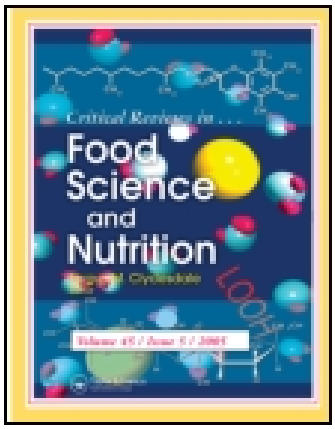

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Microbiological Aspects Related to the Feasibility of PEF Technology for Food Pasteurization

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Microbiological Aspects Related to the Feasibility of PEF Technology for Food Pasteurization

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Processing unit operations that seek to inactivate harmful microorganisms are of primary importance in ascertaining the safety of food. The capability of pulsed electric fields (PEF) to inactivate vegetative cells of microorganisms at temperatures below those used in thermal processing makes this technology very attractive as a nonthermal pasteurization process for the food industry. Commercial exploitation of this technology for food pasteurization requires the identification of the most PEF-resistant microorganisms that are of concern to public health. Then, the treatment conditions applicable at industrial scale that would reduce the population of these microorganisms to a level that guarantees food safety must be defined. The objective of this paper is to critically compile recent, relevant knowledge with the purpose of enhancing the feasibility of using PEF technology for food pasteurization and underlining the required research for designing PEF pasteurization processes.

Keywords Pasteurization, pulsed electric fields, microbial inactivation, nonthermal processing

INTRODUCTION

Traditionally, the objectives of food preservation were to extend the foods' shelf life and to provide a safe product. Today, the food industry is expected to offer safe foods, but it also prevents or reduces the negative impact of the preservation techniques on valuable food compounds (da Cruz et al., 2010). Various new food preservation technologies have recently come under investigation by the food industry to meet consumers' demands for nutritious, fresh food products with high organoleptical properties (Rajkovic et al., 2010).

The application of nonthermal processes for food preservation is a technological response to the undesirable changes induced by thermal processing in food (Arnoldi, 2002). Nonthermal technologies are processes that are capable of inactivating microorganisms at temperatures below those normally used in thermal processing (Barbosa-Cánovas et al., 2005). One such nonthermal method is pulsed electric field technology (PEF), which exposes the food to intermittent, high-intensity electric fields for short periods of time (μs). A number of researchers have demonstrated that the application of these treatments provokes an electromechanical instability in the cell membrane that modifies its permeability, causing the inactivation of vegetative forms of bacteria, yeast, and molds (Saulis, 2010). However, this technology fails to inactivate bacterial spores, probably because of the spores' envelopes, such as the coat and the cortex, prevent the permeabilization effects of PEF on the spore cytoplasmatic membrane (Pagán et al., 1998). As bacterial spores are resistant to PEF treatments, the primary applications of this technology in food preservation must focus on pasteurization. Traditionally, pasteurization has been based on thermal processing but, in recent years, it has been demonstrated that there is potential for several nonthermal technologies to obtain the same goal (Barbosa-Cánovas and Bermúdez-Aguirre, 2011). As a consequence, pasteurization has been recently redefined by the National Advisory Committee on Microbiological Criteria for Foods (NACMCF) as "*Any process, treatment, or combination thereof that is applied to food to reduce the most resistant microorganism(s) of public health significance to a level that is not likely to present a public health risk under normal conditions of distribution and storage*." (NACMCF, 2006). Therefore, this definition considers new alternatives for pasteurization of foods such as PEF.

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Table 1 Guidelines recommended by the National Advisory Committee on Microbiological Criteria for Foods (NACMCF) for developing a pasteurization process

- Identify the microorganism (s) of public health concern for the food.
- Determine the most resistant pathogen of public health concern that is likely to survive the process.
- Assess the level of required inactivation.
- Consider the impact of the food matrix on pathogen survival.
- Validate the efficacy of the pasteurization process.
- Define the specific equipment and operating parameters for the proposed pasteurization process.

Although the main objective of PEF pasteurization is to guarantee food safety, a large proportion of the population of vegetative spoilage microorganisms is also inactivated by the treatment. Therefore, PEF treatments may extend the shelf life of foods (El-Hag et al., 2006; Min et al., 2007). However, the treatment is not capable of achieving commercial sterility because spores or other nonpublic health significant microorganisms can be present; thus, other preservation techniques, such as refrigeration, atmosphere modification, the addition of preservatives, or a combination of these techniques, will be required to preserve the quality and stability of the food during its distribution and storage (Raso and Barbosa-Cánovas, 2003).

The PEF is gaining interest as a gentle method of food preservation. However, this technology has not been implemented by the food industry so far. Commercial exploitation of PEF for food pasteurization requires proof that PEF promotes a level of microbial safety that is equal to that made possible via traditional processing. Therefore, to establish the efficacy and equivalence of alternative methods of pasteurization, studies that have been conducted with microorganisms of public health concern are required (Table 1). Another aspect for the food industry to consider regarding implementation of PEF technology is that the treatment conditions needed to guarantee food safety should be applicable on a commercial scale. In recent years, different liquid foods have been treated with laboratory and pilot plant-scale, continuous-flow PEF systems to evaluate the impact of PEF treatments on microbial inactivation, food physicochemical and nutritional properties, and food shelf life (Min et al., 2003; Aguiló-Aguayo et al., 2008; Cortés et al., 2008). However, most of these studies generally do not permit an evaluation of the feasibility of PEF for food pasteurization, because they have not been conducted with target microorganisms of public health concern.

