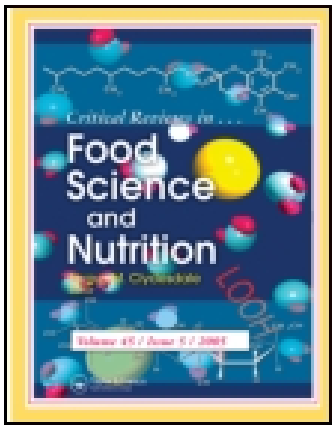

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Effects of Pulsed Electric Fields on Pathogenic Microorganisms of Major Concern in Fluid Foods: A Review

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Effects of Pulsed Electric Fields on Pathogenic Microorganisms of Major Concern in Fluid Foods: A Review

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Pathogenic microorganisms such as Escherichia coli *O157:H7,* Salmonella *spp.,* Listeria monocytogenes*,* Bacillus cereus*,* Staphylococcus aureus*,* Yersinia enterocolitica*, and* Campylobacter jejuni *have been implicated in foodborne diseases and outbreaks worldwide. These bacteria have been associated with the consumption of fresh fruit juices, milk, and dairy products, which are foodstuff, highly demanded by consumers in retails and supermarkets. Nowadays, consumers require high quality, fresh-like, and safe foods. Pulsed electric field (PEF) is a non-thermal preservation method, able to inactivate pathogenic microorganisms without significant loss of the organoleptic and nutritional properties of food. The PEF treatment effectiveness to destroy bacteria such as* Listeria innocua*,* E. coli*,* Salmonella Typhimurium*,* E. coli *O157:H7 and* E. coli *8739 at pasteurization levels ($\geq$5.0 log_{10} cycles) in some fluid foods was reported. However, data on the inactivation of some microorganisms such as* Bacillus cereus*,* Staphylococcus aureus*,* Yersinia enterocolitica*, and* Campylobacter jejuni *in fluid foods by PEF processing is very limited. Therefore, future works should be focused toward the inactivation of these pathogenic bacteria in real foods.*

Keywords pulsed electric fields, outbreaks, pathogenic microorganisms, fruit juice, milk

INTRODUCTION

The majority of foods harbor several types of microorganisms. Some of them have desirable roles in the food industry, such as in the production of fermented foods, whereas others cause food spoilage and human diseases. Bacteria such as *Escherichia coli*, *Listeria monocytogenes*, *Salmonella* spp., *Staphylococcus aureus*, *Bacillus cereus*, *Yersinia enterocolitica*, and *Campylobacter jejuni*, may be present in foods and generate public health problems.

The outbreaks associated with the consumption of fresh products in special fruit juices, milk, and dairy products have increased in the past years, due to their health-promoting image with slogans as "fresh products maintain its nutritional properties." However, those "fresh" products, which omit any effective microbial elimination step, result in foods that naturally would carry some microorganisms, including pathogenic bacteria (FDA, 2001). Thus, a minimal processing of fresh products to decrease or eliminate bacteria without significantly affecting their nourishing attributes is required. For this reason, the consumers today are demanding high quality, fresh-like, and microbially safe foods (Hoover, 1997; Aronsson, and Rönner, 2001).

Conventionally, heat is the most popular preservation technology for the elimination of microbial contamination of foods, in particular, pathogens. Although this process guarantees the safety of foods, their organoleptic, nutritional, and physicochemical properties are extensively damaged (Jeyamkondan et al., 1999; Espachs-Barroso et al., 2003). Thus, the food industry is exploring alternative preservation methods to counteract negative effects that occur during usual heat treatments (Qin et al., 1996).

Pulsed electric fields (PEF) treatment is a non-thermal technology able to inactivate microorganisms in foods without significant loss of flavor, color, and nutrients (Yeom et al., 2000; Bendicho et al., 2002; Hodgins et al., 2002; Odrizota-Serrano et al., 2006). Thus, this processing method may offer to the consumer safe, fresh-like, and nutritious food products. PEF treatment involves the application of short pulses (1 to 10 μs) with high intensity electric field (typically 20 to 80 kV/cm) to fluid foods placed between two electrodes in batch or continuous flow treatments. Nevertheless, PEF application is restricted to fluid foods that can tolerate high electric fields strength (E),

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Table 1 Most significant outbreaks of pathogenic microorganisms in fluid foods reported world wide

Causal agent	Years	Source	Country	N^r Cases	Reference
C. jejuni	2002	Unpasteurized milk	USA	46	CDC, 2002
C. jejuni	2001	Unpasteurized milk	USA	75	Harrington et al., 2002
C. jejuni	2000	Unpasteurized milk	Austria	38	Lehner et al., 2000
C. jejuni	1992	Raw milk	USA	50	CDC, 1992
C. jejuni	1985	Raw milk	USA	23	CDC, 1985
E. coli O157:H7	2005	Raw milk	USA	18	Anonymous, 2005b
E. coli O157:H7	1998	Milk	USA	28	Meng et al., 2001
E. coli O157:H7	1996	Apple juice	USA	71(1^a,14^b)	CDC, 1996
E. coli O104:H21	1994	Pasteurized milk	USA	18	CDC, 1995
E. coli O157:H7	1994	Pasteurized milk	Scotland	100 (9^b)	Upton and Coia, 1997
E. coli O157:H7	1991	Apple cider	USA	23	Besser et al., 1993
L. monocytogenes	1994	Chocolate milk	USA	56	Swaminathan, 2001
L. monocytogenes	1986	Raw milk	Austria	28	Lundén et al., 2004
L. monocytogenes	1983	Pasteurized milk	USA	49 (14^a)	Swaminathan, 2001
S. Typhimurium	2005	Orange juice	USA	31	Anonymous, 2005a
S. Typhimurium	2002	Unpasteurized milk	USA	107	CDC, 2002
S. Enteriditis	2000	Orange juice	USA	74	D'Aoust et al., 2001
S. Muenchen	1999	Orange juice	USA & Canada	220	Boase et al., 1999
S. Typhimurium	1999	Orange juice	Australia	427	D'Aoust et al., 2001
S. Enteriditis	1991	Orange juice	Germany	600	D'Aoust et al., 2001
S. Typhimurium	1985	Pasteurized milk	USA	16,284 (7^a)	D'Aoust et al., 2001
S. Dublin	1983	Raw milk	USA	123	CDC, 1983
S. Typhimurium	1981	Raw milk	Scotland	654 (2^a)	D'Aoust et al., 2001
S. Typhimurium	1976	Raw milk	Australia	500	D'Aoust et al., 2001
Bacillus cereus	1988	Milk	Canada	36	CDC, 2002
Bacillus cereus	1989	Milk	Canada	74	CDC, 2002
S. aureus	1985	Chocolate milk	USA	860	Everson et al., 1988
Y. enterocolitica	1995	Pasteurized milk	USA	10	Robins-Browne, 2001
Y. enterocolitica	1980	Milk	Japan	1,051	Robins-Browne, 2001
Y. enterocolitica	1976	Chocolate milk	USA	38	Robins-Browne, 2001

(^{a}) Numbers of people death.
(^{b}) Numbers of people with Hemolytic Uremic Syndrome (HUS).

have low electrical conductivity (σ), and do not contain or form bubbles. The particle size of the food is also a limitation (IFT, 2001). However, other factors such as frequency (f), treatment time (t), type and shape of the pulse, in addition to the self-microorganism characteristics could influence the efficacy of PEF processing (Wouters et al., 2001).

