Inactivation of *Salmonella enteritidis* in liquid egg products using pulsed electric field (PEF)

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Abstract

This study was designed to evaluate the effect of PEF parameters such as electric field intensity and number of pulses on the inactivation of *Salmonella enteritidis* suspended in liquid egg products. Liquid egg white and liquid whole egg, inoculated with $10^8$ CFU ml$^{-1}$ of *S. enteritidis* was treated continuously at 10, 20 or 30°C in factorial combination with an electric field intensity of either 20 or 30 kV cm$^{-1}$. A biphasic instant reversal PEF waveform with up to 60 or 105 pulses of 2 µs in width was employed. Bacterial inactivation increased with increasing applied electric field intensity, number of pulses and processing temperature. Maximum reductions of *Salmonella enteritidis* in egg white and whole egg obtained were 3.7 and 3.6 log cycles, respectively. The maximum input energies required to inactivate *S. enteritidis* in egg white and in whole egg were 574.76 and 914 J, respectively. The higher kinetic value of *S. enteritidis* (0.167 µs$^{-1}$) was obtained in egg white compared in whole egg (0.043 µs$^{-1}$) representing the more heat–PEF sensitivity of the bacteria.

Keywords: Pulsed electric field, liquid egg products, *Salmonella enteritidis*, Energy, Exponential decay model, Arrhenius equation.
Introduction

*S. enteritidis*, is a dangerous food-borne bacterial pathogen, may contaminate liquid whole egg preparations and cause salmonellosis in humans (Ray, 1996). The conventional heat pasteurization consist to treat whole egg and egg white to a temperature of 60°C and 56.7°C for 3.5 min, respectively (USDA, 1969). However, temperatures in excess of 60°C have well-known adverse effects on the nutritional and organoleptic properties of egg products (Cunningham, 1995; Herald and Smith, 1989; Ho and Mittal, 2000; Qin et al., 1995). In response to this challenge, innovative non-thermal pasteurization technologies, such as exposure to pulsed electric fields (PEF), have been proposed to replace or supplement heat pasteurization (Barbosa-Cánovas, et al., 1999).

PEF treatment typically involves the application of high voltage pulses (20 to 80 kV cm\(^{-1}\)) to product placed between two electrodes. It is performed at ambient, sub-ambient, or slightly above ambient temperatures for periods of less than 1 s, thus minimizing energy losses due to heating of the foodstuff (FDA, 2000). The technique is known as electroporation in the fields of genetic engineering and biotechnology, where it is used for cell hybridization (Barbosa-Cánovas, et al., 1999). In electroporation, the applied PEF is controlled to maintain cell viability while cytoplasmic contents are manipulated. However, for the pasteurization of food, in which the goal is to inactivate microbial cells, PEF is applied with sufficient intensity and duration to cause irreversible cell damage.
Numerous studies have evaluated the feasibility of using PEF for various liquid egg product-microorganism combinations (Dunn and Pearlman, 1987; Martín-Belloso et al., 1997; Barbosa-Cánovas, et al., 1999; Calderón-Miranda et al., 1999; Jeantet et al., 1999; Ma et al., 2000; Bazhal et al. 2003; Gupta et al., 2003; Amiali et al. 2004; Jeantet et al., 2004; Hermawan et al., 2004). However, the case of liquid egg products inoculated with food-borne pathogenic bacteria such as *Salmonella enteritidis* requires further study. Using four co-field continuous treatment chambers, Hermawan et al. (2004) obtained a 4.3 log reduction of viable *S. enteritidis* (ATCC 13076) in liquid whole egg subjected to a 250 µs PEF treatment at 25 kV cm⁻¹ and 55°C. Jeantet et al. (1999) reported a 3.5D reduction of *Salmonella enteritidis* in egg white by applying as little as nine exponentially decaying pulses with a peak intensity of 35 kV cm⁻¹. Much greater reductions would be required to make PEF a suitable alternative to conventional pasteurization.

