Lethal and sublethal injury and kinetics of *Escherichia coli*, *Listeria monocytogenes* and *Staphylococcus aureus* in milk by pulsed electric fields

Wei Zhao\(^a,b\), Ruijin Yang\(^a,b,*\), Xiaohan Shen\(^a,b\), Sha Zhang\(^a,b\), Xiao Chen\(^a,b\)

\(^a\) State Key Laboratory of Food Science & Technology, Jiangnan University, No. 1800 Lihu Road, Wuxi 214122, China
\(^b\) School of Food Science and Technology, Jiangnan University, No. 1800 Lihu Road, Wuxi 214122, China

**Abstract**

Lethal and sublethal injury of two Gram-positive (*Staphylococcus aureus* and *Listeria monocytogenes*) and one Gram-negative (*Escherichia coli*) bacteria in milk by pulsed electric fields (PEF) were determined using non-selective and selective media. PEF inactivation kinetics including lethal and sublethal injury fractions was also studied. The proportion of the sublethally injured microbial cells depended on the microorganisms, electric field strength and treatment time. The proportion of sublethally injured microbial cells reached maximum after a specific PEF treatment, and it kept constant or progressively decreased at greater electric field strengths and with longer PEF treatments. For the strain of *L. monocytogenes*, the proportion of sublethally injured cells increased from 18.98% to 43.64% with the increasing electric field strength from 15 to 30 kV/cm. While for the strains of *E. coli* and *S. aureus*, the proportion of sublethally injured cells achieved the maximum (40.74% and 36.51%, respectively) at 25 kV/cm and then decreased. The proportion of the sublethally injured microbial cells reached maximum at 400 μs (*S. aureus* and *L. monocytogenes*) or 500 μs (*E. coli*), and decreased at longer treatments at 30 kV/cm. The PEF inactivation kinetics including lethal and sublethally injured fractions was analyzed by the Hulsheger model, and the model parameters \(E_C, t_C, k_C, b_1\) for lethal and sublethal injury were also calculated.

© 2012 Elsevier Ltd. All rights reserved.

1. Introduction

Pulsed electric fields (PEF) processing is a non-thermal method of pasteurization for liquid foodstuff, which can inactivate spoilage and pathogenic microorganisms at or near atmospheric temperature (*Ferrer*, *Rodrigo*, *Pina*, *Klein*, *Rodrigo*, & *Martínez*, 2007; *Haughton* et al., 2012; *Moritz*, *Balasa*, *Jaeger*, *Meneses*, & *Knorr*, 2012; *Rodríguez-González*, *Walking-Ribeiro*, *Jayaramb*, & *Griffiths*, 2011; *Van Loey*, *Verachtert*, & *Hendrickx*, 2001). Recently novel combinations of PEF with other emerging physical hurdles such as manothermosonication (MST) and high intensity light pulses (HILP) were proposed to achieve food safety and quality (*Palgan*, *Muñoz*, *Noci*, *Whyte*, *Morgan*, *Cronin*, et al., 2012; *Palgan* et al., 2012). An even more exciting find was that a hurdle technology consisting of addition of nisin, moderate heat and PEF (40 kV/cm, 144 pulses) synergistically reduced *Bacillus cereus* spore count (by 3.6 log) in skim milk (*Bermúdez-Aguirre*, *Dunne*, & *Barbosa-Canoas*, 2012). Compared with conventional thermal pasteurization methods, foods were less affected initially after PEF processing and were maintained in higher quality throughout storage period (*Cortés*, *Esteve*, & *Frigola*, 2008; *Liu*, *Zeng*, & *Han*, 2010). Currently, this technology is progressing from laboratory and pilot plant scale levels to commercial scale (*Toepfl*, 2011). Dairy products as the potential application targets of PEF, have been the subject of a number of studies (*Bermúdez-Aguirre* et al., 2012; *Pérez*, *Aliaga*, *Bernat*, *Enguidanos*, & *López*, 2007; *Sobrino-López* & *Martín-Bellosillo*, 2008), where the lethal effects of PEF on common spoilage and pathogenic microorganisms in milk were evaluated.