The objective of this paper is to critically compile recent, relevant knowledge to present the applicability of PEF technology for food pasteurization according to the guidelines shown in Table 1. This work also seeks to underline the required research for designing PEF pasteurization processes. Therefore, this review will primarily focus on data obtained on the inactivation of pathogenic microorganisms that may represent a public health risk under commercially applicable conditions.

THE SELECTION OF STRAINS OF PATHOGENIC MICROORGANISMS TO DESIGN PASTEURIZATION PROCESSES BY PEF

To establish the treatment conditions for PEF food pasteurization, the first step is to identify the most PEF-resistant microorganisms that have an impact on public health. This identification depends on the intrinsic PEF resistance of the microbes. It may also be influenced by other factors that can affect microbial resistance, such as the treatment medium characteristics or the physiological state of the cells (Aronsson and Rönner, 2001; Cebrián, et al., 2007).

Researchers have observed that there is no relationship between microbial resistance and the inactivation procedure that is used for microbial destruction. For example, *Salmonella senftenberg* 775W, which is a very heat-resistant microorganism, is more sensitive to high hydrostatic pressure or PEF than other heat-sensitive *Salmonellae* (Metrick et al., 1989; Álvarez et al., 2000). Therefore, the target microorganism for PEF pasteurization is not necessarily the same as the target microorganism for thermal pasteurization or other inactivation treatments.

Efficacy of PEF against pathogenic microorganisms was generally based on single-strain studies; consequently, there was limited information available in the literature on variations of resistance to PEF among strains of the same microorganism. There were few studies which demonstrated that the PEF resistance of different strains of bacterial species may vary greatly. Inactivation of nine *Listeria monocytogenes* strains by a PEF treatment (25 kV/cm, 144 μs) ranged from 0.7 to 3.7 $\log_{10}$ CFU/ml (Lado and Yousef, 2003). Inactivation of 15 *Staphylococcus aureus* strains by a PEF treatment (22 kV/cm, 200 μs) ranged from 1.2 to 4.0 $\log_{10}$ CFU/ml (Rodríguez-Calleja et al., 2006). Saldaña et al. (2009) conducted one of the most complete studies on the variability in PEF resistance of different strains of pathogenic microorganisms to identify potential target microorganisms for designing PEF pasteurization processes. The screening study confirmed that the PEF resistance of five strains of *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli,* and *Salmonella* Typhimurium may vary significantly. As the most resistant strains at low pH are not necessarily the most resistant at neutral pH, the target microorganisms for PEF pasteurization could be expected to be different for foods, depending on their pH (García et al., 2005). Table 2 compares the resistance of the most PEF-resistant strains found in the screening study with published data and shows that these strains are especially PEF resistant. Thus, they should be considered as possible target microorganisms in future research.

To conduct challenge studies for plant validation of processes in industries, surrogate microorganisms in place of target pathogens are used. Surrogates are nonpathogenic strains that respond to a particular treatment in a manner equivalent to a pathogenic species. Although surrogates for thermal processing have been selected, at the moment few studies with this purpose for PEF processing have been published. For example, Gurtler et al. (2010) proposed the nonpathogenic *E. coli* 35128 in place

Table 2 Comparison of the PEF resistance of different pathogenic microorganisms

Pathogen	Treatment media	Treatment conditions	Log reduction	Reference
E. coli O157:H7	McIlvaine buffer pH 4.0	30 kV/cm; 150 μs; 24°C	0.5	Saldaña et al. (2009)
	McIlvaine buffer pH 7.0	30 kV/cm; 150 μs; 24°C	1.7	
	Apple juice	34 kV/cm, 166 μs, 30°C	4.0	Evrendilek et al. (2000)
	Apple juice	31 kV/cm, 202 μs, 30°C	2.6	Evrendilek and Zhang (2005)
	Apple juice	32.2 kV/cm, 35°C	4.96	Ukuku et al. (2010)
	Liquid egg yolk	30 kV/cm, 310 μs, 40°C	4.9	Amiali et al. (2007)
L. monocytogenes STCC 5672	McIlvaine buffer pH 4.0	30 kV/cm; 150 μs; 24°C	1.5	Saldaña et al. (2009)
	McIlvaine buffer pH 7.0	30 kV/cm; 150 μs; 24°C	0.6	
L. monocytogenes	Melon juice	35 kV/cm, 2000 μs, 39°C	4.3	Mosqueda-Melgar, et al. (2007)
	Milk	30 kV/cm, 300 μs	3.5	Reina et al. (1998)
S. aureus STCC 4459	McIlvaine buffer pH 4.0	25 kV/cm; 150 μs; 24°C	2.6	Saldaña et al. (2009)
	McIlvaine buffer pH 7.0	25 kV/cm; 150 μs; 24°C	2.0	
	LWE	25 kV/cm, 60 μs, 24°C	1.5	Monfort et al. (2010)
S. aureus	Skim milk	25 kV/cm, 450 μs	3.3	Evrendilek et al. (2004)
	Skim milk	31 kV/cm, 6 μs	3.0	Sobrino-López et al. (2006)
	Orange juice	20 kV/cm, 150 μs	2.0	Walkling-Ribeiro et al. (2009)
Salmonella Typhimurium STCC 878	McIlvaine buffer pH 4.0	30 kV/cm; 150 μs; 24°C	4.2	Saldaña et al. (2009)
	McIlvaine buffer pH 7.0	30 kV/cm; 150 μs; 24°C	3.6	
	LWE	30 kV/cm; 50 μs; 24°C	2.0	Monfort et al. (2010)
Salmonella Typhimurium	Juice-milk based	35 kV/cm, 350 μs	2.0	Sampedro et al. (2011)
	McIlvaine buffer pH 7.0	28 kV/cm, 150 μs; 24°C	3.2	Álvarez et al. (2003)

of *E. coli* O157:H7 for pilot plant challenge studies. As the selection of surrogate bacteria may depend on the food of interest, further studies will be required to identify nonpathogenic surrogates to validate PEF pasteurization of other products by challenge studies.