Mathematical models are needed to establish appropriate PEF processing conditions, to ensure that recommended levels of microbial inactivation, and by extension product safety, are reached without over-processing. Hülsherger et al., 1981 were the first to present a mathematical model for bacterial destruction by PEF processing of foodstuffs. Their model supposed linear relationships between the logarithm of the survival fraction and the strength of the electric field and between the logarithm of the survival fraction and the logarithm of treatment duration. While this model remains widely used to describe PEF inactivation kinetics, several studies have reported that PEF inactivation tends to follow exponential decay kinetics (Sensoy et al., 1997; Martín-Belloso et al., 1997; Geerared et al., 2001; Smelt et al., 2002; Alvares et al., 2003; Bazhal et al., 2003; Amiali et al., 2004). Bazhal et al. (2005) used exponential decay model to predict the survival fraction rate of *E. coli* O157:H7 with number of pulses (0-138 pulses) and field intensity (9-15 kV cm⁻¹) in combination with treatment temperature (50, 55 or 60°C). The kinetic rate
constant of the combined treatment varied from 0.0125 to 0.0595 µs\(^{-1}\) and from 0.017 to 0.114 µs\(^{-1}\) for 55° and 60°C, respectively. They found a synergistic effect of temperature with electric field on the inactivation of \textit{E. coli} O157:H7 within a given temperature range. Sensoy \textit{et al.} (1997) used also the exponential decay model to predict the inactivation of \textit{Salmonella dublin} inoculated in skim milk using 15-40 kV cm\(^{-1}\) electric fields. After combination of electric fields and treatment temperatures (15-40°C), the kinetic constants due to temperature effect varied from 0.044 to 0.083 µs\(^{-1}\). However, no much works have been found in the literature regarding the comparison of pathogenic bacteria in liquid egg products using heat-PEF treatment.

The objectives of the study were (i) to model \textit{S. enteritidis} inactivation in liquid egg products by using exponential decay model (ii) to evaluate the heat-PEF effect on the inactivation rate by using Arrhenius equation and (iii) to determine the energy required for inactivation of this pathogenic microorganism in liquid egg products.
Materials and methods

Liquid egg products

Pasteurized liquid whole egg and liquid egg white was supplied by a local egg processing company (Burnbrea Farm, Lyn, ON, Canada). The products was delivered in aseptic packages and stored at 4°C immediately on reception until use.

Bacterial cultures

*S. enteritidis* (ATCC 13076) was grown at 37°C with gentle agitation to the early stationary phase (about 18 hours) in 50 mL of Brain Heart Infusion Broth (BHIB, DIFCO, 0037-17-8). Cells were harvested by centrifugation at 10,000×g for 10 min (4°C). Cell pellets were washed three times by re-suspension in 10 mL of distilled water. Washed pellets were finally re-suspended in 125 mL of liquid egg products (whole egg or egg white) to achieve an initial cell concentration $10^8$ CFU mL$^{-1}$.

Bacterial enumeration

*S. enteritidis* viable cells were counted before and after PEF treatment by plating on *Salmonella-Shigella* Agar (SSA, BD, 274500). Prior to plating on the selective media, the processed egg samples were maintained at 4°C for about 4 h to repair any injured cells. This method has been shown to be an effective technique for resuscitation of injured cells compared to plating on a non-selective medium and followed by overlaying with the appropriate selective media (Mussa, Ramaswamy, & Smith, 1999). PEF-treated samples were diluted serially (10-times) in 0.1% peptone water (DIFCO 180717-4), plated (0.1 mL) in triplicate on 70 mm Petri dishes and incubated at 37°C for 18 to 24 h. Dilutions giving 30 to 300 colonies per plate were
counted using a Darkfield Quebec colony counter (Model 3327, AO Scientific Instruments, Keene, NH).

**Pulse treatment apparatus**

The continuous-flow PEF exposure chamber consisted of two parallel stainless steel electrodes enclosed in Derlin® polyoxymethylene. The distance between the electrodes was 5 mm and the total exposed electrode surface area was 141 mm². Total chamber volume was 0.71 mL and . A 30 kV cm⁻¹ pulse generator with a matched output impedance of 100 Ω was used. The output voltage had a bi-phasic instant reversal square waveform with pulse duration of 2 µs. Both voltage and current across the treatment chamber were monitored simultaneously using a 2-channel digital oscilloscope (TDS3000, Tektronix, Wilsonville, OR). The apparatus is shown in Figure 1.