Currently, it merits our attention that recent studies confirmed the occurrence of sublethally injured microbial cells under stress of PEF, indicating that electropermeabilisation was not an all-or-nothing-event (*Pina-Pérez*, *Rodrigo*, & *Martínez López*, 2009; *Somolinos*, *García*, *Condón*, *Mañas*, & *Pagán*, 2007; *Somolinos*, *García*, *Mañas*, *Condón*, & *Pagán*, 2008; *Zhao* et al., 2011). Several studies further investigated the possibility of sublethally injured cells that can recover after PEF treatment (*Somolinos* et al., 2008; *Zhao*, *Yang*, & *Wang*, 2009). PEF processing at 25–40 kV/cm with several tens to hundreds microseconds of treatment time could...
reduce 3–6 logs of spoilage and pathogenic bacteria and successfully extend the microbiological shelf-life to 3–4 weeks for milk, and 5–8 weeks for fruit juice-soymilk or milk beverages at refrigeration condition (Zhao, Yang, & Zhang, 2012). However, there is still a great challenge in PEF processing of milk. Milk is one of protein-based foods. It was found that protein components could protect the micro-organisms from the action of PEF treatment (Jaeger, Schulz, Karapetkov, & Knorr, 2009; Martin-Belloso et al., 1997). A concentration-dependent protective effect of milk protein fraction on PEF inactivation of *L. rhamnosus* was observed (Jaeger et al., 2009), which might favor the occurrence of sublethally injured microbial cells in PEF processing. Moreover, milk possesses higher pH value compared with vegetable and fruit juices, which is beneficial to the recovery of sublethally injured microbial cells during the storage period. However, the investigations on the microbial inactivation of PEF processing on milk taking into account the sublethal injury of microorganisms are far from enough. It is necessary for the further research on how to increase the microbial inactivation of PEF processing in milk through reduction of sublethal fractions of microorganisms and/or inhibition of their recovery.

Moreover, several mathematical models have been proposed to explain the decrease in microbial counts as a function of PEF treatment (Castro, Barbosa-Canovas, & Swanson, 1993; Hülshgeger, Potel, & Niemann, 1981; Peleg, 1995). The basic model used for interpreting survival curves focuses on first-order relationships, in which a semi-log plot of inactivation data against treatment time (or number of pulses) yields a straight line (Calderón-Miranda, Barbosa-Cánovas, & Swanson, 1999; Castro et al., 1993; Sensoy, Zhang, & Sastry, 1997). However, inactivation curves obtained from PEF treatments are usually not linear. Many hypotheses have been proposed to explain different types of deviations from linearity (Martin, Qin, Chang, Barbosa-Cánovas, & Swanson, 1997), and models different from first-order kinetics have been proposed. The goodness-of-fit of the Hülshgeger model may be affected by the occurrence of a sublethally injured fraction. However, few studies have been carried out to investigate the kinetic model with regard to sublethal injury potentially caused by PEF treatment.

The aims of the present study were to investigate the lethal and sublethal injury after PEF treatments in two Gram-positive (*Staphylococcus aureus* and *Listeria monocytogenes*) and one Gram-negative (*Escherichia coli*) bacteria in milk, and develop inactivation models taking into account the lethal and sublethal injury of microorganisms in milk following PEF processing.

2. Materials and methods

2.1. Materials

Raw whole milk purchased from a local farm (Tianzi, Wuxi, China) was heated at 100 °C for 10 min prior to PEF treatment. The raw whole milk could be sterilized as no viable bacteria could be found after 48 h of incubation at 37 °C on plate count agar. The electrical conductivity of the milk was 4.8 ms/cm at 25 °C as measured with a conductivity meter (FE30, Mettler Toledo Inc, Shanghai, China).