FOOD SAFETY OBJECTIVE, PERFORMANCE CRITERION, AND PROCESS CRITERION CONCEPTS TO DESIGN PEF PASTEURIZATION PROCESSES

Once the target microorganisms have been identified, the next step in designing a pasteurization process is to establish the frequency or maximum concentration of the microorganism that is considered acceptable for public protection. This concept is called *Food Safety Objective* (FSO) (International Commission on Microbiological Specifications for Foods [ICMSF], 2002). The FSO should be established by regulatory agencies, and it is based on the infectious dose of the target microorganism, as well as its ability to grow in the food under normal distribution and storage conditions.

Reducing the target microorganism to an appropriate level to protect public health is dependent upon its initial cell number in raw materials before pasteurization, taking into consideration the normal variation in concentration and the FSO. This reduction in the target microorganism is known as *performance criterion*, which is the required outcome of a step or a combination of steps that can be applied to ensure an FSO (ICMSF, 2002). An example of a performance criterion is the required 5 Log_{10} reduction of the most resistant microorganism of public health significance established by the US Food and Drug Administration (FDA) concerning juice pasteurization (FDA, 2001). Traditionally, different reduction values have been defined for thermal pasteurization of various foods, depending on the pathogen of concern (IFT, 2004). Because studies have demonstrated that these reduction values afford an appropriate level of public health protection, they could also be used as a reference for pasteurization by PEF or other novel technologies. FSO and/or *performance criterion* that should be established by regulatory agencies for food/pathogen combination could be useful to judge the equivalency when a new technology is proposed as an alternative to traditional pasteurization (NACMCF, 2006).

To achieve a successful use of PEF as a pasteurization process, it is critical to demonstrate that it is possible to reach the performance criterion in the target microorganisms. The control parameters that must be applied to achieve a performance criterion are referred to as *process criterion* (ICMSF, 2002). Before defining *process criterion* it is necessary to identify the control parameters that influence the efficacy of the inactivating technology.

Despite the fact that many studies have been done, the numerous critical process factors involved broad experimental conditions used for researchers and diversity of equipment operating in different laboratories have made difficult obtaining general conclusions on the main control parameters affecting microbial inactivation by PEF (Wouters et al., 2001).

Presently, it is known that electric field strength, treatment time, specific energy, pulse shape, and frequency are the main processing parameters that potentially may influence the microbial resistance to PEF (Min et al., 2007; Ortega-Rivas, 2011).

There is general agreement in published literature that electric field strength and treatment time are the most important processing parameters that characterize PEF technology. To induce microbial inactivation, it is necessary to apply an electric field

with strength above a critical value. Microbial inactivation is strengthened by increasing the intensity of the electric field and treatment time when treatments are applied above the critical value. The specific energy of the PEF treatment depends on the voltage that is applied, treatment time, and electrical resistance of the treatment chamber, which varies according to its geometry and the electrical conductivity of the food. This parameter reveals the energy cost of the process and comparison of the energy requirements of different preservation systems (Heinz et al., 2003). The use of this parameter instead of the treatment time has been proposed as a process criterion because it is difficult to define the treatment time when exponential decay pulses are used (Heinz et al., 2001; Álvarez et al., 2003b).

There is some controversy concerning the influence of the pulse shape, width, and frequency on PEF microbial inactivation. Presently, it is generally accepted that square wave pulses are better than exponential decay ones. Although some authors have observed a slightly higher inactivation with longer pulses and higher frequencies (Wouters et al., 1999; Aronsson and Rönner, 2001), these two parameters apparently exert no influence when the temperature rise of the medium caused by the application of longer pulses of higher frequencies is avoided (Álvarez et al., 2003a). Therefore, the most suitable key process parameters related to the pulse generation system to describe PEF treatments are electric field strength and treatment time or specific energy. These parameters should be considered in the development of predictive models that are useful tools in the product development and process design to define the *process criterion* with a similar inactivation effect to achieve the *performance criterion* and to establish the requirements that the PEF equipment must meet to apply the treatment on a commercial scale. This information is very useful to conduct cost analysis of the processing options so that the production can run as economically as possible while delivering a microbiologically safe product to the consumer (Bull et al., 2005).

Some of the first investigations on microbial inactivation by PEF suggested a primary model to describe the survival curves the first-order kinetics (Martín-Belloso et al., 1997; Heinz et al., 1999; Reina et al., 1998; Amiali et al., 2007; Pérez et al., 2007). However, these linear relationships were observed for short treatment times. When the treatment time is prolonged to achieve higher inactivation, the shape of the survival curves is generally concave upward. Over the years, several equations have been proposed to describe these survival curves that correspond to PEF inactivation, such as Hülsheger's model (Hülsheger et al., 1981; Zhong et al., 2005; Rivas et al., 2006; San-Martin et al., 2007) and Fermi's model (Sensoy et al., 1997; San-Martin et al., 2007). However, an equation based on the Weibull distribution is the most frequently used due to its simplicity and flexibility (Rodrigo et al., 2001; Alvarez et al., 2003c; Rivas et al., 2006; Sampedro et al., 2006; Fox, 2007; Monfort et al., 2010).