For PEF treatments, liquid egg product was exposed to 20 or 30 kV-cm⁻¹ pulses at a frequency of 2 Hz for a total of 0 to 60 or 105 pulses at 10, 20 or 30°C. Liquid flow rate through the chamber was set at 6 mL min⁻¹ using a peristaltic pump (Masterflex 77521-40, Cole-Parmer Instruments Co., Vernon Hills, IL) and a cooling system was used to maintain constant temperature. Treatment chamber contents turned over every 15 pulses. After exposure to the desired number of pulses, 2-3 mL of liquid were aseptically bled from the circuit, transferred to a sterile test tube and kept in an ice-water bath until analysis. Treatment temperature was monitored at the inlet and outlet of the treatment chamber (T_{inlet} - T_{outlet}) using K type thermocouples (OMEGA™, Stamford, CT). The apparatus was thoroughly cleaned with alcohol (70%) and rinsed with sterile distilled water after each experiment.
Kinetic modeling

The reduction of bacterial survival fraction as a function of treatment time at each electric field treatment may be expressed by a first-order kinetic model (Equation 1). The rate constants are dependent on electric field strength and temperature. Effect of temperature was described using the Arrhenius model (Equation 2).

\[ s(t) = e^{-k_T \cdot t} \]  
\[ k_T = k_0 \cdot e^{\frac{E_a}{RT}} \]

where \( s(t) \) is the fraction of total survivors, \( t \) is the treatment duration (\( \mu s \)), \( k_T \) is the kinetic rate constant (\( \mu s^{-1} \)), \( T \) is the treatment temperature (\( ^\circ K \)), \( E_a \) is activation energy (\( J \text{ mol}^{-1} \)), \( R \) is gas constant (8.31 J \( ^\circ K^{-1} \text{ mol}^{-1} \)), and \( k_0 \) is a constant (\( \mu s^{-1} \)).

Input energy

The input energy \( Q \) (J) during PEF treatment was calculated as follows:

\[ Q = V \cdot I \cdot n \cdot \tau \]

where \( V \) is the voltage across the treatment chamber (V), \( I \) is the current applied (A), \( n \) is the number of pulses, and \( \tau \) is the pulse width (sec).

Data analysis

Regression analyses were conducted using Sigmaplot software (Sigmaplot, Version 6.00, 2000, SPSS Inc, Chicago, Illinois). Analysis of variance (ANOVA) was performed using the General Linear Models procedures (GLM) of the Statistical Analysis System (SAS, Version 8.02, 2001, Cary, NC, USA). Experiments were duplicated and the means of the two data sets are presented.
Results and discussion

Inactivation of Salmonella enteritidis

The effects of temperature, treatment duration and applied field intensity, were significant ($P \leq 0.0001$). The influence of temperature, duration of PEF application, and strength of the applied electric field on bacterial inactivation in egg products has been noted in similar studies (Barbosa-Cánova et al., 1999; Barsotti et al., 2002; Barbosa-Cánova and Rodríguez, 2002; Espachs-Barroso et al., 2003; Rastogi, 2003). Some have also reported a synergistic effect of temperature with PEF (Barbosa-Cánova et al., 1999; Wouters et al., 1999; Hermawan et al., 2004; Bazhal et al., 2005).

In liquid egg products (whole egg and egg white) containing 8 log units of Salmonella per mL, the survival fraction decreased with increasing number of pulses, electrical field intensity and treatment temperature (Figures 2 and 3). Exposure to 60 pulses (120 µs) of 30 kV cm$^{-1}$ at 30°C produced maximum reductions of 3.7 log cycles for S. enteritidis in egg white while only 3.6 log cycles were obtained in whole egg after 105 pulses (210 µs) of treatment. This shows that this bacterium was more sensitive in egg white compare to in whole egg. In addition, these results corroborate reports of a treatment temperature that play a hurdle effect for PEF pasteurization of liquid egg products (Raso and Barbosa-Cánova, 2003; Hermawan et al., 2004; Bazhal et al., 2005). At low temperatures, the decreased inactivation of S. enteritidis could be attributed the closely packed rigid gel structure of the lipid bilayer microbial cell membrane. This structure yields to a less ordered liquid crystalline structure as temperature increases, which makes the bacterial cells more susceptible to breakdown (Mitchell and Slaughter, 1989). Food components such as lipids become more gel-like in structure as temperature decreases, which may have
protected the microbial cells against the electric field. Moreover, both proteins and fats in liquid egg are important nutrients for microbial growth (Banwarts, 1989) and may decrease the effectiveness of PEF treatment. The fat portion is protective for microorganisms while proteins may absorb active radicals and ions resulting from the discharges (Allen and Siokem 1966; Gilliland and Speck, 1967).