The Electroporation's Theory of Coster and Zimmermann (1975) elucidates the microbial inactivation mechanism, when pulses of electric fields are applied. This theory explains the formation of pores in the cellular membrane, which is able to generate cellular lysis with subsequent leakage out of intracellular compounds due to the induced electric field (Sale and Hamilton, 1967). However, this phenomenon has demonstrated to be reversible or irreversible, depending on the level of electric fields intensity (Barbosa-Canovas et al., 1999) and/or the membrane organizational changes (Weaver et al., 1988; Tsong, 1990). Mild electric fields intensity conditions form reversible pores in the cellular membrane, whereas drastic electric field intensity conditions lead to the irreversibility of this phenomenon, which results in cellular death (Tsong, 1990; Ho and Mittal, 1996; Barbosa-Canovas et al., 1999).

The PEF application as a non-thermal method to assure the elimination of pathogenic microorganisms frequently responsible for outbreaks by consumption of fluid foods was the main motivation for this review.

ESCHERICHIA COLI O157:H7

Enterohemorrhagic *E. coli* O157:H7 is a Gram-negative rod, which possesses several characteristics uncommon to most others. *E. coli* is unable to grow properly at 44.5°C and produce β-glucuronidase (Doyle et al., 1997). Enterohemorrhagic *E. coli* O157:H7 is recognized as an important foodborne pathogen, since it has been implicated in several outbreaks involving fluid foods, such as juices, and dairy products (Feng, 2001) (Table 1). The Center for Disease Control and Prevention (CDC) reported an outbreak in the United States(1996) due to *Escherichia coli* O157:H7 in unpasteurized apple juice. In that case, the pathogenic microorganism affected 71 people, and among them 14 suffered from the hemolytic uremic syndrome (HUS) and one died. The contamination may have occurred by using poor quality and/or dropped apples and the localization of apples orchard near cattle/deer. Most of the microbial contaminations in juices reported in the literature are due to fruits inadequately washed, brushed, and pressed, the use of contaminated rinsing water, and poor manufacturing practices applied during processing.

However, nowadays there are several methods for controlling the presence of microorganisms in these fluid foods, such as PEF treatment.

Evrendilek et al. (1999) inactivated pathogenic microorganisms such as *E. coli* O157:H7 and *E. coli* 8739 at pasteurization levels in apple juice treated by PEF. They reached 5.0 $\log_{10}$ and 5.4 $\log_{10}$ reductions of *E. coli* O157:H7 and *E. coli* 8739, respectively, applying 29 kV/cm electric field during 172 μs in a bipolar mode and a continuous flow treatment (Table 3). Likewise, Iu et al. (2001) achieved 5.35 $\log_{10}$ and 5.91 $\log_{10}$ reductions of *E. coli* O157:H7 in apple cider by PEF treatment when using 80 kV/cm and 90 kV/cm during 60 μs and 20 μs of treatment time, respectively. They kept the treatment temperature at 42°C, since this pathogenic microorganism is likely to be sensitive to heat above 42°C. In addition, when they combined the PEF treatment (80 kV/cm and 60 μs) with antimicrobial agents such as cinnamon or nisin at 2.0%, 6.23 $\log_{10}$ and 8.78 $\log_{10}$ reductions were reached, respectively.

On the other hand, Evrendilek and Zhang (2005) studied the effect of pulse polarity and pulse delaying time on *E. coli* O157:H7 in apple juice (σ: 2.3 mS/cm; pH 3.70 $\pm$ 0.24) and skim milk (σ: 6.2 $\pm$ 3.4 mS/cm; pH 6.7 $\pm$ 0.65). Both apple juice and skim milk were processed at 20 μs pulse delaying time, 700 Hz of frequency, and 30°C outlet temperature in a continuous flow system that discharged square wave pulses. They found reductions up to 2.6 $\log_{10}$ cycles without significant differences among bipolar and monopolar pulses for the inactivation of the microorganism in apple juice when used 31 kV/cm by 202 μs treatment time and 4.0 μs pulse length. However, in skim milk, the polarity exerted a significant influence on the effectiveness of the PEF treatment, using 24 kV/cm by 141 μs treatment time and 2.8 μs pulse length. They reported a reduction of 1.27 $\log_{10}$ and 1.88 $\log_{10}$ cycles in skim milk using monopolar and bipolar pulses, respectively. Generally, bipolar pulses are slightly more efficient than monopolar pulses on the destruction of microorganisms (Qin et al., 1994; Ho et al., 1995; Elez-Martinez, 2004, 2005). The PEF applications cause movement of charged molecules in the cell membranes of microorganisms, thus a reversal in the orientation of polarity of the electric field causes a change in the direction of movement of the charged molecules. The alternating changes in the direction of the movement of the charged molecules may cause a stress in the cell membrane that enhances electric breakdown of the cell membrane and microbial inactivation (Qin et al., 1996; Jeymkondan et al., 1999; Everndilek and Zhang, 2005). Moreover, the bipolar pulses offer the advantage of reducing the solid deposits on the electrode surface by more than 80% and a minimum energy consumption in comparison with monopolar pulses.

McDonald et al. (2000) and Martín-Belloso et al. (1997) reported from 5.0 to 6.0 $\log_{10}$ reductions of nonpathogenic *E. coli* strains, which showed behavior and characteristics very similar to the pathogenic strain under PEF treatment, in orange juice and liquid egg respectively. McDonald et al. (2000) applied six pulses per unit of volume of 2.0 μs pulse width at 30 kV/cm and 54°C outlet temperature to orange juice inoculated with the microorganism. Whereas, Martín-Belloso et al. (1997) applied 100 pulses of 4.0 μs pulse width at 26 kV/cm electric field and a maximum 37.2°C temperature to liquid egg also inoculated with *E. coli* (Fig. 1). However, in the study made by McDonald et al. (2000), the high inactivation rate might be mainly attributed to thermal effects, since *E. coli* is sensible to heat above 46°C. Likewise, Martín-Belloso et al. (1997) working with a much higher number of pulses and lower temperature (37.2°C maximum), reached the same inactivation of *E. coli*, ensuring that the inactivation of the microorganisms was achieved by the PEF treatment and not by heat.