Generally, the secondary models that are used to describe the microbial inactivation by PEF are based on quadratic equations whose complexity increases with the number of processing variables investigated and the experimental range considered. Recently, the combination of experimental design techniques with multiple regression analysis and Monte Carlo simulation have been used to establish the most influential factors on the inactivation of pathogenic microorganisms by PEF (Mosqueda-Melgar et al., 2007; Marsellés-Fontanet et al., 2009; Sampedro et al., 2011; Walkling-Ribeiro et al., 2011). As these approaches also generate complex equations, the development of user-friendly software applications could facilitate the employment of mathematical models to design PEF pasteurization treatments by technicians in the food industry.

Mathematical models are especially useful for defining treatment conditions when the influence of several factors on the microbial behavior is investigated under a wide range of treatment conditions. From a practical point of view, PEF treatments should be evaluated while considering their commercial applicability. Figure 1 compares, as an example, the resistance of four PEF-resistant pathogenic strains under treatment conditions commercially applicable in media of different pH. The inactivation achieved in the two more resistant strains, *L. monocytogenes* 5672 and *E. coli* O157:H7 (0.5 to 3 Log_{10} cycles), was too low to reach an appropriate level of public health protection. Therefore, approaches such as considering thermal effects on PEF lethality or combining PEF with other preservation methods are required to achieve the sufficient microbial destruction needed to design PEF pasteurization processes.

STRATEGIES TO INCREASE THE LETHALITY OF PEF TREATMENTS

Influence of Temperature on Microbial Inactivation by PEF

Results reported in the literature show that microbial inactivation by PEF is usually enhanced when the temperature of the treatment medium is increased, even in ranges of temperatures that are not lethal for microorganisms (Sepúlveda et al., 2005; Sampedro et al., 2006; Amiali et al., 2007; Fox et al., 2008; Gurtler et al., 2010; McNamee et al., 2010). This effect has been attributed to changes in the phospholipid bilayer structure of the cell membranes, from a gel-like consistency to a liquid crystalline state that is caused by the temperature increase. The increased membrane fluidity reduces its stability and facilities the electroporation caused by PEF in the cell membrane (Jayaram et al., 1991).

Generally, investigations into the influence of temperature on microbial inactivation by PEF have been performed in continuous flow treatment chambers (Heinz et al., 2003; Sepúlveda et al., 2005; Amiali et al., 2007). In these studies, the outlet temperature was variable and depended on the inlet temperature and total specific energy applied to the product into the treatment chamber. Although the PEF treatment is considered a nonthermal food processing technology, temperature may increase significantly during high intensity PEF treatments due

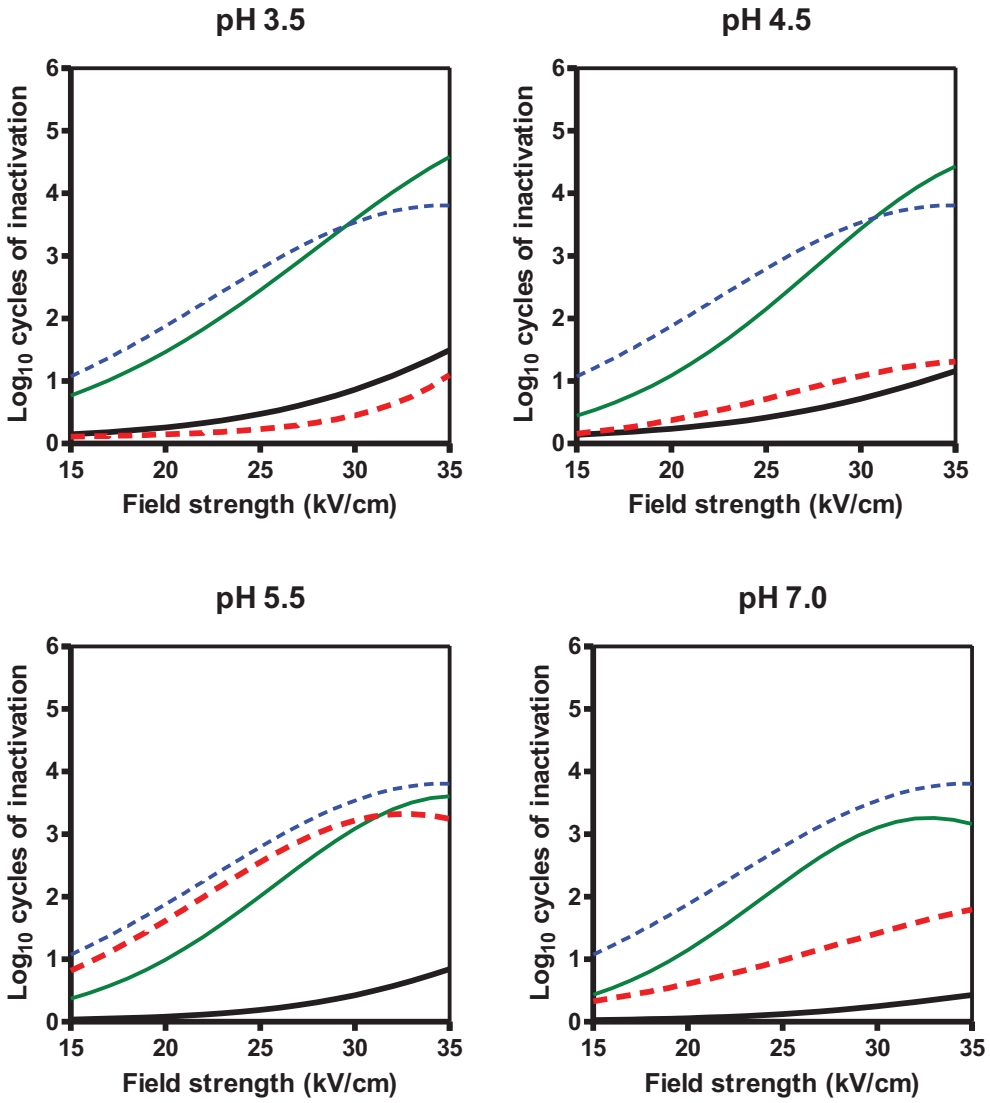

Figure 1 Log_{10} cycles of microbial inactivation by PEF treatments of 100 μs at different field strengths in media of distinct pH estimated by tertiary models. *L. monocytogenes* 5672 (▬), *E. coli* O157:H7 (▬ ▬), *S. aureus* 4459 (—), *Salmonella* Typhimurium 878 (…). (Color figure available online.)