2.2. Microbial growth conditions and analysis

The strains of *E. coli* (ATCC8739), *S. aureus* (ATCC6538) used in this study were obtained from the American Type Culture Collection (ATCC, Rockville, USA). *L. monocytogenes* (GIMI1230) was purchased from Guangdong Microbial Culture Collection Center (Guangzhou, China). During this investigation, the strains were maintained on slants of tryptic soy agar (TSA) (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China). For each strain, one single colony of culture was inoculated from the TSA into a flask containing 100 mL of sterile tryptic soya broth (TSB) (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China). It was then incubated at 37 °C in a well-shaken (150 r.p.m.) water bath to achieve the stationary phase. It has been reported that bacteria cells in this phase of growth are more resistant to PEF, so all experiments were conducted with cells at the stationary phase of growth. From the growth curves of the three strains conducted in our lab (data not shown), the time to reach the stationary phase of growth was 18 h for the strain of *E. coli*, 24 h for *S. aureus* and 18 h for *L. monocytogenes*. After the incubation to reach the stationary phase, 10 mL broth of each strain were transferred to a 50 mL sterile centrifugal tube. The tubes were then centrifuged at 4 °C (10 min, 5000 g) to harvest bacterial cells. Cells then washed three times by re-suspension in 0.1% sterile peptone water (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China). Finally, cells were suspended in milk with a cellular concentration of approximately 10⁷ CFU/mL. Colony counts of cells were determined using a drop plating method on TSA (Baumgart, 1986).

2.3. Bench scale PEF treatment system

A bench scale PEF continuous system (OSU-4L, The Ohio State University, Columbus, Ohio, USA) with square-wave pulsed was used in this study. Milk was subjected to PEF processing at 15, 20, 25 and 30 kV/cm for various treatment time from 0 to 600 µs. The pulse repetition rate and pulse width were set at 200 Hz and 2 µs, respectively. The diagram of the PEF bench-scale processing unit and temperature measurement were clearly described in our previous study (Yang, Li, & Zhang, 2004; Zhao & Yang, 2008). The highest temperature achieved in the test was lower than 35 °C. The temperatures of the samples before and after PEF treatment were 15 and 25 °C, respectively.

2.4. Enumeration of viable and sublethally injured cells

One milliliter of each sample was serially diluted in sterile peptone water, and then 1 mL of appropriated dilution was poured plated and well mixed with 15 mL of molten TSA. In order to obtain estimates of sublethally injured cells, treated samples were also plated on selective medium, supplemented TSA with 2% (w/w) sodium chloride (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) for *E. coli*, 7% sodium chloride for *S. aureus* and 2% sodium chloride for *L. monocytogenes*. Increasing concentrations in several ranges of sodium chloride in agars were tested with regard to their impact on growth of the microorganisms: 1–4% of sodium chloride for *E. coli*, 1–9% for *S. aureus* and 1–4% for *L. monocytogenes*. Undiminished growth up to a concentration of 2%, 7% and 2% for *E. coli*, *L. monocytogenes* and *S. aureus* were observed, respectively, and these concentrations were therefore chosen to assure that injured cells were not able to recover whereas vital cells without membrane damage were growing. On the non-selective medium without the addition of sodium chloride, both vital cells and injured cells were able to grow. The plates were incubated at 37 °C for 48 h under atmospheric conditions. Each experiment was carried out on three independent cultures.

The inactivation of microorganisms was evaluated by calculating the log₁₀ reduction in the treated sample compared to the untreated sample. The difference between selective and non-selective media was expressed as the injured survivors. The percentage of injured cells was calculated according to the following equation:
The percentage injured cells \( (\%) \)

\[
= 1 - \frac{\text{CFU/mL}_{\text{selective}}}{\text{CFU/mL}_{\text{nonselective}}} \times 100
\]  

(1)

where CFU/mL_{selective} was the counts in selective medium; and CFU/mL_{nonselective} was the counts in nonselective medium.

2.5. Statistical analyses

Each experiment was carried out at least in triplicate. The variance and regression analysis was conducted with SAS software (Version 8.0, 2000, Cary, NC, USA). Significance was assigned to comparisons with a \( P \)-value of less than 0.05 (\( P < 0.05 \)).

3. Results and discussion

3.1. Occurrence of sublethal injury after PEF treatments

The inactivation of \( E. \text{coli} \), \( S. \text{aureus} \) and \( L. \text{monocytogenes} \) inoculated in milk by PEF at different electric fields and treatment time followed by plating onto selective and non-selective media are shown in Figs. 1 and 2, respectively. In each case non-selective medium and selective medium supplemented with sodium chloride were employed, and the survivors at selective medium supplemented with sodium chloride could be thought as undamaged cells, whilst those at un-supplemented medium included both undamaged cells and sublethally damaged cells.

