MODELLING THE EFFECT OF pH AND PECTIN CONCENTRATION ON THE PEF INACTIVATION OF *Salmonella enterica* serovar Typhimurium BY USING THE MONTE CARLO SIMULATION

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NOMENCLATURE

\( S \): Survival fraction of microorganisms after the treatment.

\( a \): Kinetic parameter of Weibull model

\( n \): Shape parameter of Weibull model

\( E \): Electric field (kV/cm)

\( t \): Treatment time (µs)

\( a_{\text{ref}}(E, pH, \%) \): a value of Weibull model at reference conditions (15 kV/cm, pH 4.5 and 0.3% pectin concentration)

\( N_0 \): Initial number of microorganisms

\( N \): Final number of microorganisms after the treatment

\( z_E \): Rate change of a value of Weibull model with E

\( z_{pH} \): Rate change of a value of Weibull model with pH

\( z_{\%) \): Rate change of a value of Weibull model with pectin concentration
ABSTRACT

The effects of pH (3.5, 4 and 4.5), electric field (15, 25, 35 and 40 kV/cm) and pectin concentration (0.1, 0.3 and 0.6%) on the inactivation kinetics of *Salmonella typhimurium* in an orange juice-milk beverage treated by pulsed electric fields (PEF) were studied. A secondary model, based on Weibull distribution function, was used together with a Monte Carlo simulation to establish the most influential factors on the final number of *Salmonella* cells after PEF treatment. The Monte Carlo simulation can be a useful and practical tool for the industry to predict how the process parameters and product formulation will influence the food safety of the product.

Keywords: *Salmonella*, orange juice, pulsed electric fields, Monte Carlo simulation
1. INTRODUCTION

Quantitative Microbial Risk Assessment (QMRA) studies, predictive modeling and Hazard Analysis and Critical Control Points (HACCP) systems have gained increased attention in food microbiology in recent years, as they offer structures and tools to enhance food safety (Nauta, 2002). Although the three systems can be integrated, there are some important differences. HACCP system has become the premier system for evaluating and controlling foodborne hazards in food industries while QMRA as a part of risk analysis has been demonstrated to be an effective tool for foodborne diseases in order to design, develop and evaluate control measures to protect public health of a country or region (Zwietering & Nauta, 2007). HACCP systems do however have some limitations, such as the inability to relate to public health goals and the incapability to deal with the variability inherent in food systems due to its qualitative nature (Buchanan & Whiting, 1998; Hoornstra et al. 2001). These limitations can be overcome by combining QMRA and HACCP systems. However, less attention has been paid to the application of a QMRA study at industrial level and only a few studies are available in the open scientific literature (Hoornstra et al. 2001; Membré & Lambert, 2008; Shorten et al. 2006; Syposs et al. 2005; Voysey et al. 2007).

To assess the impact of a new food preservation technology on the food safety of an existing or new product, the process level exposure assessment of an IQMRA (Industrial Quantitative Microbiological Risk Assessment) study could be an effective tool in order to detect and quantify the probability that microbial hazards occur. The use of predictive modeling is essential to perform such quantitative studies describing the microorganism inactivation rate associated with particular food formulation and/or process conditions (Membré & Lambert, 2008). That is the case of pulsed electric field (PEF) technology and a beverage made of a mixture of orange juice and milk. Traditionally kinetic data have been adjusted to deterministic models to obtain the kinetic parameters. However, the development of probabilistic models that actively consider product (ingredients or formulation) and process (new technology) variability considering the whole
distribution (from minimum to maximum with all modes and percentiles) and the possibility to carry out a sensitivity analysis through the use of computer simulation techniques such as Monte Carlo simulation has gained importance in recent years (Baert et al. 2009; Buchanan & Whiting, 1998; Hoornstra et al. 2001). The use of the Monte Carlo simulation where input parameters are described as frequency distributions is an example of stochastic or probabilistic analysis (van Gerwen & Gorris, 2004). However, the application of the Monte Carlo simulation in a process level exposure assessment study is still scarce and only a few studies are available (den Aantrekker et al. 2003; Ferrer et al. 2006, 2007).