to the thermal energy that is dissipated in the product because of its resistance to the current flow. The application of numerical simulation techniques have demonstrated that an extensive distribution of temperatures can exist in the treatment chamber during continuous processing due to inhomogeneities in the electric field distribution, especially when colinear treatment chamber configurations are used (Buckow et al., 2010, 2011). So, in a continuous process, it is difficult to know the exact process temperature and, as a consequence, quantify the effect of temperature. A better understanding of the influence of the temperature on PEF microbial inactivation has been obtained by designing a static parallel-electrode treatment chamber with tempered electrodes that permitted researchers to obtain data at different temperatures under quasi-isothermal conditions and uniform electric field strength (Saldaña et al., 2010a). Using this treatment chamber, it has been demonstrated that the microbial inactivation by PEF is highly dependent on the temperature, even when the treatments are applied at temperatures that are not lethal for the microorganisms (Fig. 2). Therefore, the temperature during treatment is a critical parameter that should be considered in future studies to characterize microbial PEF resistance and to obtain reliable kinetic data to design predictive models.

A new processing concept that is based on using the ohmic heating due to the electric energy dissipation that occurs during the PEF processing to increase the microbial lethality has been proposed for apple juice (Heinz et al., 2003) and milk (Guerrero-Beltrán et al., 2010) pasteurization. In this approach, treatment conditions are selected to keep treatment temperatures above those that have lethal effects on microorganisms (e.g., up to 70–72°C). Although temperatures similar to those used in thermal processing are reached during the treatment, the main

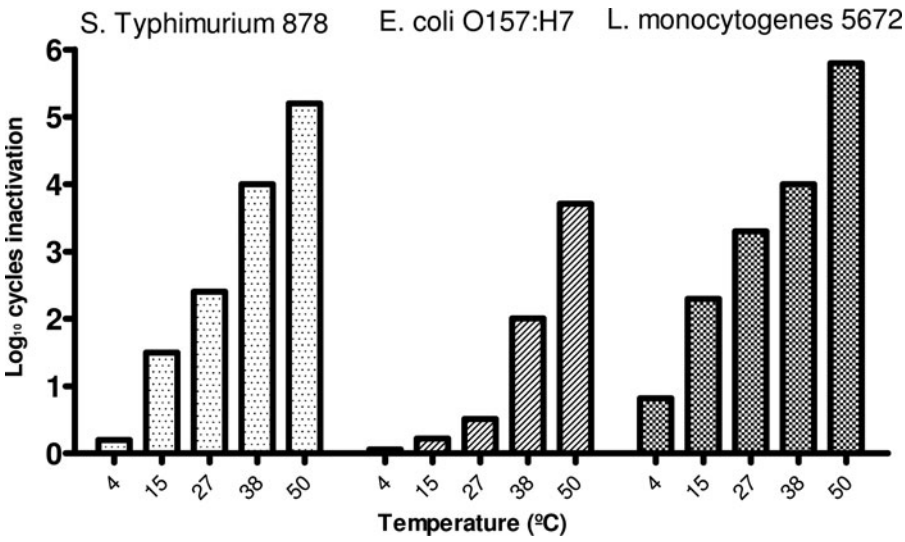

Figure 2 Influence of the treatment temperature on the inactivation of *Salmonella* Typhymurium 878, *E. coli* O157:H7, and *L. monocytogenes* 5672 after PEF treatments at quasi-isothermal conditions. PEF conditions: 30 kV/cm, 99 μs, pH of the treatment medium 3.5.

advantage of this process is that the extremely short residence times required to reach the temperatures that are joined to the inactivation effect caused the PEF treatment results in a drastic reduction of processing time and thermal load. Although the efficacy of this PEF processing concept has been investigated for microbial inactivation studies, it is necessary to identify the advantages of this process against traditional thermal processing in terms of impact on nutritional compounds and sensorial properties.

Combining PEF with Other Preservation Methods in the Design of Pasteurization Processes

Combining PEF with other preservation methods has been widely investigated to increase the lethal effect of PEF treatments to levels that guarantee food safety and to reduce electrical energy costs. PEF, in combination with other physical methods of microbial inactivation based on thermal and nonthermal effects (i.e., high hydrostatic pressure, high-pressure carbon dioxide, ultrasound, ultraviolet radiation, or high-intensity light pulses), has proven to be effective to enhance microbial inactivation (Martín-Belloso and Sobrino-López, 2011). Generally, the combination of these treatments consisted in a successive application of hurdles (Noci et al., 2009; Walkling-Ribero et al., 2009) but, in some cases, PEF has been applied simultaneously with high hydrostatic pressure (Heinz and Knorr, 2000). Inactivation reported by combining PEF with these other inactivation techniques has proven to cause microbial reductions higher than 5 Log_{10} cycles in different pathogenic microorganisms. However, the use of PEF treatments of very long duration (i.e., a high number of pulses) at very high electric field strengths (i.e., >30 kV/cm) generally restricts applicability at the commercial scale of these combinations. For example, Walkling-Ribeiro et al. (2008) obtained 9.5 Log_{10} reductions in the population of *S. aureus* in apple juice by combining a PEF treatment at 40 kV/cm for 100 μs followed by a UV treatment (i.e., 30 W, 30 minutes).