Synergism between temperature and field intensity may strongly influence bacterial inactivation obtained by PEF. Bazhal et al. (2005) found that inactivation of *E. coli* O157:H7 in liquid whole egg was increased by two log cycles with combined PEF-heat treatment compared to heat treatment alone. Under static conditions, they obtained up to four log reductions of viable *E. coli* O157:H7 subjected to a 15 kV cm⁻¹ electric field at 55°C. Hermawan et al. (2004), treating whole egg inoculated with *S. enteritidis* for 250 µs under continuous conditions, obtained reductions of 4.3 log cycles at 25 kV cm⁻¹ and 55°C. Similar or higher values have been reported in the literature (Martin-Belloso et al., 1997; Ma et al., 2000; Góngora-Nieto et al. 2001; Bazhal et al., 2003; Hermawan et al., 2004). These have been attributed to the combination of high electric field intensity and high treatment temperature and/or to the sensitivity of the bacteria to one or more factors. Martin-Belloso et al. (1997) obtained six log reductions of *E. coli* ATCC 11229 added to liquid whole egg with a 400 µs PEF treatment at 26 kV cm⁻¹ and 37.2°C, using a continuous coaxial treatment chamber and exponentially decaying pulses (2 and 4 µs pulse duration), in re-circulating mode. Góngora-Nieto et al. (2001) reported inactivation rates of up to 3.5 log reductions for three *Pseudomonas* strains (ATCC 17400, ATCC 13252 and WSU-07) suspended in liquid whole egg, by using an electrical field of 48 kV cm⁻¹ and a 230 µs treatment duration at 32°C. Temperature alone in some cases (Bazhal et al., 2003; Hermawan et al., 2004) may have determined the inactivation of *E. coli* and *Salmonella*, since mesophilic bacteria begin
to be heat-sensitive at temperatures as low as 46°C (Doyle and Schoeni, 1984; Frank, 1992). Strain-specific resistance to heat-PEF could contribute to decreasing inactivation rates.

**Inactivation kinetics**

Survival curves obtained over a range of treatment times were fitted to equations (1) in order to determine the kinetic constants of *S. enteritidis* in liquid egg products (Tables 1). The first order kinetic constant values ($k_T$) of *S. enteritidis* ranged from 0.016 µs$^{-1}$ to 0.167 µs$^{-1}$ and from 0.009 µs$^{-1}$ to 0.045 µs$^{-1}$ in egg white and whole egg, respectively, and the increase in treatment temperature involved an exponential raise in the first order $k_T$ values. Sensoy *et al.* (1997) after inactivated *S. dublin* in skim milk using 25 kV cm$^{-1}$ field intensity at temperatures varied from 10 to 50°C, obtained $k_T$ values varied from 0.044 to 0.083. At temperature of 37°C and electric field of 26 kV cm$^{-1}$ using batch recirculation treatments at different pulse rate and pulse duration. Although different $k_T$ values were reported in the literature due to the different of treatment conditions (medium, treatment chamber and setup), the results of kinetic constants of this study were almost in the same range. The kinetic constant for *S. enteritidis* in egg white at 30°C and 20 kV cm$^{-1}$ was 0.055 µs$^{-1}$, which is almost similar than the one reported by Sensoy *et al.* (1997) ($k_T = 0.059$ µs$^{-1}$) for *S. dublin* in skim milk after heat-PEF inactivation using 25 kV cm$^{-1}$ and 30°C treatment parameters.

Change in kinetic rate constants with respect to temperature was modelled using the Arrehnius equation. The data for *S. enteritidis* in egg white yielded Equation 5 and 6 for electric field 20 and 30 kV cm$^{-1}$, respectively:

$$k_T = 6.474 \cdot e^{-\frac{-14.23 \cdot 10^4}{8.31 \cdot T}}$$  \hspace{1cm} (5)
whereas, Equation 7 and 8 was obtained for *S. enteritidis* in whole egg at electric field strength of 20 and 30 kV cm\(^{-1}\), respectively:

\[
k_T = 1.544 \times 10^7 \cdot e^{\left[-\frac{46.53 \times 10^3}{8.31 \cdot T}\right]} \quad (6)
\]

\[
k_T = 1.28 \times 10^3 \cdot e^{\left[-\frac{25.79 \times 10^3}{8.31 \cdot T}\right]} \quad (7)
\]

\[
k_T = 5.1 \times 10^6 \cdot e^{\left[-\frac{46.82 \times 10^3}{8.31 \cdot T}\right]} \quad (8)
\]

As expected, the rate constant increased with increasing temperature of treatment. Except at 10 and 20°C for electric field strength of 20 kV cm\(^{-1}\), higher values of inactivation rate constants of *S. enteritidis* in egg white were obtained compared to the bacterium in whole egg indicating therefore the vulnerability of this bacterium to the heat-PEF treatment.