Salmonella is a microorganism of special concern when setting up proper pasteurization processes. The latest report from the US Center for Disease Control and Prevention (CDC, 2009) indicated that in 2008, Salmonella was the most frequent microorganism in relation to the number of infections and incidence per 100,000 persons in USA representing 16.20% of the total outbreaks. Among the Salmonella isolates serotyped, Salmonella enterica serovar Typhimurium accounted for 16.0%. Salmonella was, as in previous years, the most commonly reported cause of foodborne outbreaks in the EU reporting 2,201 Salmonella outbreaks and affecting 8,922 people, hospitalized 1,773 cases and caused ten reported deaths (EFSA, 2009). In addition, several Salmonella outbreaks in orange juice based products have been reported (Cook et al. 1998; Khan et al. 2007) and studies show the acid-tolerance of different Salmonella strains including S. typhimurium (Pao et al. 1998; Parish et al. 1997; Yuk & Schneider, 2006), demonstrating that the acid pre-adaptation of Salmonella in diverse fruit juices could increase the probability of infection developing.

The juice physicochemical parameters have a great variability during the fruit harvest season. In the case of orange juice, pH can vary during the harvest season (3.4-3.8) and could have a great impact on microbial inactivation rates and food safety. On the other hand, foods based on the mixture of different fruit juices and milk (pH 3.5-4.5) need the addition of an adequate stabilizer (pectin
derivatives) to maintain the product physical stability with the pectin concentration therefore becoming a parameter that could affect the food safety of the product by its impact on the inactivation rate of microorganisms. Although several authors have studied PEF inactivation of *S. typhimurium* (Álvarez et al. 2003; Liang et al. 2002) and the effect of the pH in several *Salmonella* strains (Álvarez et al. 2000) by using deterministic models, no work has been published applying a process level Monte Carlo simulation to establish the most influential parameters on PEF inactivation of *S. typhimurium*.

In the present work a Monte Carlo simulation was carried out to predict the final load of *Salmonella* cells treated by PEF in a juice-milk based beverage as influenced by electrical field intensity, pH and pectin concentration. This kind of methodology, when used in an industrial environment, could help industrial managers in knowing how safe their products are.

2. MATERIALS AND METHODS

2.1 Food Sample

The beverage contained the following ingredients: 50% (v/v) commercial pasteurized orange juice from squeezed oranges kept frozen until use (the pulp was removed), 20% (v/v) commercial UHT skimmed milk, 0.3% (w/v) high methoxyl citrus pectin as stabilizer (Unipectine AYD 250, Cargill, USA), 7.5% (w/v) sugar, and 30% (v/v) distilled water. The pH of the beverage was modified by adding commercial citric acid. The orange juice-milk beverage physicochemical characteristics were reported in a previous article (pH 4.5 and 14.3 °Brix) (Sampedro et al. 2007). The beverage was prepared immediately before use.

2.2 *Salmonella enterica* serovar Typhimurium

The culture of *S. typhimurium* CECT 443 was provided by the Spanish Type Culture Collection. Cells were obtained according to Sampedro et al. (2006). For that, the frozen microorganism (-
80ºC) was placed in 2 mL vials with Tryptic Soy Broth (TSB) (Scharlab SL, Spain) and 20% (v/v) sterile glycerol with an initial concentration of $6 \times 10^8$ CFU/mL in stationary growth stage. Immediately before the PEF treatment, the beverage (700 mL) was inoculated with the thawed microorganism reaching a final concentration of $3.5 \times 10^6$ CFU/mL.

2.3 PEF treatment

An OSU-4D bench-scale continuous unit was used to treat the food sample. Eight co-field chambers with a diameter of 0.23 cm and a gap distance of 0.293 cm between electrodes were connected in series. One cooling coil was connected before and after each pair of chambers and submerged in a circulating bath (Polystat, Cole Parmer, USA) to maintain the selected temperature of 5ºC. The temperature was recorded by thermocouples (K type) at the entrance of the first treatment chamber (initial temperature) and at the exit of the last treatment chamber (final temperature). The values were recorded with a data logger (Control Company, USA). Pulse waveform, voltage, and current in the treatment chambers were monitored with a digital oscilloscope (Tektronix TDS 210, Tektronix, USA). The flow rate was set at 30 mL/min with a peristaltic pump (XX 80002 30, 6-600 rpm, Millipore, USA). A bipolar square-wave of 2.5 µs was selected. Treatment time ($t$) ranged from 0 to 2500 µs, and the electric field ($E$) was set at 15, 25, 35 and 40 kV/cm. A negative control was carried out immediately after the microorganism was added to the beverage and after the treatment to ensure no inactivation took place due to the acid environment. Samples were collected after each treatment time. The different treatments were serially diluted in sterile 0.1% peptone water, plated in Tryptic soy agar (TSA) and incubated for 24 h at 37 ºC. The experiments were performed until three valid repetitions (experimental error less than 10%) were obtained. The effect of pH (3.5, 4 and 4.5) and pectin concentration (0.1, 0.3 and 0.6%) was studied at the treatment conditions commented above.

