}(E * t) + \beta_{23}(T * t) \tag{22}$$

where $k_N$ is the first-order inactivation constant, $T$ is the temperature ( °C), and $\beta$ terms represent regression coefficients estimated by the least squares method with a confidence level of 95%.

Through these models the authors were able to conclude that $E$ was the primary variable affecting the inactivation of LOX. Another study carried out by Elez-Martínez et al. [26] compare the application of a first-order kinetic model, a first-order fractional conversion model (Eq. 23) and the Weibull distribution function to kinetically describe the inactivation of orange juice peroxidase (POD) by HIPEF at different treatment conditions ($E$ = 5–35 kV/cm, $t$ = 1,500 μs, $f$ = 50–450 Hz, $\tau$ = 1–10 μs in mono and bipolar fashion).

$$\frac{\mathrm{RA} - \mathrm{RA}_\infty}{\mathrm{RA}_o - \mathrm{RA}_\infty} = \exp(-k \cdot P) \tag{23}$$

where $\mathrm{RA}_\infty$ (%) is the residual enzyme activity after a prolonged time of treatment, $\mathrm{RA}_o$ is the initial residual activity (100%), $k$ is a first-order rate constant ($\mu^{-1}$), and $P$ is the treatment time ($t$, μs), the pulse frequency ($f$, Hz), the pulse width ($\tau$, μs), or the electrical energy density input ($Q$, MJ/m$^3$).

Their results showed that the first-order kinetic model appeared to be insufficient to model the inactivation of the enzyme as a function of $t$. The first-order fractional conversion model fitted the experimental data with good agreement and predicted POD inactivation by PEF with acceptable accuracy. However, the Weibull distribution function displayed high determination coefficients and fit the experimental data better than the other models as a function of the majority of PEF parameters evaluated. Moreover, the authors concluded that reduction of POD activity related to $E$ could also be well described by de Fermi's model. Later on, Elez-Martínez and Martín-Belloso [27, 28] showed that the first-order fractional conversion (Eq. 23), Fermi's (Eq. 19), and Hülsheger's (Eq. 18) models described with enough accuracy the orange juice PME inactivation by PEF as a function the individual $t$, $E$ and the combination of $t$ and $E$, respectively. In addition, the authors observed that orange juice PME activity was well related to $f$, $\tau$ or energy density ($Q$) by the first-order fractional conversion model (Eq. 23). Based on the characteristics parameters of Weibull distribution function, Fermi's and Hülsheger's models, the authors confirmed that enzymatic inactivation by PEF is mainly affected by PEF treatment conditions and the enzyme source.

Giner et al. [41] proposed a Bayesian framework to solve overfitting problems found in the traditional empirical models used up to now and to extract more information from the data. Bayesian modeling deals with uncertainty in an inherent way and provides a flexible framework for choosing one of a number of models. In addition this new method is able to describe satisfactorily most of the observed variability and measurement uncertainties for PEF parameters and influencing factors in a unique stage [41]. The authors stated that the Bayesian procedure and a bi-exponential model based on a kinetic mechanism involving two consecutive first-order steps could become helpful tools to analyze data and model the evolution of the enzymes under PEF [42]. Recently, these authors [43] used the Bayesian framework (Fig. 4) to study the inactivation of PME in *gazpacho* (Mediterranean vegetable soup) treated by PEF. The results showed that PEF was greater when $t$ and $E$ increased, and bipolar pulses were more effective inactivating PME than monopolar ones. The kinetic evolution of the enzyme was explained using 3-parameter bi-exponential model (Eq. 24) based on a mechanism involving two irreversible consecutive steps.

$$\mathrm{RA} = \mathrm{RA}_0 \left[ e^{k_1 t} - \frac{k_1 \cdot \Lambda}{(k_1 - k_2)} (e^{-k_1 t} - e^{-k_2 t}) \right] \tag{24}$$

where $\mathrm{RA}_0$ is the RA at initial time ($\mathrm{RA}_0$ = 100), $t$ is the total treatment time (μs), $k_1$ and $k_2$ (μs$^{-2}$) are the kinetic constants for the first and second steps, respectively, and $\Lambda$ is the ratio between the activities of the intermediate forms of enzyme and the native ones.