The activation energies (*E_a*) obtained in the range of 10 to 30°C at 20 and 30 kV cm\(^{-1}\) were 14.23 and 46.53 kJ mol\(^{-1}\) in egg white and 25.79 and 46.82 kJ mol\(^{-1}\) in whole egg. The Arrhenius equation at 30 kV cm\(^{-1}\) in egg white (Equation 6) was almost the same as the equation in whole egg (Equation 8), showing the same inactivation kinetic behaviour.

**Energy**

The energy required to achieve a given level of microbial inactivation by PEF depends on treatment chamber volume, product flow rate, number of pulses or treatment time, and system configuration. Tables 2 and 3 show mean input energies for the inactivation of *S. enteritidis* in egg white and whole egg, respectively, using pulses of 2 µs duration at 20 and 30 kV cm\(^{-1}\) and 10, 20 and 30°C. *S. enteritidis* inactivation increased with energy applied during PEF treatment. The energies input required to inactivate *S. enteritidis* in whole egg were higher than that
required to inactivate the same bacteria in egg white. For *S. enteritidis* in whole egg, the highest required energy input occurred at 30°C and 30 kV cm⁻¹, whereas the lowest was obtained at 10°C and 20 kV cm⁻¹ in egg white. The energy required to achieve the maximum inactivation of 3.7 and 3.6 log reductions were 574.76 J and 914 J for *S. enteritidis* in egg white and whole egg, respectively. The input energy used to achieve 3.7 log cycles was lower than those reported in the literature when inactivated *E. coli* species in liquid whole egg. Martin-Belloso *et al.* (1997) used 6000 kJ input energy to achieve 5 log reductions of *E. coli* (ATCC 11229) in liquid whole egg after 100 of continuous pulses (2 µs pulse width) and using 26 kV cm⁻¹ and 37°C electric field and treatment temperature, respectively. Amiali *et al.* (2004) used energy input of 708.4 J to obtained only 3.1 log reductions of *E. coli* O157:H7 in dialysed liquid whole egg. The treatment parameters were 15 kV cm⁻¹, 60 ms and 0°C of electric field strength, treatment time and treatment temperature, respectively.

**Conclusion**

PEF treatment inactivates *S. enteritidis* in liquid egg products. The extent of bacterial inactivation is a function of electric field intensity, treatment time and treatment temperature and may reach 3.7 and 3.6 log cycle reductions of *S. enteritidis* in egg white and whole egg, respectively. The data of the study accurately fit the exponential decay model. To evaluate the temperature effect with PEF treatment, Arrhenius equation was examined. Except at 20 and 30°C for an electric field of 20 kV cm⁻¹, lower kinetic constant values (*k*₇) of *S. enteritidis* were obtained in whole egg in comparison with the same bacteria in egg white. In egg white, only 60 pulses (120 µs) were used to achieve 3.7 log *S. enteritidis* inactivation. In whole egg, 105 pulses were needed to achieve the same amount of inactivation. Therefore, this bacterium is less resistant to heat-PEF treatment when is inoculated in egg white. This study indicates that the
energy required to inactivate *S. enteritidis* during the treatment varies depending on process conditions and media.
References


Figure 1: Diagram of experimental apparatus for PEF treatment of liquid whole egg