The survived cells in the non-selective and selective media should be the same if no sublethal damage was caused by PEF treatment. As shown in Fig. 1, a higher level of inactivation was achieved for all strains tested when using the selective recovery medium, indicating that a certain proportion of survivors to PEF treatment were sublethally injured, which could not recover in the selective medium. Especially PEF treatment at 25 kV/cm for 200 \( \mu \)s resulted in a 2.1 \( \log_{10} \) cycles reduction of \( E. \text{coli} \) when using the selective medium, higher than the 1.6 \( \log_{10} \) cycles reduction obtained using non-selective medium. Similar behavior was observed for the other two strains. It is known that pores on the microbial cell membrane caused by PEF can be reversible or irreversible (Weaver & Chizmadzhev, 1996). Some of the microorganisms surviving PEF treatment might be sublethally injured, and developed sensitivity to physical or chemical environments to which normal cells were resistant (Somolinos et al., 2008; Zhao et al., 2009). García et al. (2005) detected a large proportion of sublethally injured cells in both Gram-positive (\( B. \text{subtilis} \) ssp. \( \text{niger} \), \( L. \text{monocytogenes} \)) and Gram-negative (\( E. \text{coli} \), \( E. \text{coli} \) O157:H7, \( P. \text{aeruginosa} \), \( S. \text{aureus} \) serotype \( \text{Senftenberg} 775W \), \( S. \text{salmonella} \) serotype \( \text{Typhimurium} \), \( Y. \text{enterocolitica} \)) bacteria in citrate-phosphate McIlvaine buffer after PEF. More than 90% of surviving \( S. \text{cerevisiae} \) cells and 99% of surviving \( S. \text{aureus} \) are means \( \pm \) standard deviations (error bars).

3.2. Effect of electric field strength, treatment time and microbial strains on the lethal and sublethal injury of microorganisms in milk

As shown in Figs. 1 and 2, the electric field strength and the treatment time are the main factors affecting the lethal and sublethal inactivation of microorganisms. The higher the electric fields applied, the greater the number of inactivated cells was achieved, and the maximum inactivation level was reached at 30 kV/cm after 200 \( \mu \)s (Fig. 1). Three strains showed different PEF resistance when survivors were recovered in both selective and non-selective media. \( E. \text{coli} \) cells (Gram-negative) were slightly more sensitive to PEF treatment than \( L. \text{monocytogenes} \) and \( S. \text{aureus} \) (Gram-positive) when using the selective medium. Especially PEF treatment at 25 kV/cm for 200 \( \mu \)s in milk, recovered in the non-selective (white bars) and the selective (gray bars) media. Data are means \( \pm \) standard deviations (error bars).
S. aureus cells (Gram-positive). L. monocytogenes was the most PEF resistant strain among the three test strains. Only less than 2 log10 cycles of reduction were inactivated by PEF at 30 kV/cm for 200 ms. However, the same PEF treatment killed around 2.8 log10 cycles of S. aureus and more than 3 log10 cycles of E. coli. This is probably explained by the different size of the cells or by the higher susceptibility of Gram-negative cells to PEF. A higher degree of inactivation was attained after PEF treatments (30 kV/cm) for all the three strains with the increase of the treatment time (200–600 μs), and the maximum inactivation level was achieved after 600 μs (Fig. 2). Compared with L. monocytogenes, E. coli and S. aureus were a bit more sensitive to PEF, reaching approximate 5 log10 cycles of reduction in non-selective medium. The results presented here were similar to the results obtained by other researchers (Calderón-Miranda et al., 1999; Evrendilek, Zhang, & Richter, 2004).