Another approach that is considered to improve microbial lethality is the combination of PEF with antimicrobials such as bacteriocins (e.g., nisin, enterocin AS-48), enzymes (e.g., lysozyme), organic acids (e.g., citric, lactic, acetic, malic), or essential oils (e.g., clove, carvacrol, citral). Several authors reported additive or synergistic effects on microbial inactivation when antimicrobials were added to the treatment medium, including buffers and liquid foods (Martín-Belloso and Sobrino-López, 2011). However, it is well known that some antimicrobials used in these combinations, such as nisin or lysozyme, are ineffective or scarcely effective against gram-negative bacteria. For example, McNamee et al. (2010) found that adding 2.4 IU/ml of nisin to orange juice (40 kV/cm, 100 μs) increased the PEF inactivation of *Listeria innocua* by 1.7 Log_{10} cycles but of *E. coli* less than 1 Log_{10} cycle. On the other hand, the presence of nisin (i.e., 200 ppm) in the treatment medium did not increase the lethality of *E. coli* O157:H7 and *Salmonella* Typhimurium 878 (Saldaña et al., 2011a), so it seems that the impermeability of the outer membrane of gram-negative bacteria does not allow nisin to reach its site of action—the cytoplasmatic membrane—even when the cells are treated by PEF. The lack of synergy between some antimicrobials and PEF to inactivate gram-negative bacteria reduces the effect of these combinations on pasteurizing foods in which both gram-positive and negative microorganisms are present.

A synergistic effect between N^{α}-lauroyl ethylester (ethil lauroil arginate, LAE) and PEF was observed for gram-positive and gram-negative PEF resistant strains (Saldaña et al., 2010b). At temperatures between 40 and 50°C, around 5 Log_{10} cycles of inactivation in the population of *E. coli* O157:H7 and *L. monocytogenes* 5672 were obtained at 30 kV/cm for 100 μs when LAE was present in the treatment medium at a concentration

of 50 ppm. Therefore, the application of PEF in the presence of compounds such as LAE at moderate temperatures has the great potential to achieve effective control of pathogenic microorganisms.

CONTINUOUS PEF TREATMENTS FOR FOOD PASTEURIZATION

Treatments in batch with a static treatment chamber with parallel electrodes permit the processing of small samples and strictly controlled treatment conditions (Raso et al., 2000). This approach is required for a better understanding of the critical parameters that affect microbial inactivation by PEF, the prevention of experimental artifacts, and the avoidance of misinterpreting results. However, as commercialization of PEF processing for liquid food pasteurization requires the application of continuous flow treatments, the results obtained in a batch treatment need to be validated in a continuous flow installation before they can be successfully implemented on a large scale.

Microbial inactivation by PEF in continuous flow treatment has been extensively reported in the literature (Mosqueda-Melgar et al., 2008). However, as most of these studies have not been conducted with target microorganisms of public health concern, *process criteria* that demonstrate that it is possible to reach an extensive microbial inactivation in the target microorganisms have not been proposed (El-Hag et al., 2010).

Recently, there has been considerable progress in the development of continuous flow treatment chambers that are essential for scaling up the technology for industrial applications (Huang and Wang, 2009). A detailed knowledge of the values of the critical process parameters affecting microbial inactivation inside the treatment chamber during processing is necessary for validation. The small dimensions of the treatment chambers make it impossible to perform adequate measurements of the process parameters inside the treatment chamber with the corresponding probes without perturbation of the flow, temperature, and electric field distribution (Jaeger et al., 2009).

Gerlach et al. (2008) reviewed investigations of applying numerical simulation techniques to provide information on the spatial and temporal distribution of the electric field strength and temperature inside the treatment chambers with different electrode configurations. A uniform treatment can be achieved using a parallel plate electrode configuration rather than a colinear configuration (Fig. 3). However, for some applications, a colinear configuration is selected because the load resistance of the treatment chamber is higher and the energetic requirements are consequently lower. To improve the treatment uniformity in colinear configurations, generating a turbulent flow by modifying the treatment chamber geometry or by inserting a grid before the treatment zone is suggested (Jaeger et al., 2009; Buckow et al., 2011; Meneses et al., 2011a). Although these approaches produce more uniform treatment conditions, inhomogeneities in the distribution of the electric field strength and temperature in treatment zone are still observed. As uniform processing of food when passing through the treatment chamber is one of the most important criteria for a successful design in terms of assuring food safety, a parallel electrode treatment chamber would be more appropriate for validating pasteurization treatments.

Another aspect for consideration to implement the PEF technology by the food industry is that the treatment conditions needed to guarantee food safety should be applicable at commercial scale. Studies to evaluate the application of PEF for microbial decontamination at laboratory scale have been generally conducted with long treatments (i.e., >100 μs) at high electric field strengths (i.e., >30 kV/cm) (Álvarez et al., 2003a, 2003b; Gomez et al., 2005; Sepúlveda et al., 2005; Sampedro et al., 2006; Mosqueda-Melgar et al., 2007; Zhao et al., 2008; Walkling-Ribeiro et al., 2009). At commercial scale, technical and economical limitations exist in applying these intense treatments. Long treatments require recirculation or the use of several treatment chambers. The food must also be cooled between chambers to maintain the temperature below those used in thermal processing (Min et al., 2003). Consequently, in addition to the high total specific energy required in these long treatments, an extra cost is necessary to control food temperature. On the other hand, technical limitations and risks of arching exist in applying an electric field strength that exceeds 30 kV/cm in the treatment chambers required for commercial application of PEF technology (Barbosa-Cánovas and Altunakar, 2006).