2.4 Data analysis
2.4.1 Primary kinetic model

Different mathematical models have been used to describe the microbial behavior when non log-linear experimental survivor curves were obtained. Previous works carried out by the same authors using PEF technology, pointed out the feasibility of using the Weibull distribution function to characterize the survival curves obtained (Rivas et al. 2006; Rodrigo et al. 2001, 2003):

\[
\log(S) = -\left(\frac{t}{a}\right)^n 
\]

Equation 1

where \( S \) is the survival fraction after the treatment. In most cases a kinetic constant has been considered as \( a \) parameter since this represents the microbial resistance to the PEF treatment. Shape parameter \( n \) gives an idea of the form of the curve, if \( n > 1 \) the curve is convex (it forms shoulders), if \( n < 1 \) the curve is concave (it forms tails) and if \( n = 1 \) the curve is a straight line and can be described by linear models. In most PEF survival curves values were lower than 1 indicating that the microbial population had a distribution and reflected the progressive elimination of sensitive members of the population with the most resistant cells remaining.

2.4.2 Secondary kinetic model

A global model proposed by Leguerinel et al. (2005) explained the dependence of primary kinetic parameters to environmental factors:

\[
\log a = \log a_{\text{ref}} + \left(\frac{A - A_{\text{ref}}}{z_A}\right) + \left(\frac{B - B_{\text{ref}}}{z_B}\right) - \left(\frac{C - C_{\text{ref}}}{z_C}\right) 
\]

Equation 2

where \( a \) is the kinetic parameter expressed in time units, \( a_{\text{ref}} \) is the \( a \) value at reference conditions, \( z_A, z_B \) and \( z_C \) are the conventional \( z \) values representing the environmental factors variation which leads a ten-fold reduction in \( a \) value. \( z_A, z_B \) and \( z_C \) values can be calculated by plotting the log of \( a \) values against the environmental factor value as the negative inverse of the slope of the regression line.
2.4.3 Monte Carlo simulation

Input parameters were characterized by probability distributions using the Best fit tool (Palisade Corporation, NY) according to the Chi-Squared test. Once the input parameters were characterized, a one-dimensional Monte Carlo simulation was carried out by Microsoft Excel 2003 (Microsoft Corporation) spreadsheet software and @Risk 5.5 for Excel (Palisade Corporation, NY). The analysis was performed using 1000 iterations with Latin-hypercube method.

2.4.4 Goodness of fit and accuracy

The corrected $R^2$ was calculated to check how well the models fit to the experimental data and the accuracy factor parameter ($Af$) was obtained to externally validate the models with a replicate not used to build them. This factor gives an idea of the model accuracy to predict experimental data and can be defined as follows (Ross, 1996):

$$ Af = 10^n \sum |\text{Log(predicted/observed)}| $$

Equation 3

where $n$ is the number of observations and the predicted and observed values are referred to the survival fraction. The meaning of this statistic is the closer to 1 the $Af$ values, the better the model fit the data.

3. RESULTS AND DISCUSSION

The survivor curves show the relationship between the survivor’s fraction of *S. typhimurium* in the beverage (pH=4.5 and % pectin=0.3) with treatment time at different electric field intensities and are characterized by a rapid decrease in the number of microorganisms followed by a tail (Figure 1). They were fitted to Weibull distribution function (Equation 1) in order to interpret this concave upwards behavior. Table 1 shows the model parameters and $Af$ values for Weibull distribution function fitted to the survival curves at different electric fields and reference conditions (pH=4.5 and % pectin=0.3). Values of the $n$ parameter were always less than 1, indicating the tailing effect
of the survival curves. An analysis of variance of the $n$ values did not show evidence for a statistically significant difference ($p > 0.05$), and therefore the shape factor was considered constant (0.33) in the range studied. Values of the $a$ parameter correlated well with the variation of electric field so it was considered as the kinetic constant. $A_f$ values ranged from 1.006-1.011 indicating good predictions (6.0-11.0% error in the predictions). $a$ values were then calculated for the different process conditions (variable pH, E and pectin concentration) maintaining the $n$ value constant (Table 2). Decreasing the pH and increasing the E values decreased the microbial resistance yielding lower $a$ values.