The population surviving PEF treatment contained different proportion of injured cells when exposed to a range of electric field strengths. For further quantitative analysis of sublethally injured microorganisms in PEF treatment, the number of sublethal cells was calculated according to the equation (1). Percentages of injured cells of E. coli, S. aureus and L. monocytogenes treated by PEF at several levels of electric field strengths for 200 μs were given in Fig. 3a. With the exception of the lower electric field strength studied, less than 20% of survivors of L. monocytogenes were sublethally injured at 15 kV/cm, and the proportion increased drastically with the increase of the electric field strength, up to 47.38% at 30 kV/cm. However, E. coli and S. aureus showed different behaviors. The proportion of sublethally injured cells achieved the maximum (40.74% and 36.51%) at 25 kV/cm and then decreased at higher electric field strength. This might be due to the higher PEF sensitivity of the two strains. These strains are more susceptible to PEF at higher electric field strength to form dead cells rather than sublethally injured cells, which was consistent with the results of Fig. 1a and c that the level of sublethally injured cells decreased by increasing the electric field strength.

Percentages of injured cells of E. coli, S. aureus and L. monocytogenes treated by PEF at 30 kV/cm for different treatment time are illustrated in Fig. 3b. The number of dead cells increased with the duration of the PEF treatments, whereas the proportion of

![Fig. 2. Log10 cycles of inactivation of E. coli (a), S. aureus (b) and L. monocytogenes (c), treated by PEF treatments at 30 kV/cm for different time in milk, recovered in the non-selective (white bars) and the selective (gray bars) media. Data are means ± standard deviations (error bars).](image)

![Fig. 3. The proportion of sublethally injured cells after PEF treatments at different electric field strengths for 200 μs (a), and at a fixed electric field strength of 30 kV/cm for different time (b). E. coli (○), S. aureus (□) and L. monocytogenes (△).](image)
sublethally injured E. coli cells increased steady up to 61.46% with the treatment time from 200 to 500 µs, and decreased at 600 µs. Similar behaviors were observed for L. monocytogenes and S. aureus, the proportion of sublethally injured cells achieved the maximum (72.92% and 64.05%) at 400 µs and decreased with the longer PEF treatment time (500–600 µs). Similarly, Somolinos et al. (2007) discovered the proportion of sublethally injured yeast cells was maximum after a specific PEF treatment, and it kept constant or progressively decreased at higher electric field strength and longer treatment time.

L. monocytogenes showed a different behavior to PEF treatment from E. coli and S. aureus. L. monocytogenes was more PEF resistant than the others under the same treatment parameters (Fig. 2). However, the percentage of sublethally injured cells was the highest at any given treatment time (Fig. 3b), suggesting that survivors of L. monocytogenes in milk after PEF treatment contained much larger proportion of damaged cells. Therefore, methods to inhibit the repair process and recovery of sublethally injured cells in milk are expected to be proposed to enhance bactericidal effect and extend shelf life of PEF-treated milk.

Under the PEF treatment conditions investigated, the proportion of sublethally injured cells of E. coli caused by PEF treatment (20 kV/cm, 200 µs) was 33.68%. According to García et al. (2005), there was 99.9% injury in E. coli cells when cells were suspended in citrate-phosphate buffer after PEF treatment at 19 kV/cm for 400 µs. The discrepancy may arise from different strains and suspension medium being used, which played a role in the effectiveness of PEF (Somolinos et al., 2008). Milk has protective effect during PEF inactivation of microorganisms due to the presence of proteins and fats (Jaeger et al., 2009). It was observed that less inactivation of E. coli by PEF was achieved in skim milk than in a buffer solution when exposed to similar treatment conditions (Bendicho, Barbosa-Cánovas, & Martín, 2002; Martín-Belloso et al., 1997). Considering this, components of milk, such as protein and fat, may have some effect on the proportion of the sublethally injured cells.

3.3. Kinetics model including lethal and sublethal injury fractions

It is important to have a reliable mathematical model accurately describing the kinetics of inactivation of microbial population in real food systems to establish appropriate PEF processing. Hülsheger et al. (1981) proposed a mathematical relation that described the kinetics of the survival curves of different microorganisms assuming a linear relationship between the logarithm of survivor fraction and the electric field intensity for a given treatment time, and a linear relationship between the logarithm of survivor fraction and the logarithm of treatment time for a given electric field intensity. According to this model the inactivation is given by

\[ \ln S = -k_E (E - E_C) \]  \hspace{1cm} (2)

\[ \ln S = -b_l \ln(t/t_C) \]  \hspace{1cm} (3)

Where \( S \) is the ratio between the number of survivors and the number of initial microorganism (\( N/N_0 \)) after PEF treatment, \( E \) is the electric field strength, \( E_C \) is the critical electric field strength, \( t \) is the treatment time, \( t_C \) is the critical treatment time, \( k_E \) is the regression coefficient and \( b_l \) is the independent constant. The parameters \( E_C \), \( t_C \), \( k_E \), and \( b_l \) are proposed to be solely dependent on the chosen microorganisms, if certain limits of the experimental variables are observed.