The authors concluded that despite this model is more complex than others with less parameters, it adds more flexibility and accuracy. In addition, more useful information is obtained for the establishment of treatment conditions and PEF equipments design than the information provided by traditional models.

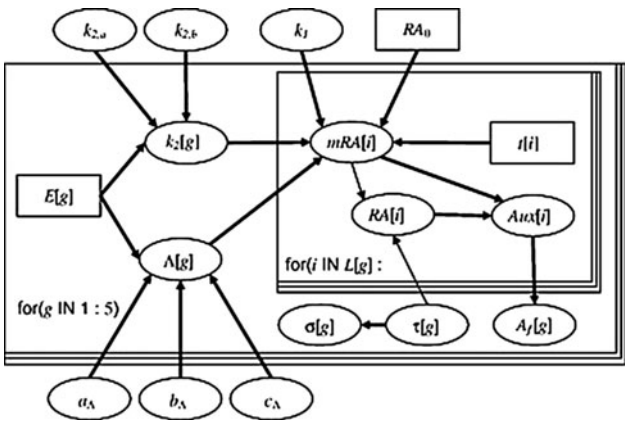


**Fig. 4** Directed acyclic graph model for the proposed kinetic mechanism. From Guiner-Seguí et al. [43]

Taking into account all of these results, it can be said that modeling PEF effectiveness against enzymes mainly depend on processing conditions, the enzyme itself, and media and source characteristics. Similar to the studies reported for microorganisms, most of the models applied for predicting enzyme inactivation kinetics have contributed to the development of PEF technology in the last years. However, some occurrences cannot be adequately explained through all of these models or do not grasp satisfactorily the mechanism by which PEF inactivation enzymes takes place and therefore other approaches and models should be explored [42].

### Modeling Effect of PEF on Bioactive Compounds

Being a non-thermal process, it is believed that PEF can be a feasible way of assuring pasteurization treatments without substantially affecting the nutritional composition of foods [85]. During the last few years, interesting research has been carried out about how PEF processing affects the content of bioactive compounds in different foods such as fruit and vegetable juices, milk, and mixed beverages containing fruit juices and milk or soymilk, among others [13, 20–22, 62, 67, 68, 79, 90, 98]. The results obtained from these researches have demonstrated that PEF technology allows high retention of several bioactive compounds after processing, leading to processed foods with high nutritional values. Nevertheless, bioactive compound retention levels depend on treatment intensity, the food intrinsic characteristics as well as the specific compound under study.

Considering consumers' claims for healthy food products, the retention of bioactive components in a PEF-processed food can also be a limiting factor when defining processing conditions. Hence, the application of mathematical models for predicting the changes on the concentration of health-related compounds as a function of PEF process has been analyzed during the last years. However, these studies are few compared to the works focused on microbiological or enzymatic inactivation,

Earliest studies about modeling changes on the concentration of bioactive compounds in PEF-treated foods were carried out by Bendicho et al. [11]. The authors reported that retention of ascorbic acid in PEF-treated milk fitted a first-order kinetic model (Eq. 25). Their results showed that the decrease in ascorbic acid content after applying PEF treatment was more significantly affected by the increase in $E$ than the increase in other PEF parameters. They observed that at any $E$ applied, a higher $t$ resulted in greater reduction of ascorbic acid. Similarly, Zhang et al. [96] reported that the degradation kinetic of cyanidin-3-glucoside exposed to PEF was well fitted to a first-order reaction. Later on, Odriozola-Serrano et al. [69] applied different mathematical models to evaluate the changes of anthocyanins, vitamin C, and antioxidant capacity of strawberry juices subjected to $E$ from 20 to 35 kV/cm for up to 2,000 μs applying 1 μs bipolar pulses at 232 Hz. Their results showed that the Weibull kinetic model (Eq. 26) was the most accurate to predict the health-related compound changes in strawberry juice based on $t$ (Fig. 5). On the other hand, the combined effect of $E$ and $t$ was successfully predicted by secondary expressions. In addition, the authors reported that a model previously used to describe moisture sorption processes (Eq. 27) was the most accurate for describing lycopene changes during PEF processing of tomato juices.