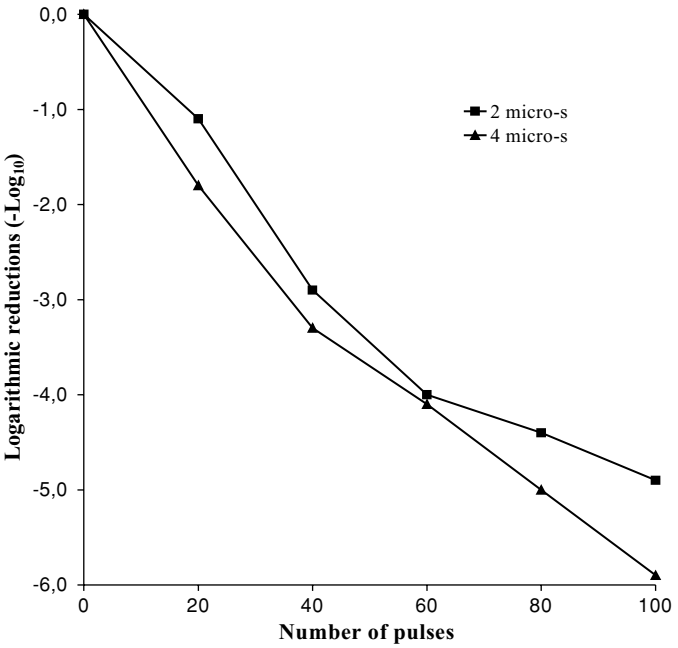

Figure 1 Inactivation of *E. coli* in liquid egg by PEF treatment at 26 kV/cm and 37°C, using different number and width pulses (Adapted from Martín-Belloso et al., 1997).

On the other hand, Malicki et al. (2004) found a reduction of 4.7 $\log_{10}$ cycles of *E. coli* in liquid whole egg by PEF, using 180 pulses for 30 μs at 20°C (Table 3). The inactivation found by those researchers is lower than the one reported by Martín-Belloso et al. (1997), due probably to the lower treatment temperature. Hence, the effect of the medium temperature could significantly influence the membrane fluidity properties, because at a low temperature (10 to 20°C) the phospholipids of the lipidic bilayer are closely packed into a rigid gel structure, while at a higher temperature (30 to 40°C) the phospholipids are less ordered and the cellular membrane has a liquid-crystalline structure (Aronsson and Rönner, 2001). Therefore, choosing the PEF treatment temperature is very important, given that a high temperature (up 30°C) increases the susceptibility of the cellular membrane to the electroporation and the antimicrobial efficiency of the PEF treatment. In the same direction, Bazhal et al. (2006) achieved up to 4.0 $\log_{10}$ reductions of *E. coli* O157:H7

in liquid whole egg by PEF treatment using 40 pulses subject to 11 kV/cm and 60°C outlet temperature (Table 3). In spite of the high temperature applied, the PEF treatment was not enough for inactivating the microorganism at pasteurization levels. It could be ascribed to the low electric field value applied during the treatment, since higher electric field values generate larger microbial inactivation. In addition, the thermal treatment (60°C) applied to *E. coli* O157:H7 in liquid egg reduced to 2.3 $\log_{10}$ cycles, given that this microorganism is sensible at temperatures above 46°C. Therefore, the PEF treatment only inactivated approximately 2.0 $\log_{10}$ cycles of the pathogenic bacterium.

Dutruex et al. (2000) applied PEF to skim milk (σ: 4.8 mS/cm; pH: 6.8) and buffer phosphate (σ: 4.8 mS/cm; pH: 6.8) inoculated with *E. coli*. They found a reduction of 4.0 $\log_{10}$ and 4.6 $\log_{10}$ cycles for skim milk and the buffer phosphate respectively, using 63 pulses of 2.5 μs length each (Table 3). The experiments were carried out using different media to test their effects on the reduction of the microbial load when PEF is applied. They concluded that the medium composition did not seem to influence on inactivation of *E. coli* by PEF application. However, Grahl and Märk (1996), Martín et al. (1997) and Martín-Belloso et al. (1997) encountered that the presence of fats and proteins in UHT-milk (1.5 and 3.5% fat), skim milk, and liquid egg constrained the effectiveness of the PEF treatment. Grahl and Märk (1996) subjected UHT-milk 1.5 or 3.5% fat inoculated with *E. coli* to PEF. They observed that UHT-milk with 1.5% fat had a lower inactivation constant (B_E) than UHT-milk with 3.5% fat. This behavior indicates that the fat particles of milk seem to protect the bacteria against the induced electric field. However, they reported a critical electric field (E_C) value more elevated for UHT-milk with 1.5% fat than for UHT-milk with 3.5% fat. It appears to be contradictory to the shown B_E value, since an elevated E_C value indicates a higher resistance to cellular death. Thus, the E_C value in the UHT-milk with 1.5% fat should be lower regarding UHT-milk 3.5% fat based on the B_E value. On the other hand, Martín-Belloso et al. (1997) and Martín et al. (1997) mentioned that proteins decrease the lethal effect of PEF on microorganisms by absorbing free radicals and ions, which are active in the cell breakdown. Moreover, the inactivation of bacteria by PEF is a function of the solution resistivity, which is inversely proportional to ionic strength (Martín-Belloso et al., 1997). Thus, the microbial inactivation is more difficult in real foods than buffer solutions and model foods, due to the complex composition of food.

On the other hand, Martín-Belloso et al. (1997) did not find significant differences between a continuous flow system and a stepwise flow treatment in the inactivation of *E. coli* in liquid eggs by PEF treatment. Similarly, Martín et al. (1997) reported the same behavior in skim milk, but they achieved a reduction near to 3.0 $\log_{10}$ cycles of *E. coli*, using 64 pulses at 45 kV/cm, 15°C and 1.8 μs duration pulse for stepwise treatment, and over 2.0 $\log_{10}$ cycles using 25 pulses at 25 kV/cm, 15°C and 1.8 μs duration pulse for a continuous flow treatment.

LISTERIA MONOCYTOGENES

The *Listeria monocytogenes* specie is widely distributed in the environment and may survive and grow in temperature, pH and oxygen harsh conditions (Table 2), but it is less competitive within populations with different bacteria. This pathogenic microorganism is able to grow in various food products such as milk, dairy products, fruit, and vegetable juices among others. Recently, listeriosis outbreaks have been associated with dairy products manufactured from raw milk (Table 1) in the United States of America (Swaminathan, 2001) and Europe (Lundén et al., 2004) leading to a big concern in the dairy industry. Although outbreaks of listeriosis associated with consumption of fruit and vegetable juices have not been reported, Sado et al. (1998) isolated *L. monocytogenes* from two samples of unpasteurized apple juices sold in retail stores in the US. Thus, the ability of *Listeria* spp. to grow at refrigeration temperatures and survive at a wide range of pH values, confirm the importance of the inactivation of this microorganism during food processing (Calderón-Miranda et al., 1999a).