In order to study PEF inactivation kinetics including both lethal and sublethal injury fractions, experimental data of survivors after PEF treatment from non-selective and selective media were both analyzed using the Hülsheger model. Figs. 4 and 5 illustrated the fit of the Equations (2) and Equation (3) to the experimental data of E. coli, L. monocytogenes and S. aureus. The goodness of fit of the experimental data to the model was measured with the regression coefficient \( R^2 \) (Figs. 4 and 5). The Hülsheger model yielded a better fit for the inactivation date from selective medium (including lethally and sublethally injured cells) with respect to treatment time as well as electric field strength. Some studies have been carried out to evaluate the adequacy of this model to describe PEF inactivation, and observation of the numerous data using non-selective medium.

![Graphs showing inactivation kinetics of E. coli (a), S. aureus (b) and L. monocytogenes (c) in PEF treatments at various electric field strength for 200 µs. Data from non-selective medium (○), data from selective medium (●).](image-url)
might because the sublethally injured cells were immediately inactivated after PEF treatment in selective medium, however, which recovered in the incubation in the non-selective medium.

Hülsheger model assumes a linear relationship between the log of the survivor fraction and the electric field strength above a critical level. The critical electric field strength can be obtained by extrapolating the straight line according to Equation (2) to the intersection with \( \ln S = 0 \), and the critical treatment times are obtained by extrapolating the straight line according to Equation (3) to \( \ln S = 0 \). The model parameters including \( E_C, t_C, k_E \) and \( b_t \) can be used to compare the lethality of different treatments. The kinetics constants \( k_E \) and \( b_t \) from the selective medium were higher for all the three strains. It was obvious in the Table 1 that the value of \( k_E \) from selective medium for \( E. coli \) was 0.4106 \( \mu s^{-1} \), higher than that from the non-selective medium (0.3833 \( \mu s^{-1} \)). It was reasonable to get higher \( k_E \) and \( b_t \) values since sublethally injured cells were included in the inactivation in the selective medium. In contrast, \( E_C \) and \( t_C \) are lower when non-selective medium were used. \( E_C \) values decreased from 13.27 kV/cm to 12.33 kV/cm, and \( t_C \) values also declined from 155.52 \( \mu s \) to 146.80 \( \mu s \) for \( S. aureus \) when selective medium were used. Similar trends were observed for \( E. coli \) and \( L. monocytogenes \). \( E_C \) and \( t_C \) values depend on the PEF treatment conditions, below which no remarkable lethal of target microorganism occurs. The results indicated that the critical electric field strength and critical treatment time causing sublethal injury were lower than that causing lethal injury.

4. Conclusions

The proportion of sublethally injured microbial cells in milk after PEF treatments depended on the type of bacteria, electric field strength and PEF treatment time. According to the results obtained in this investigation, the most PEF resistant bacteria in milk corresponded to the strain of \( L. monocytogenes \), which also showed the largest proportion of sublethally injured cells. In addition, this study provided the PEF inactivation kinetics including lethal and sublethal injury fractions by the Hülsheger model, and the model parameters \((E_C, t_C, k_E, b_t)\) were calculated. Knowledge about lethal and sublethal injury and kinetics of microorganisms in milk by PEF would be useful for designing food pasteurization processes by PEF technology and the use of additional hurdles which interfere with injury repair to inhibit the repair process and recovery of sublethally injured micro-organisms in milk.

Acknowledgments

The authors gratefully acknowledge the financial support provided by National “863” project (2011AA100801-02, Project 31000829 and 31101376 of the National Natural Science Foundation of PR China), the Project BK2010148 of Jiangsu Provincial Natural

Table 1: Parameters for kinetic model.