Heinz et al. (2003), using a parallel electrode treatment chamber, demonstrated that, by modifying the inlet temperature in the range of 35 to 65°C, the energy consumption of a PEF treatment (i.e., 35 kV/cm) required for reduction of 6 Log_{10} cycles on the population of *E. coli* suspended in apple juice could be reduced from above 100 to less than 40 kJ/kg. Although the increment of the initial temperature of the untreated juice would increase the energetic cost of the PEF process, these authors proposed that the thermal energy dissipated in the product because the resistance to the current flow during the PEF treatment could be recovered in a heat exchanger by preheating the untreated product. PEF processing at temperatures over 53°C when using a thermal regeneration system could be an effective treatment to extend the shelf life of whole milk at low energy-consumption rates (Sepúlveda et al., 2009).

Saldaña et al. (2011b), following the same strategy, investigated the *process criterion* to achieve the *performance criterion* established by the FDA to pasteurize apple juice by PEF when using a parallel electrode treatment chamber. The target microorganisms that were used for this study were four PEF-resistant strains, such as *Listeria monocytogenes* 5672, *Staphylococcus aureus* 4459, *Escherichia coli* O157:H7, and *Salmonella* Typhimurium 878. A treatment of 25 kV/cm for 63 μs that corresponds with an inlet temperature of 35°C, an outlet temperature of 65°C, and an input energy of 125 kJ/kg was required to achieve more that 5 Log_{10} cycles in the four strains. The addition of LAE reduced the treatment time required to obtain an equivalent inactivation in the four microorganisms to 38.4 μs, the outlet temperature to 55°C, and the input energy to

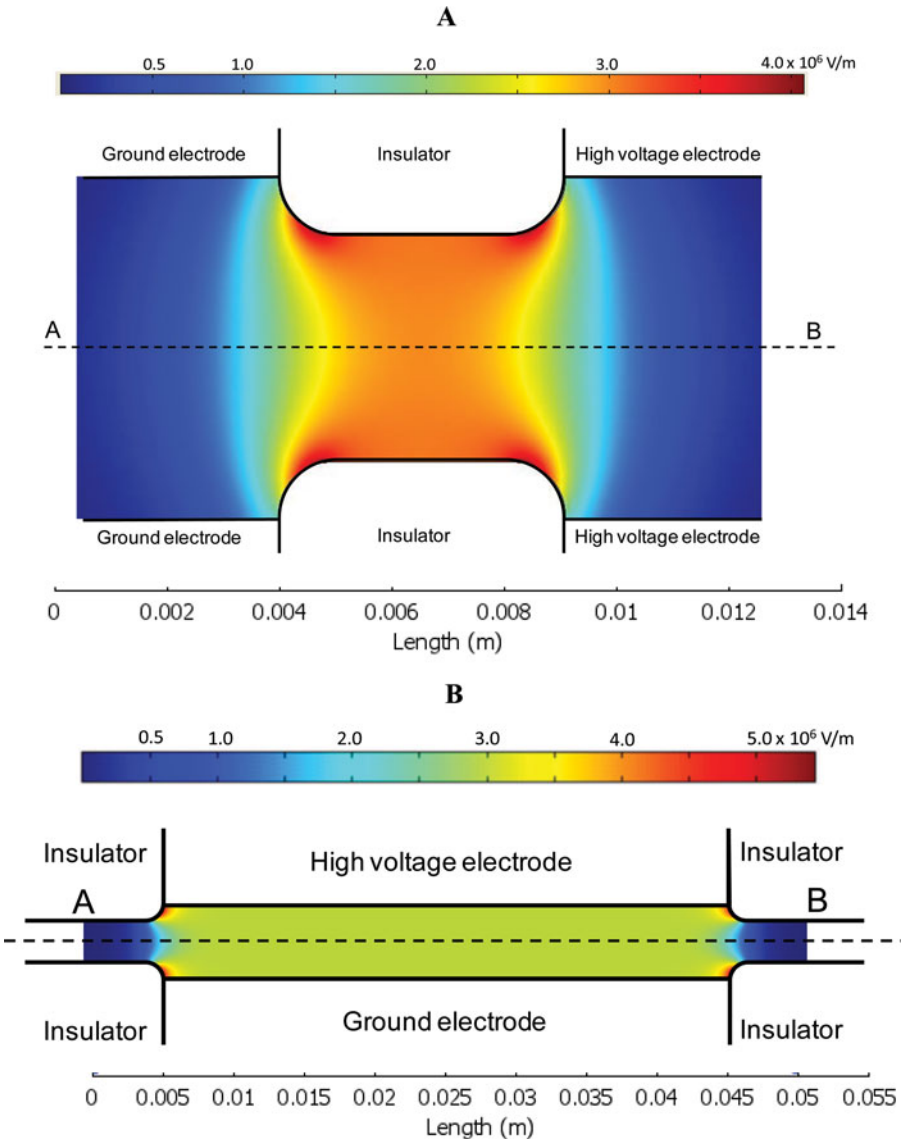

Figure 3 Sectional views of the electrode configuration and the resulting electrical field in the treatment zones of a colinear (A) and parallel-electrodes (B) treatment chambers, calculated for: 6-mm electrode inner diameter, 4-mm insulator inner diameter, 5-mm gap length, 19.5-kV charging voltage, 2-ms/cm fluid conductivity for the colinear chamber and 4-cm electrode length, 5-mm gap length, 15-kV charging voltage, 2-ms/cm fluid conductivity for the parallel-electrode chamber. (Color figure available online.)