In order to study the electric field, pH and pectin concentration dependence of the $a$ value of Weibull distribution function, Collado et al. (2003) suggested the following global model based on equation 1 and equation 2:

$$\log(S) = -\left( a_{\text{ref}}(E, \text{pH}, \%) \times 10^t \right)^n$$

Equation 4

where $a_{\text{ref}}(E, \text{pH}, \%)$ is the $a$ parameter of Weibull model at reference conditions ($E=15$ kV/cm, pH=4.5 and $\%=0.3$) and is expressed in $\mu$s. $z_E$, $z_{\text{pH}}$ and $z_\%$ represented the electric field change (kV/cm), pH change and variation of pectin concentration respectively which led a ten-fold reduction in the $a$ value. As $n$ was independent of the electric field intensity applied, the $n$ value of equation 4 was substituted by the mean value (0.33).

Once the relation between all the input parameters was established, $a_{\text{ref}}(E, \text{pH}, \%)$, $z_E$, $z_{\text{pH}}$, $z_\%$ values and the initial number of microorganisms in each experiment ($N_0$) were used as input parameters in the Monte Carlo simulation model and characterized by distribution functions (Table 3). The generalized Beta distribution function gave good predictions for $a_{\text{ref}}(E, \text{pH}, \%)$, $z_E$, $z_{\text{pH}}$, $z_\%$ while logistic distribution function fitted best $N_0$ parameter as can be seen in table 3. This is in agreement
with Ferrer et al. (2006, 2007) where a deviation from normal distribution was also observed to characterize input parameters in the thermal inactivation kinetics of *B. stearothermophilus* and PEF inactivation kinetics of *E. coli*. This shows that care must be taken when assuming that input data is normally distributed.

The distribution functions were entered into @Risk Simulation Software (Palisade Corporation, NY) and Monte Carlo simulations were carried out to predict the final number of microorganisms (output parameter) under the different process conditions (electric field, pH, pectin concentration and treatment time) (Table 4). Predicted data were compared with a new set of experimental data not used to build the model and $Af$ values were calculated. $Af$ values were in all cases close to 1 (1.06-1.35) indicating a good prediction of final number of microorganisms as compared with experimental ones. Slight deviation from experimental data was observed in the most severe conditions (high E and low pH) corresponding to the higher $Af$ values.

A sensitivity analysis (Palisade Corporation, NY) was performed to establish the influence of input parameters on the output parameter (final number of microorganisms) through correlation coefficients (Figure 2). Values closer to 1 indicate a higher influence of the input parameter on the output and values closer to zero indicates no influence on the output. A positive number indicates that an increase in the input parameter produces an increase in the output whereas a negative value indicates that an increase in the input parameter produces a decrease in the output.

It can be deduced from the figure 2-(a) that at reference conditions (low electric field, medium pH and 0.3% pectin concentration) the most influential parameter on final number of microorganisms was the initial microbial load. The initial microbial load is intimately related to the overall process hygiene and quality of raw material. In addition at low process conditions the microorganism death rate was lower being more influenced by the initial microbial load. An increase in electric field...
strength (figure 2-b) had a great influence in the output producing a decrease in the final number of microorganisms (due to its negative form in equation 4). An increase of pectin concentration had no significant effect on the output value (figure 2-c) with the initial microbial load being the most influential parameter. This may be important information for the industry regarding the impact of changes in product formulation on the food safety of the product. A decrease in the pH value produced a decrease in the output due to the additional cell stress produced by the acidic environment followed by the initial microbial load at low treatment conditions (figure 2-d) and electric field at higher treatment conditions (figure 2-e). This fact demonstrates that the pH of the orange juice has a great influence on the food safety of the product. These results are in agreement with those obtained by Ferrer et al. (2007) where the PEF inactivation of *E. coli* cells was studied in an orange-carrot juice. They observed that at low treatment intensity, the initial microbial load was the most influential factor whereas with increasing treatment intensity, the electric field gained importance as well as the percentage of juice.