Reina et al. (1998) studied the effect of the PEF treatment over *L. monocytogenes* inoculated in milk samples with different fat content (whole milk, 2% milk, and skim milk). They reached approximately 3.0 $\log_{10}$ reductions in each case, when treating the inoculated samples with 400 square wave pulses of 1.5 μs length each (Table 3) in a continuous flow treatment. They did not observe significant effects of the composition (fat content) of medium when the PEF treatment was applied. The same behavior was reported by Dutruex et al. (2000) in skim milk and buffer phosphate inoculated with *L. innocua* when applied 63 pulses of 2.5 μs length each (Table 3). Nevertheless, Picart et al. (2002) studied the influence of the fat content and the frequency of pulsation on *L. innocua* in UHT sterilized whole milk (3.6% fat), skim milk (0% fat), and sterilized liquid dairy cream (20% fat) by PEF treatment. They reported a higher inactivation rate at 100 Hz than at 1.1 Hz for whole (2.0 $\log_{10}$ cycles and 0.67 $\log_{10}$ cycles respectively) and skim milk (1.25 $\log_{10}$ cycles and 0.95 $\log_{10}$ cycles respectively), whereas they did not find a significant difference on the inactivation rate of microorganisms in dairy cream, since 2.0 $\log_{10}$ reduction was achieved at both frequencies 100 Hz and 1.1 Hz. Curiously, when whole milk and skim milk were treated at 100 Hz, a higher microbial inactivation in whole milk than in skim milk was observed (Fig. 2) when a 29 kV/cm electric field strength was applied. However, they observed a protective effect of the fat content on *L. innocua* inactivation in dairy cream when it was treated by PEF at 38 kV/cm. In general, the protective effect of the fat content on *L. innocua* when it is submitted to PEF treatment is not very clear. Thus more studies about the influence of fat particles, as well as other components on the effectiveness of PEF to inactivate this pathogenic microorganism, are necessary.

On the other hand, Reina et al. (1998) found that increasing the treatment temperature from 25°C to 50°C in whole milk (Table 3), the inactivation of *L. monocytogenes* reached maximum values around 4.0 $\log_{10}$ reductions when 30 kV/cm for

Table 2 General characteristics of pathogenic microorganisms

Characteristics	*E. coli* O157:H7	*Listeria monocytogenes*	*Salmonella* spp.	*S. aureus*	*Bacillus cereus*	*C. jejuni*	*Yersinia enterocolitica*
Shape	Rod	Short rod	Rod	Spherical	Rod	Spiral rod	Rod
Diameter (μm)	0.9 to 1.5	0.5 to 0.8	1.0	0.5 to 1.0	1.0 to 2.0	0.2 to 0.9	0.5 to 1.0
Length (μm)	2.0 to 6.0	1.0 to 2.0	4.0	—	3.0 to 5.0	0.5 to 5.0	1.0 to 2.0
Type	Gram-negative	Gram-positive	Gram-negative	Gram-positive	Gram-positive	Gram-negative	Gram-negative
Temperature[1] (°C)	7 to 46 (37*)	−1.5 to 45 (37*)	2 to 50 (37*)	7 to 48 (37*)	4 to 55 (35*)	30.5 to 45 (42*)	4 to 44 (29*)
Oxygen conditions	Aerobic or facultatively anaerobic	Aerobic or facultatively anaerobic	Aerobic or facultatively anaerobic	Aerobic or facultatively anaerobic	Aerobic or facultatively anaerobic	Facultatively anaerobic	Aerobic or facultatively anaerobic
pH[2]	4.0 to 9.0 (7.0*)	4.3 to 9.6 (7.0*)	3.6 to 9.6 (7.0*)	4.2 to 9.5 (7.0*)	4.3 to 9.3 (7.0*)	4.9 to 9.0 (7.0*)	4 to 10 (7.3*)
Water activity[3]	0.95	0.92	0.93	0.86	0.912	0.987	0.95
Spore-forming	Not	Not	Not	Not	Yes	Not	Not

[1]Growth temperature range.
[2]Growth pH range.
[3]Minimal water activity of growth.
*Optimum values of growth.

Table 3 Process parameters used for the inactivation of pathogenic microorganisms in fluid foods by PEF treatment

Microorganism	Fluid Food	E (kV/cm)	n[a]	τ^b (μs)	t_t^c (μs)	f (Hz)	T (°C)	Log_{10} reductions	References
L. innocua	Orange juice	30	6	2.0	12	—	54	6.0*	McDonald et al., 2000
L. monocytogenes	Whole milk	30	400	1.5	600	1700	50	4.0	Reina et al., 1998a
L. innocua	Skim milk	41	63	2.5	157.5	3	37	3.9	Dutruex et al., 2000
L. innocua	Liquid egg	50	32	2.0	64	3.5	36	3.4	Calderon-Miranda et al., 1999b
L. innocua	Whole milk	29	312	0.8	250	100	36	2.0	Picart et al., 2002
L. innocua	Dairy cream	37.5	250	1.0	250	100	36	2.0	Picart et al., 2002
L. monocytogenes	Skim milk	20	10	3.25	32.5	—	35	1.0	Fleischman et al., 2004
E. coli	Liquid egg	26	100	4.0	400	2.5	37	6.0*	Martín-Belloso et al., 1997
E. coli	Orange juice	30	6	2.0	12	-	54	6.0*	McDonald et al., 2000
E. coli O157:H7	Apple cider	90	10	2.0	20	—	42	5.91*	Iu et al., 2001
E. coli 8739	Apple juice	29	43	4.0	172	1,000	42	5.4*	Evrendilek et al., 1999
E. coli O157:H7	Apple juice	29	43	4.0	172	1,000	42	5.0*	Evrendilek et al., 1999
E. coli	Liquid egg	32.89	180	0.17	30	—	20	4.7	Malicki et al., 2004
E. coli O157:H7	Skim milk	41	63	2.5	157.5	3	37	4.0	Dutruex et al., 2000
E. coli O157:H7	Liquid egg	11	40	2.0	80	1	60	4.0	Bazhal et al., 2006
E. coli	Milk (1,5% fat)	23	20	—	—	—	45	4.0	Grahl and Märkl., 1996
Bacillus cereus	Skim milk	31	20		6.0		25	0.7	Sobrino et al., 2001
S. aureus	Raw milk	40	40	—	—	3.5	—	4.0	Raso et al., 1999
S. aureus	Skim milk	35	124	3.7	459	250	40	3.7	Evrendilek et al., 2004
S. aureus	Skim milk	31	35		6.0		25	3.0	Sobrino et al., 2001
S. aureus	Skim milk	35	600	4.0	2,400	100	25	1.0	Sobrino-López and Martín-Belloso, 2006
S. Typhimurium	Orange juice	90	50	2.0	100	—	55	5.9*	Liang et al., 2002
S. Dublin	Skim milk	35	164	1.0	164	2,000	50	4.0	Sensoy et al., 1997
S. Enteriditis	Eggs white	35	8	—	—	900	—	3.5	Jeantet et al., 1999

a: number of pulses.
b: pulse width.
c: treatment time (μs).
−: No reported.
*: log_{10} reductions at pasteurization levels.