<table>
<thead>
<tr>
<th>Strain</th>
<th>( k_E (\mu s) )</th>
<th>( E_C (\text{kV/cm}) )</th>
<th>( t_C (\mu s) )</th>
<th>( b_t )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( E. coli )</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( S )</td>
<td>0.41</td>
<td>10.14</td>
<td>158.03</td>
<td>8.70</td>
</tr>
<tr>
<td>( NS )</td>
<td>0.38</td>
<td>10.32</td>
<td>167.41</td>
<td>8.38</td>
</tr>
<tr>
<td>( S. aureus )</td>
<td>0.38</td>
<td>12.33</td>
<td>146.80</td>
<td>8.04</td>
</tr>
<tr>
<td>( NS )</td>
<td>0.36</td>
<td>13.27</td>
<td>155.52</td>
<td>7.65</td>
</tr>
<tr>
<td>( L. monocytogenes )</td>
<td>0.24</td>
<td>10.62</td>
<td>129.54</td>
<td>4.91</td>
</tr>
<tr>
<td>( NS )</td>
<td>0.22</td>
<td>11.17</td>
<td>150.01</td>
<td>4.57</td>
</tr>
</tbody>
</table>

* data from selective medium.

* data from non-selective medium.

Fig. 5. The inactivation kinetics of \( E. coli \) (a), \( S. aureus \) (b) and \( L. monocytogenes \) (c) in PEF treatments at 30 kV/cm for various treatment time. [data from non-selective medium (○), data from selective medium (●)].

suggested that the model might not be adequate in describing all the experimental data (Arroyo, Cebrián, Pagán, & Condón, 2008; Sagarazau, Cebrián, Pagán, Condón, & Mañas, 2010; San Martín et al., 2007; Wouter, Alvares, & Raso, 2001). Many hypotheses have been proposed to explain different types of deviations from linearity observed in the behavior of a wide variety of microorganisms inactivated by PEF (Baranyi & Roberts, 1994; Cole, Davies, Munro, Holyoak, & Kilby, 1993; Xiong, Xie, Edmondson, Linton, & Sheard, 1999), such as mixed bacterial populations in samples where each strain has its first-order inactivation kinetics, the variability of sensitivity to lethal agents in the bacterial population. In this study, the kinetics curves including lethal and sublethal injury fractions were obtained for the first time. The Hülsheger model yielded a better fit when sublethal injury fraction was considered. This
Science Foundation. This study was also supported by Jiangsu Provincial union innovation fund among industries, universities and research institutes (SBY201020121) and 111 project-B07029 Program for Changjiang Scholars and Innovative Research Team in University.

References


Bendicho, S., Barbosa-Cánovas, G. V., & Martín, O. (2002). Milk processing by high intensity pulsed electric fields. *Trends in Food Science & Technology*, 13, 195–204.


Castro, A. J., Barbosa-Cánovas, G. V., & Swanson, B. G. (1993). Microbial inactivation of foods by pulsed electric fields. *Journal of Food Processing and Preservation*, 17, 47–73.


Ferrer, C., Rodrigo, D., Pina, M. C., Klein, G., Rodrigo, & M., Martínez, A. (2007). The Monte Carlo simulation is useful to establish the most influential parameters on the final load of pulsed electric fields coli cells. *Food Control*, 18, 934–938.


Martín, O., Qin, B. L., Chang, F. J., Barbosa-Cánovas, G. V., & Swanson, B. G. (1997). Inactivation of *Escherichia coli* in skim milk by high intensity pulsed electric fields. *Journal of Food Process Engineering*, 20, 317–336.


Pina-Pérez, M. C., Rodrigo, D., & Martínez López, A. (2009). Sub-lethal damage in *Cronobacter sakazakii* subsp. sakazakii cells after different pulsed electric field treatments in infant formula milk. *Food Control*, 20, 1145–1150.


Zhao, W., Yang, R., & Wang, M. (2009). Cold storage temperature following pulsed electric fields treatment to inactivate sublethally injured microorganisms and extend the shelf life of green tea infusions. *International Journal of Food Microbiology*, 129(2), 204–208.

Zhao, W., Yang, R., & Zhang, H. Q. (2012). Recent advances in the action of pulsed electric fields on enzymes and food component proteins. *Trends in Food Science & Technology*, 27, 83–96.