HV: High voltage
HC: High current
GND: Ground

30 kV, 300 A, 18 J/p
Figure 2: Survival fraction of *S. enteritidis* in egg white as a function of PEF treatment time, electric field strength and temperature. (o) 20 kV cm\(^{-1}\) and 10°C; (●) 30 kV cm\(^{-1}\) and 10°C; (Δ) 20 kV cm\(^{-1}\) and 20°C; (▲) 30 kV cm\(^{-1}\) and 20°C; (◇) 20 kV cm\(^{-1}\) and 30°C; (♦) 30 kV cm\(^{-1}\) and 30°C.
Figure 3: Survival fraction of *S. enteritidis* in whole egg as a function of PEF treatment time, electric field strength and temperature. (o) 20 kV cm$^{-1}$ and 10°C; (●) 30 kV cm$^{-1}$ and 10°C; (△) 20 kV cm$^{-1}$ and 20°C; (▲) 30 kV cm$^{-1}$ and 20°C; (◇) 20 kV cm$^{-1}$ and 30°C; (♦) 30 kV cm$^{-1}$ and 30°C.
Table 1: Exponential decay model rate constant \([k_T \, (\mu \text{s}^{-1})]\) for inactivation of *Salmonella enteritidis* in liquid whole egg and liquid egg white by pulsed electric field at different electric field intensity and temperatures.

<table>
<thead>
<tr>
<th>Electric field (kV cm(^{-1}))</th>
<th>Temperature (°C)</th>
<th>Egg white</th>
<th>(R^2)</th>
<th>Whole egg</th>
<th>(R^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>10</td>
<td>0.0162</td>
<td>0.960</td>
<td>0.00818</td>
<td>0.966</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>0.0194</td>
<td>0.966</td>
<td>0.02341</td>
<td>0.999</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0.0229</td>
<td>0.967</td>
<td>0.04516</td>
<td>0.999</td>
</tr>
<tr>
<td>30</td>
<td>10</td>
<td>0.0411</td>
<td>0.983</td>
<td>0.01962</td>
<td>0.997</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>0.0546</td>
<td>0.999</td>
<td>0.04057</td>
<td>0.998</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0.1670</td>
<td>0.999</td>
<td>0.04306</td>
<td>0.999</td>
</tr>
</tbody>
</table>

Table 2: Energy required for the inactivation of *S. enteritidis* in liquid egg white at pulsed electric field intensities of 30 and 20 kV cm\(^{-1}\) and temperatures of 10, 20 and 30°C.

<table>
<thead>
<tr>
<th>Electric field (kV cm(^{-1}))</th>
<th>Parameter</th>
<th>30</th>
<th>20</th>
<th>30</th>
<th>10</th>
<th>20</th>
<th>30</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>(Q_a) (J)</td>
<td>348.37</td>
<td>486.80</td>
<td>574.76</td>
<td>193.07</td>
<td>247.99</td>
<td>290.05</td>
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<tr>
<td></td>
<td>(Q_b) (J p(^{-1}))</td>
<td>5.81</td>
<td>8.11</td>
<td>9.58</td>
<td>3.22</td>
<td>4.13</td>
<td>4.83</td>
</tr>
<tr>
<td></td>
<td>(Q_1) (J)</td>
<td>23.22</td>
<td>32.45</td>
<td>38.32</td>
<td>12.87</td>
<td>16.53</td>
<td>19.37</td>
</tr>
</tbody>
</table>

\(Q_a\): energy input after 60 pulses; \(Q_b\): energy input per pulse; \(Q_1\): energy input after 15 pulses.

Table 3: Energy required for the inactivation of *S. enteritidis* in liquid whole egg at pulsed electric field intensities of 30 and 20 kV cm\(^{-1}\) and temperatures of 10, 20 and 30°C.

<table>
<thead>
<tr>
<th>Electric field (kV cm(^{-1}))</th>
<th>Parameter</th>
<th>30</th>
<th>20</th>
<th>30</th>
<th>10</th>
<th>20</th>
<th>30</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>(Q_a) (J)</td>
<td>575.59</td>
<td>665.50</td>
<td>914.00</td>
<td>308.91</td>
<td>402.05</td>
<td>538.86</td>
</tr>
<tr>
<td></td>
<td>(Q_b) (J p(^{-1}))</td>
<td>5.52</td>
<td>6.33</td>
<td>8.70</td>
<td>2.94</td>
<td>3.83</td>
<td>5.13</td>
</tr>
<tr>
<td></td>
<td>(Q_1) (J)</td>
<td>82.80</td>
<td>94.95</td>
<td>130.50</td>
<td>44.10</td>
<td>57.45</td>
<td>76.95</td>
</tr>
</tbody>
</table>

\(Q_a\): energy input after 105 pulses; \(Q_b\): energy input per pulse; \(Q_1\): energy input after 15 pulses.