600 μs treatment time were applied. Similarly, Fleischman et al. (2004) observed that increasing the temperature from 35°C to 55°C in skim milk with gellam gum inoculated with *L. monocytogenes* gave a reduction of 1.0 to 4.5 log_{10} cycles respectively, using 10 pulses of 3.25 μs pulse width (Table 3). However, they demonstrated that temperatures above 50°C were sufficient to inactivate *L. monocytogenes* up to 4.0 log_{10} cycles, since it is sensible at a temperature up to 45°C. Thus, this reduction was due to heat and not due to the PEF treatment. Finally, they concluded that *L. monocytogenes* seems not to be easily destroyed by PEF as a unique treatment but a combination of the treatment with heat would be adequate for its inactivation.

There are other species included within the genus *Listeria*, such as *L. innocua*, *L. grayi*, *L. ivanovii*, *L. seeligeri*, and *L. welshimeri* (Donnely, 2001). Nonetheless, *L. innocua* is often selected for inactivation studies because it is not a pathogenic microorganism and its behavior is closely related to *L. monocytogenes* (McLaughin, 1987).

Fernández-Molina et al. (2001) reported a reduction of 2.7 log_{10} cycles of *L. innocua* in raw skim milk at 50 kV/cm for 60 μs treatment time, 2 μs pulse width, 4 Hz, and 28°C outlet temperature in a continuous flow system with exponential decay pulses. Calderón-Miranda et al. (1999a) observed the same behavior when they treated skim milk inoculated with *L. innocua* by PEF. They achieved a reduction of 2.4 log_{10} cycles of the microorganism at 50 kV/cm, 32 pulses of 2 μs pulse duration, and 34°C and 3.5 Hz in a stepwise process with exponential decay pulse waveforms. In addition, when they exposed *L. innocua* to nisin after a PEF application, a significant synergistic effect between PEF and the nisin was observed, reaching 3.4 and 3.8 log_{10} reductions at 10 IU/ml and 100 IU/ml respectively. In the same way, Calderón-Miranda et al. (1999b) achieved a reduction on the microbial population of 3.4 log_{10} cycles applying only PEF treatment in liquid whole egg when inoculated with *L. innocua* and processed in the same conditions of electric field strength, pulse duration, number of pulses, and frequency (Table 3) than skim milk, but at 36°C. Moreover, they observed that the combination of PEF with nisin at 10 IU/ml and 100 IU/ml produced a higher microbial inactivation (4.1 log_{10} and 5.5 log_{10} reductions, respectively) than PEF alone. The difference in the inactivation levels between skim milk and liquid whole egg are attributed to electrical conductivity, ionic strength, and the composition of each medium. Likewise, Dutruex et al. (2000) achieved a higher reduction of *L. innocua* (3.9 log_{10} cycles) by PEF treatment than the one reported by Calderón-Miranda et al. (1999a), due to a higher pulse number applied and consequently a higher treatment time. They discharged 63 pulses of 2.5 μs pulse length at 41 kV/cm (Table 3) in a continuous flow system to skim milk.

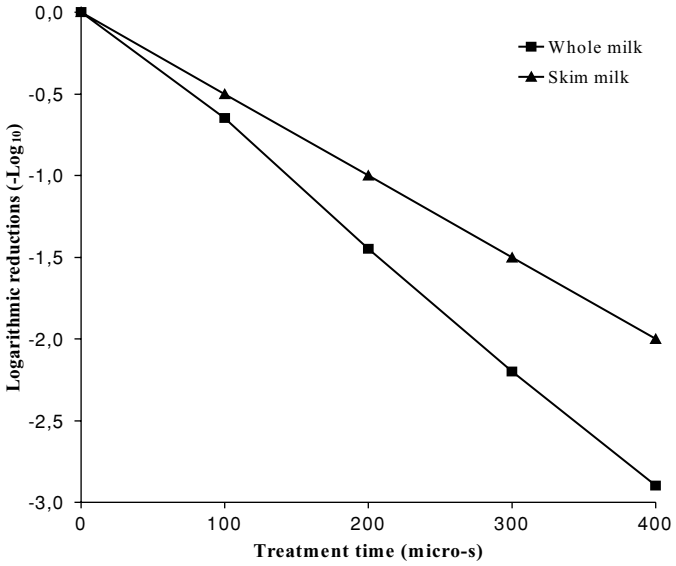

Figure 2 Influence of the fat content on the inactivation of *Listeria innocua* as a function of treatment time at 29 kV/cm electric field and 0.86 μs pulse duration with 100 Hz pulse repeat frequency and 45°C outlet temperature (Adapted from Picart et al., 2002).

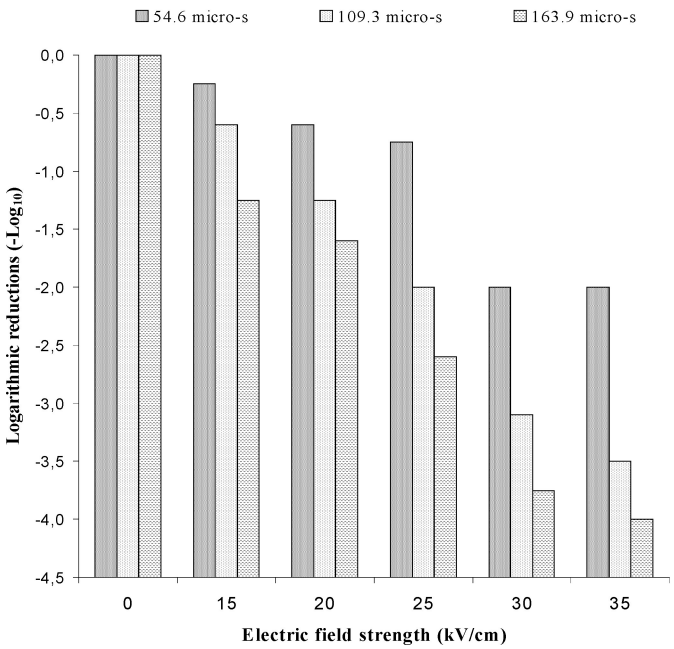

Figure 3 Effect of electric field strength on survival fraction (-$\log_{10}$)of *Salmonella serovar Dublin* treated at different treatment times in skim milk, using 1 μs pulse length with 2,000 Hz of frequency in continuous flow mode (Adapted from Sensoy et al., 1997)

On the other hand, McDonald et al. (2000) reported the maximum levels of *L. innocua* inactivation in fluid foods. They achieved reductions from 5.0 to 6.0 $\log_{10}$ cycles for *L. innocua* suspended in orange juice after PEF treatment with six pulses of 2.0 μs duration (Table 3) in a continuous flow system with exponential decay pulses and outlet temperature of 54°C. However, this great inactivation may be attributed to the high treatment temperature applied during the processing and not only to PEF treatment, since *Listeria* spp. is sensible at temperature over 45°C.