$$\mathrm{RBC} = \mathrm{RBC}_0 \cdot \exp(-k \cdot t) \tag{25}$$

$$\mathrm{RBC} = \mathrm{RBC}_0 \exp\left[-\left(\frac{t}{\alpha}\right)^{\gamma}\right] \tag{26}$$

$$\mathrm{RC} = \mathrm{RC}_0 + \frac{t}{K_1 + K_2 + t} \tag{27}$$

where RBC (%) is the relative bioactive compound, $\mathrm{RBC}_0$ (%) is the intercept of curve, $k$ is the first-order kinetic constant ($\mu^{-1}$), $t$ is the treatment time (μs), $\alpha$ is the scale factor (μs), $\gamma$ is the shape parameter that indicates concavity (tail-forming) or convexity (shoulder forming) of the curve when it take values below and above 1, respectively, RC (%) is the relative lycopene content, $\mathrm{RC}_0$ (%) is the intercept of the curve, $K_1$ (μs/%) is the Peleg rate constant indicating the lycopene formation rate at initial treatment time, and $K_2$ is the Peleg capacity constant, which is related to the steady value reached for prolonged treatment times.

According to Bendicho et al. [11], Zhang et al. [96] and Odriozola-Serrano et al. [69], the proposed model could be very useful to predict the variation of the antioxidant potential of food products with the key parameters involved in PEF treatments. Nevertheless, more mathematical models are needed to be evaluated to achieve a better understanding on the mechanisms of the health-related compound concentration changes during PEF processing.

## PEF Scale-Up Challenges

In order to scale-up PEF technology at industrial levels, process evaluation considering engineering, microbiological, and quality aspects, in addition to a deeply study related to PEF implementation costs and investment are necessary. This kind of analyses requires accurate data about the process conditions needed to obtain microbiologically safe products and avoid overprocessing and its related detrimental effects on product quality. In addition, other aspects such as product characteristics as well as the available facilities for plant installation are also important