83.2 kJ/kg. Gurtler et al. (2011) demonstrated that strawberry juice inoculated with *E. coli* O157:H7 and added with sodium benzoate (750 ppm) and potassium sorbate (350 ppm) followed the current FDA regulations for fruit juices after applying a PEF treatment of 18.6 kV/cm for 150 μs and an outlet temperature of 55°C. An inactivation of around 7 Log_{10} cycles was obtained when, under the same conditions, 2.7% of citric acid was added to the juice before the PEF treatment.

Therefore, these studies confirm that the application of PEF at moderate temperatures provides the possibility of obtaining substantial microbial inactivation of pathogenic microorganisms that are particularly PEF resistant at lower temperatures than those used in conventional heat treatment with a short residence time (less than 1 s). Combining the PEF treatment at moderate temperatures with the presence of antimicrobials effective against gram-positive and gram-negative pathogenic bacteria would reduce the number of pulses and, consequently, the energy costs required to obtain safe foods. These treatment conditions should be considered in the future during studies of the impact of PEF on shelf life and quality attributes of foods.

CONCLUSIONS AND FUTURE RESEARCH DEMANDS

The PEF is one of the most promising methods for food pasteurization because of its potential to inactivate microorganisms at temperatures that avoid the harmful effects of heat on

the organoleptic properties and nutrient values of liquid foods. However, before the technology can be commercialized and used for the pasteurization of liquid foods, it is critical to demonstrate that PEF treatments promote an equivalent or, preferably, an enhanced safety level when compared with the commercially available products processed with conventional technologies.

Recent research confirmed that there is a considerable variation in the susceptibility of different strains of pathogenic microorganisms to PEF treatments. This must be taken into consideration in studies that aim to define a process criterion to achieve a given performance criterion. If studies are not conducted with the most PEF-resistant strains selected in previous screening studies, the results should be validated with a cocktail of different strains. On the other hand, when operating at room temperature, PEF process conditions that are commercially applicable are insufficient to obtain substantial inactivation in the most PEF-resistant strains of pathogenic microorganisms.

Recent investigations have confirmed that the lethality of PEF is highly dependent on the temperature of the treatment medium. Therefore, kinetic studies on microbial inactivation by PEF at different temperatures are required to evaluate the real potential of this technology for food preservation because the incremental lethality of PEF treatments at moderate temperatures introduces the possibility of pasteurizing liquid foods by using short treatments at moderate electric field strengths.

There is a substantial variety in PEF equipment operating in different laboratories around the world, as well as alternative methods of presentation of inactivation data, which makes the comparison of the efficiency of these systems rather challenging. As it is very difficult to standardize experimental procedures used in different laboratories, the development of suitable PEF-temperature-time indicators could be a very useful tool to asses PEF process. By analogy with others integrators developed in other fields such as thermal or high hydrostatic pressure processing (Van der Plancken et al., 2008), a PEF integrator could be defined as a small, wireless device that shows PEF, temperature and time dependent, easily and accurately measurable, irreversible readout to the PEF treatment. On the other hand, it has been demonstrated that the application of numerical simulation techniques may also contribute to the development and establishment of standards for the proper design, performance, and analysis of PEF processing (Knoerzer et al., 2011).

The implementation of PEF technology in the food industry as an alternative to food pasteurization requires guaranteeing that during the process, all parts of the food receive the established treatment to achieve an inactivation level that assures food safety. To demonstrate this fact it would be desirable to have homogeneous conditions of electric field strength and temperature during PEF. However, the application of numerical simulation techniques has demonstrated inhomogeneity in the electric field strength and temperature distribution in the treatment chambers during PEF processing in continuous flow (Meneses et al., 2011b). The lack of uniformity in the distribution of the temperature and electric field strength can impair the evaluation of the treatment lethality. A strategy to estimating the microbial inactivation in a continuous PEF treatment is to assume that the inactivation in an inhomogeneous PEF process is a function of the momentary electric field strength and temperature in the different zones of the treatment chamber. To validate this approach, the integration of numerical simulation techniques to discover the distribution of temperature and electric field strength in continuous treatment chambers with the development of predictive models generated from microbial inactivation data obtained under uniform conditions of temperature and electric field strength should be required. Another approach for getting microbial safety confidence in PEF processing could be based on the determination by numerical simulation of the "coldest point" in the treatment chamber. This point would correspond with the zone of the treatment chamber where the electric field strength and temperature would be the lowest during processing. This point should be determined experimentally for each situation because probably it is dependent upon the treatment chamber configuration and the characteristics of the food product. Once this point is determined treatment conditions required to reach the process criteria in this location should be selected.

The objective of food preservation technologies used by the food industry is to control microorganisms once they have contaminated the foods. Because it is estimated that the infection dose of some pathogenic microorganisms is low (Blackburn and McClure, 2002), the application of treatments aiming at microbial destruction is of primary importance to produce safe foods. PEF at moderate temperatures and treatments conditions commercially applicable has proven effective to obtain substantial inactivation of PEF-resistant strains in continuous treatments of fruit juices. These *process criteria* should be considered in the future in studies on the impact of PEF on quality attributes of foods. The definitions of performance standards and criteria such as FSO, *performance criteria*, and *process criteria* for food pasteurization by PEF can help implement this technology in the food industry as an effective tool to improve food safety and to meet consumer demand for freshness and convenience. These definitions must be based on scientifically validated and verifiable data, which must be continuously improved.

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