Salmonella SPP

The genus *Salmonella* groups two species, enterica and bongori, and currently encompasses approximately 2,700 serovars. These Gram-negative microorganisms can exhibit psycrotrophic properties by their ability to grow in foods stored between 2 and 4°C (D'Aoust et al., 2001), Moreover, they can survive and proliferate at low pH and water activity values (Table 2). Raw meats, eggs, and dairy products are the most common source of human foodborne salmonellosis, but new products such as fruit and fresh juices have been incriminated in recent years as vehicles of human salmonellosis infections (D'Aoust, 2001). Some outbreaks of salmonellosis associated with the consumption of pasteurized and raw milks and fruits juices are showed in Table 1. In the United States of America (2000) and Germany (1991), outbreaks of salmonellosis caused by *Salmonella Enteritidis* associated with unpasteurized orange juice affected 74 and 600 persons, respectively. Thus, studies on the inactivation of *Salmonella* spp. at pasteurization levels are very important for avoiding these outbreaks.

Sensoy et al. (1997) studied the effect of PEF processing on the inactivation of *Salmonella Dublin* suspended in skim milk. About 4.0 $\log_{10}$ reductions were reached using an electric field of 35 kV/cm with 163.9 μs treatment time of 1 μs pulse duration and 2000 Hz (Fig. 3). In addition, they observed that an enhancement in microbial inactivation from 3.0 to 4.0 $\log_{10}$ reductions was obtained when increasing the treatment temperature from 25 to 50°C.

Jeantet et al. (1999) were able to inactivate *Salmonella* Enteritidis about 3.5 $\log_{10}$ cycles in diultrafiltered egg white ($\sigma =$ 2 mS/cm), when used at an electric field strength of 35 kV/cm with 8 exponential decay pulses during 9 ± 1 μs decay time and 900 Hz frequency in a continuous flow system at 30°C.

On the other hand, Liang et al. (2002) reduced *Salmonella* Typhimurium at pasteurization levels (5.9 $\log_{10}$ cycles) in fresh squeezed orange juice (without pulp) when PEF was applied. They used 90 kV/cm and 50 pulses at 55°C treatment temperature. However, the high temperatures applied in this experiment could be the main cause of cellular death, since *Salmonella* spp. is heat sensitive at temperatures over 50°C. Therefore, PEF in combination with moderate thermal treatment may lead to reaching pasteurization levels desirable in the food industry.

STAPHYLOCOCCUS AUREUS

This Gram-positive microorganism of spherical shape is an important pathogenic microorganism that has been associated with food poisoning when found in the nourishment at

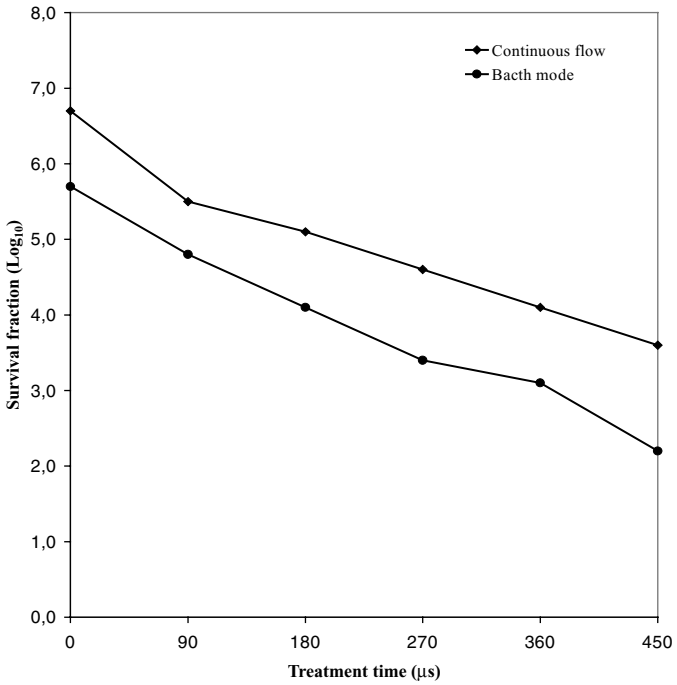

Figure 4 Inactivation of *Staphylococcus aureus* micro cells inoculated in skim milk by both continuous flow and batch mode. Treatment condition: 35 kV/cm electric field strength, 3.7 μs pulse width, 250 Hz, 40°C outlet temperature, bipolar square wave pulses (Adapted from Evrendilek et al., 2004)

concentrations that exceed 10^5 CFU/g (FDA, 2005). At these levels, the bacterium is able to generate an enterotoxin which is highly heat-stable. However, *Staphylococcus aureus* is not regarded as a good competitor against other bacteria and therefore rarely causes food poisoning in raw products. In addition, this pathogen is very susceptible to destruction by thermal treatment. The fluid foods that have been frequently incriminated in staphylococcal food poisoning include milk, dairy products, and egg derivates (Jay et al., 2005).

Raso et al. (1996) observed that *S. aureus* and coagulase negative *Staphylococus* spp. were inactivated in 4.0 and 2.0 $\log_{10}$ cycles, respectively, on raw milk when used at 40 kV input voltage, 40 exponential decay pulses, and 3.5 Hz. However, Evendrilek et al. (2004) achieved 3.7 $\log_{10}$ reductions of *S. aureus* in skim milk at 35 kV/cm, 124 pulses, and 250 Hz (Table 3) by both continuous flow and batch mode (Fig. 4).

On the other hand, Sobrino et al. (2001) reduced the survival fraction of *S. aureus* inoculated in skim milk up to 3.0 $\log_{10}$ cycles, using 35 exponential decay pulses at 31 kV/cm and room temperature. Likewise, Sobrino-López and Martín-Belloso (2006) found a reduction of 1.0 $\log_{10}$ cycle of *S. aureus* in skim milk at its natural pH (6.8), using 600 square wave pulses of 4.0 μs length at 35 kV/cm (Table 3) in a bipolar mode. However, when they combined the PEF treatment with 20 ppm nisin, it reached 6.0 $\log_{10}$ reductions of the pathogenic microorganism in the food.

Those results suggest that the PEF application itself is not enough for inactivating *S. aureus* at pasteurization levels. Nonetheless, PEF treatment applied in combination with nisin has demonstrated to be effective in the *S. aureus* inactivation.

OTHER PATHOGENIC MICROORGANISMS

Pathogenic bacteria such as *Bacillus cereus*, *Yersinia enterocolitica* and *Campylobacter jejuni* have been associated with fluid foods such as milk and dairy products (Table 1). Different research has been made about the effect of the PEF processing against these microorganisms in buffer and model solutions. However, studies applied directly on real fluid food are scarce.