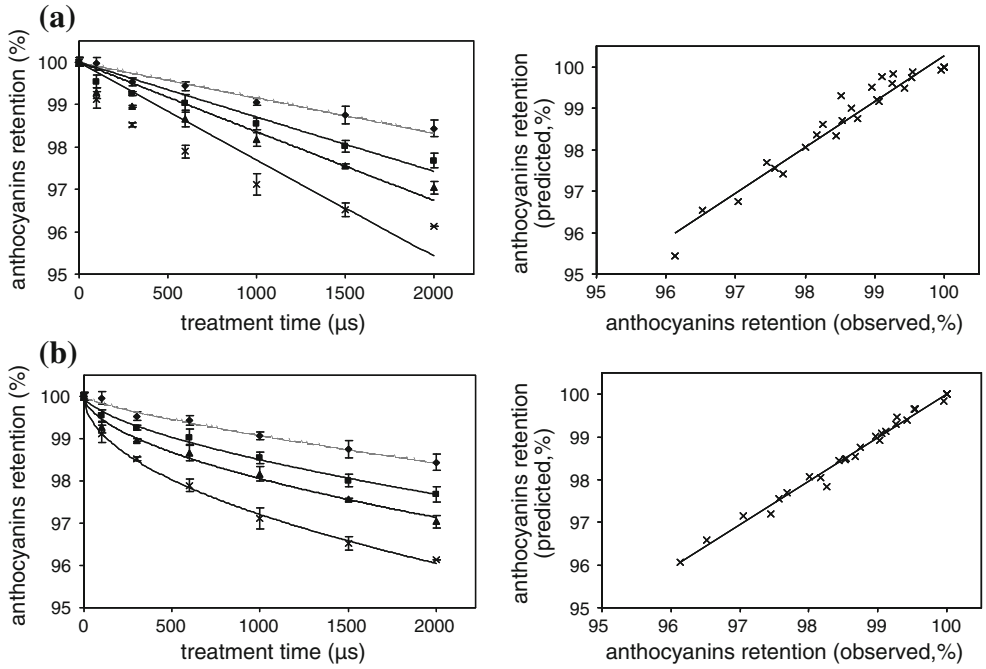


**Fig. 5** Effect of treatment time and electric field strength on the anthocyanin retention of strawberry juice (mean ± SD) as modeled by the first-order model (**a**) and Weibull approach (**b**). From Odriozola-Serrano [67]

to carry out the evaluation. Up to now, a lot of research in the field of PEF engineering has been done; however, existing information focused on the development of PEF units at industrial levels and economical analysis of its implementation is scarce. In this aspect, Hoogland and de Haan [49], Barbosa-Cánovas and Altunakar [9] and Toepfl et al. [87, 88] have performed some interesting analyses.

At present, PEF systems for food preservation at industrial scale are not in the market. In this way, PEF equipments are rare and expensive, resulting in elevated investment costs compared with other processes. One of the main limitations for PEF processing large quantities of fluid is the generation of high-voltages pulses with sufficient peak power to obtain safe products. Therefore, this is one important challenge for researchers in order to make commercial processing available in the near future.

As industrial systems are not available, costs can only be estimated based on data accessible from other pulse power applications, or scaled up from pilot-scale equipments [87, 88]. Hoogland and Haan [49] reported an economic comparison of PEF process and an in-line thermal treatment. A PEF system design was made for a unit of 5,000 l/h. It was concluded that the installation of the above-mentioned PEF system would be roughly 1 million Euros, which is more expensive than a standard thermal processing plant. In a previous study, Braakman [16] reported an investment cost in the range of 2 million Euros for a flow capacity of 5 ton/h or 4 million Euros for 10 ton/h capacity. Barbosa-Cánovas and Altunakar [9] affirmed that the major concern for commercialization of PEF technology is the initial investment cost. Nonetheless, if there were more companies dedicated to the design of PEF equipments and a high number of industrial equipments installed, the cost of each system would dramatically decrease, making its implementation more accessible.

Moreover, according to the different studies carried out up to now, it could be considered that with proper equipment, PEF is an energy efficient process compared to thermal pasteurization. Operation cost for a liter of PEF-treated product was estimated around 0.8 Eurocents [49]. These operation costs are proved to be lower than traditional thermal processing technologies [8]. In addition, as PEF is nearly a non-thermal process, the cost for cleaning and downtime will be less. Furthermore, one of the main advantages of this novel process is the consumer perception of the food quality improvement by applying the right process. Therefore, better quality characteristics of PEF-processed foods than those heat treated are offered to consumers. In this way, while investments for a PEF industrial process are relatively large, the benefits may outweigh the depreciation costs, especially by the reduction of downtime and value added products.

## Final Remarks

PEF technology offers potential alternatives to the food industry to accomplish preservation process with optimal

results and could serve as a complementary process in food preservation treatments. This fact has motivated researchers from both academy and industry to explore PEF treatment as a food preservation process. High PEF efficiency can be attained by optimal design of treatment chambers. The strength of applied numerical simulation to investigate PEF process lies in its capability to provide detailed information about the effects inside the treatment chambers, which allow the optimization of the process. Modeling the effects of PEF over microbial or enzyme inactivation, as well on the content of health-related compounds, has become a helpful tool that enables the establishment of optimal processing variables to obtain the best results. Nevertheless, the scaling up of PEF systems, their high initial cost, and the availability of commercial units are the major constrain confronting the usage of this technology. However, it is considered as an attractive process for its lower operation costs and value added products. Hence, more research on the use of PEF for food preservation to overcome hurdles should be faced to make commercial units available and ensure that the process is completely safe at the same time that economically convenient.

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