Bacillus cereus is a spore forming bacterium that has a ubiquitous distribution in the environment and can be isolated from a variety of both raw and processed foods (Bennet, 2001). However, its presence in foods is not a significant hazard to consumer health unless it is able to grow above 10^5CFU/g and produce toxins. The stability and resistance of their spores and the increased number of psychrotolerant strains on foods has led to surveillance of this opportunistic foodborne pathogen, specifically in the dairy industry, in recent years (Granum, 2001).

Sobrino et al. (2001) achieved a reduction of 0.7 $\log_{10}$ cycles of *B. cereus* in skim milk by PEF, using 20 exponential decay pulses at 31 kV/cm and room temperature. However, when a thermal treatment of 10 min. at 75°C was applied in combination with PEF technology, a maximum of 6.2 $\log_{10}$ reductions were obtained. In this case, the thermal treatment itself inactivated 5.8 $\log_{10}$ cycles, since *B. cereus* is susceptible to temperatures over 55°C. Therefore, the combination of PEF with moderate heating may be useful to optimize milk processing to obtain a high quality product.

Yersinia enterocolitica is a Gram-negative psychotropic microorganism, which tolerates acidic conditions (Table 2). However, it may be quickly destroyed by thermal pasteurization. Several food-poisoning outbreaks of the pathogenic bacterium linked to the consumption of milk and dairy products have occurred in recent years (Table 1). Nowadays, several works have been carried out in buffer solutions to evaluate the inactivation levels against this emerging pathogen by PEF treatment (Alvarez et al., 2003; Virto et al., 2005). However, no study has ever been made over real food systems.

Campylobacter jejuni is a pathogenic bacterium that causes human gastroenteritis, and is transmitted primarily through foods of animal origin. This pathogenic microorganism is fragile by nature and could be a health problem only if the foods are prepared using poor hygienic conditions or when the foods are consumed without being thoroughly cooked (Stern, 2001). In addition, *C. jejuni* has a minimum growth temperature of about 30°C. On the other hand, this microorganim has been associated with the consumption of unpasteurized milk (Table 1). Therefore, it is being considered as an emerging pathogen of great importance for the dairy industry. However, there is no data available on its inactivation by PEF treatment in neither fluid foods nor buffer or model solutions.

COMPARISON OF THE PEF EFFECTIVENESS ON PATHOGENIC MICROORGANISMS

The numerous processing factors, variety of equipment, and the wide range of experimental conditions applied by different researchers, limit the comparison of the effectiveness of PEF processing on pathogenic microorganisms in diverse fluid foods. However, some conclusions from studies carried out under the same experimental conditions can be made.

Dutruex et al. (2000) observed 3.4 $\log_{10}$ reductions of *Escherichia coli* and 2.3 $\log_{10}$ reductions of *Listeria innocua* in skim milk when 35 pulses at 41 kV/cm were applied (Table 4). In this case, the different levels of microbial inactivation could be attributed to the type of microorganism and/or cellular diameter. *E. coli* and *L. innocua* are Gram-negative and Gram-positive microorganisms, respectively. Several authors have demonstrated that, within bacteria, the Gram-negative are more susceptible to PEF treatment than those Gram-positive (Castro et al., 1993; Mazurek et al., 1995; Pothakmury et al., 1995; Qin et al., 1998; Dutruex et al., 2000). This fact might be attributed to the composition of their membranes, since the cellular membranes of the Gram-positive bacteria are more rigid and thicker than the Gram-negative bacteria, which could constitute an additional protection to PEF treatment (Aronsson et al., 2001). On the other hand, *E. coli* have a higher cellular diameter than *L. innocua* (Table 2). Thus, *E. coli* is more sensible to PEF than *L. innocua*, since the induced voltage across the cell membrane is proportional to the geometric size (Qin et al., 1998). Pothakamury et al. (1995) also observed this behavior, when inactivated 4.0 $\log_{10}$ cycles of *Escherichia coli* and 3.0 $\log_{10}$ cycles of *Staphylococcus aureus* in simulated milk ultrafiltrate (SMUF), a model food. Both microorganisms were exposed to the same experimental conditions (Table 4).

On the other hand, *E. coli* O157:H7 was inactivated by 5.0 $\log_{10}$ (Evrendilek et al., 1999) and 4.0 $\log_{10}$ reductions (Dutruex et al., 2000) in apple juice and skim milk, respectively (Table 3). Those fluid foods (juice and milk) are very different in composition and properties. Therefore, the degree of inactivation of this pathogenic microorganism by PEF treatment is unlikely to occur in each food. Electrical conductivity and pH are influential parameters in PEF effectiveness, simple to be measured and thus, always included in the characterization of fluid foods to be treated by PEF. Apple juice has lower conductivity (2.6 mS/cm) and pH (3.70) than skim milk (4.8 mS/cm and 6.80, respectively) and thereby, the microorganisms present in the juice are more easily inactivated by PEF than in milk. A low food conductivity increases the difference in conductivity between the microorganism cytoplasm and the medium, which causes an additional pressure on the microorganism membrane due to osmotic forces, and makes it more sensitive to the PEF treatment (Sensoy et al., 1997). According to Dutruex et al. (2000), the conductivity might be one of the most important parameters influencing the inactivation of a microorganism by PEF. In addition, the pH of the medium also could affect the inactivation levels of the microorganism, since it is related to the ability of the microorganism to maintain the cytoplasm pH near the neutrality. Nevertheless, when an electric field is induced during the PEF treatment, the formation of pores on the cell membrane occurs and an osmotic imbalance around the cell is produced. Both acid and alkaline pH values induce additional stress to cells, and consequently increase their susceptibility to physical and chemical preservation treatments (Aronsson and Rönner, 2001).

DESCRIBING THE MICROBIAL INACTIVATION OF PATHOGENS IN fLUID FOODS BY PEF TREATMENT USING MATHEMATICAL MODELS

Mathematical models are important tools that can describe and predict the growth, survival, and inactivation responses of foodborne microorganisms under specific environmental conditions. In food microbiology, these mathematical models usually are empirical. Nevertheless, the models should be based on reliable experiments and on the understanding of the physiological mechanism of the microorganism inactivation. Likewise, it is very important to have a consistent model that accurately expresses the behavior of the bacteria when they are submitted at different environmental conditions (Raso et al., 2000). In addition, the mathematical models should be validated in a continuous process and in real food systems (Wouters et al., 2001).

Some models have been suggested to describe the microbial inactivation by PEF in fluid foods. Hülsheger and

Table 4 Influence of cell size, shape and type of bacteria on microbial inactivation by PEF treatment

Microorganism	Log_{10} reductions	Food	Φ	Cell shape	E	n	Type	T	Reference
E. coli	4.0	SMUF[a]	1.0	Rod	16	50	Gram negative	30	Pothakamury et al., 1995
S. aureus	3.0	SMUF[a]	0.9	Spherical	16	50	Gram negative	30	Pothakamury et al., 1995
E. coli	3.5	SMUF[a]	1.0	Rod	60	10	Gram negative	40	Qin et al., 1998
S. aureus	2.5	SMUF[a]	0.9	Spherical	36	10	Gram positive	40	Qin et al., 1998
E. coli	3.4	Skim milk	1.0	Rod	41	35	Gram negative	37	Dutruex et al., 2000
L. innocua	2.3	Skim milk	0.8	Rod	41	35	Gram positive	37	Dutruex et al., 2000

E: electric field strength (kV/cm).
n: number of pulses.
T: treatment temperature (°C).
[a]SMUF: simulate milk ultrafiltrated.
Φ:cellular diameter (μm).

Niemann (1981) and Hülsheger et al. (1980) proposed various mathematical models for inactivation of *E. coli* K12 in aqueous suspensions using PEF treatment (Equations [1–3]). Those models are based on the dependence of the survival ratio, S, defined as the microbial load after PEF treatment over the cell initial count before PEF processing ($N/N_{\circ}$), on the electric field intensity, E, treatment time, t, and the combination of both, E and t, according to the expressions 1, 2, and 3:

$$In(S) = -B_E(E - E_c) \tag{1}$$

$$In(S) = -B_t * In\left\{\frac{t}{t_c}\right\} \tag{2}$$

$$S(E,t) = \left\{\frac{t}{t_c}\right\}^{\left\{-\frac{E-E_C}{K}-\right\}} \tag{3}$$

where B_E and B_t are regression coefficients of the straight survival curves dependent on treatment time and electric field strength respectively, E, is the electric field strength (kV/cm) applied and, E_c, is the critical field strength value (kV/cm) which are threshold values where the inactivation occurs, t, is the treatment time (μs), and, t_c, is the critical treatment time value (μs). The E_c has been found to be a function of cell size, since it is much lower for bigger cells due to the transmembrane potential experienced by the cell, which is proportional to the cell size (Grahl and Märkl, 1996; Qin et al., 1998; Barbosa-Cánovas et al., 1999). Lower E_c values would indicate less resistance to the PEF treatment. The survival rate can be measured as a combined function of the electric field strength (Equation [1]) and treatment time (Equation [2]) in a double-logarithmic relationship (Equation [3]), where the symbol k represents an independent constant factor (cm/kV) for a specific microorganism. Therefore, a small value of the kinetic constant k indicates a wide span in the inactivation rate curve and lower sensitivity to PEF, whereas a large value implies a steep decline or higher susceptibility to PEF.

Equations (1–3) fitted very well the experimental data of Grahl and Märkl (1996), Martín et al. (1997) and Martín-Belloso et al. (1997) in UHT milk, liquid egg, and skim milk, respectively, for PEF inactivation of *E. coli*.

Sensoy et al. (1997) proposed a first-order kinetic model to describe the effect of the treatment temperature on *Salmonella* Dublin inactivation in skim milk (Equation [4])

$$S = e\{-K_E \times t\} \tag{4}$$

where, S, is the survival ratio, k_E, a constant that first was evaluated as a function of the electric field (μs^{-1}) and then as a function of the medium temperature (°K) following Arrhenius' model (Equation [5]) and, t, the treatment time (μs).

$$K = K_E \times e^{-\left\{\frac{E_A}{R\times T}\right\}} \tag{5}$$

where, k,is the survival fraction rate constant (μs^{-1}), E_A, is the activation energy (J/Kg.mole), R, is the universal gas constant (1.9872 J/Kg.mole.°K) and, T, is the medium temperature (°K).

Peleg (1995) also proposed a model (Equation [6]) based on Fermi's equation that describes the percentage (%) of survival microorganisms which reads as follows:

$$S(E,n) = \frac{1}{1+e^{\left\{\frac{E-E_c(n)}{a(n)}\right\}}} \tag{6}$$

being, S, a function of the electric field strength E (kV/cm) and number of pulses n, E_c, is the critical electric field strength value (kV/cm) where the survival level is 50%, and a, the parameter that indicates the steepness of the survival curve around E_c. Both parameters, E_c and a,are exponentially related to the number of applied pulses, n. Sensoy et al. (1997) used this model to describe the inactivation of *Salmonella serovar Dublin* by PEF in skim milk.

The other mathematical model based on the Weibull distribution (Equation [7]) has been used to fit the survival curves, relating $\log_{10}$ of the microbial survival with treatment time (Van Boekel, 2002; Gómez, et al., 2005).

$$Log(S) = -\left\{\frac{1}{2.303}\right\}\left\{\frac{t}{b}\right\}^n \tag{7}$$

where t, is the treatment time, b and n are the scale and the shape factors, respectively; n, factor interprets the shape of the survival curve, so that when $n < 1$ the survival curve is upward concave, $n > 1$ the survival curve is downward convex, and $n = 1$ indicates a linear survival curve on a log-scale. This model accurately predicted the inactivation of *Listeria monocytogenes* in apple juice (Gómez et al., 2005)

FINAL REMARKS

Human disease linked to the consumption of unpasteurized fruit juices, milk, and dairy products due to pathogenic microorganisms is affecting various countries worldwide. Several publicized outbreaks of foodborne infections and intoxications in recent years have enhanced public awareness of this risk. For this reason, the consumers today are demanding high quality, minimally processed, and microbially safe foods. So now, new processing technologies may counteract or destroy the presence of pathogenic bacteria in fluid foods. The PEF treatment is a nonthermal method that offers the advantages of maintaining the organoleptic and nutritional properties of foods, which results in a fresh-like product. Moreover, this technology has demonstrated that it can to inactivate some microorganisms such as *Listeria innocua*, *E. coli*, *E. coli* O157:H7, *E. coli* 8739, and *Salmonella Typhimurium* at pasteurization levels (over 5.0 $\log_{10}$ cycles) in some fluid foods. Nowadays, the PEF application at industrial level is a reality. Genesis Juice Corporation is a food industry that has initiated the application of PEF treatment to

some juices and blends in the Portland-USA market demonstrating the feasibility of the industrial application of the technology (Clark, 2006).

However, more emphasis in inactivation of pathogenic microorganisms in fluid foods are required to standardize the experimental proceedures. The few studies on PEF inactivation of vegetative cells and spores of *Bacillus cereus* in fluid products have shown little effectiveness against them, but further research is needed to prove if this phenomenon occurrs generally. In addition, studies of inactivation by PEF on emerging pathogens such as *Yersinia enterocolitica* and *Campylobacter jejuni* should also be carried out to evaluate if their presence in foods may be avoided through a PEF treatment, since these bacteria have caused several problems related to public health.

On the other hand, the relevant processing parameters used for the inactivation of microorganisms should be clearly highlighted to allow comparisons and scale up the technology at the industrial level. Thus, processing conditions, and the intrinsic and extrinsic factors of both fluid foods and microorganisms should be reported to facilitate the optimization and standardization of the PEF treatment. Moreover, the knowledge and understanding of these factors would help in the development of new mathematical models that should adequately predict the microbial behavior under PEF and improve the food safety and quality.

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