This study showed that the Monte Carlo simulation permitted to estimate the final number of microorganisms after PEF treatment of an orange juice and milk beverage with variable physicochemical characteristics (pH and pectin concentration). Moreover, through sensitivity analysis, it allowed to evaluate the influence of each of the variables of the process (*N₀*, *E*, pH and % pectin) on the final number of *Salmonella* cells. With these processes in place, the industry can quantitatively simulate, what the impact will be for a new product formulation or processing with a new technology on the food safety of the product and predict the final number of microorganisms after treatment at different food production steps. Lately, the Monte Carlo simulation can be a useful and practical tool for the industry to verify, that changes introduced meet their HACCP plan and current legislation.

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Figure 1: Survival curves of *Salmonella enterica* serovar Thyphimurium in juice-milk based beverage at different electric field strengths [(●) 15 kV/cm, (●) 25 kV/cm, (○) 35 kV/cm, (◇)] 40 kV/cm] and reference conditions (pH=4.5 and % pectin=0.3) adjusted to the Weibull model. The deviation standard was expressed by error bars.

Figure 2: Sensitivity analysis for input parameters. (a) Reference conditions [0.3%, E=15 kV/cm, t=40 µs, pH=4.5]; (b) Influence of E [0.3%, E=40 kV/cm, t=15 µs, pH=4.5]; (c) Influence of pectin concentration [0.6%, E=15 kV/cm, t=40 µs, pH=4.5]; (d) Influence of pH [0.3%, E=15 kV/cm, t=40 µs, pH=3.5]; (e) Influence of pH and E [0.3%, E=40 kV/cm, t=100 µs, pH=3.5].
Table 1: Model parameters and $Af$ values of Weibull distribution function for *S. typhimurium* survivor curves after PEF treatment at reference conditions (pH=4.5 and 0.3% pectin).

<table>
<thead>
<tr>
<th>E (kV/cm)</th>
<th>$a$ (µs)</th>
<th>$n$</th>
<th>Corr. $R^2$</th>
<th>$Af$</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>1629.31±120.52</td>
<td>0.40±0.09</td>
<td>0.986</td>
<td>1.006</td>
</tr>
<tr>
<td>25</td>
<td>235.60±62.67</td>
<td>0.39±0.05</td>
<td>0.972</td>
<td>1.010</td>
</tr>
<tr>
<td>35</td>
<td>56.23±24.37</td>
<td>0.25±0.04</td>
<td>0.978</td>
<td>1.010</td>
</tr>
<tr>
<td>40</td>
<td>25.43±9.32</td>
<td>0.28±0.03</td>
<td>0.989</td>
<td>1.011</td>
</tr>
</tbody>
</table>
Table 2: Estimates of parameter $a$ and confidence intervals (95%) by fitting Weibull model for *S. typhimurium* after PEF treatment varying the pH and pectin concentration.

<table>
<thead>
<tr>
<th>pH</th>
<th>% pectin</th>
<th>E (kV/cm)</th>
<th>15</th>
<th>25</th>
<th>35</th>
<th>40</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.5</td>
<td>0.1</td>
<td>496.38±59.03</td>
<td>-</td>
<td>-</td>
<td>11.48±1.89</td>
<td></td>
</tr>
<tr>
<td>4.0</td>
<td>0.1</td>
<td>972.46±138.56</td>
<td>-</td>
<td>-</td>
<td>26.72±5.42</td>
<td></td>
</tr>
<tr>
<td>4.5</td>
<td>0.1</td>
<td>1116.82±734.54</td>
<td>223.36±36.25</td>
<td>98.58±16.06</td>
<td>53.74±33.75</td>
<td></td>
</tr>
<tr>
<td>3.5</td>
<td>0.3</td>
<td>418.86±74.54</td>
<td>123.15±22.74</td>
<td>25.75±3.92</td>
<td>7.85±2.06</td>
<td></td>
</tr>
<tr>
<td>4.0</td>
<td>0.3</td>
<td>1043.44±208.29</td>
<td>201.96±17.38</td>
<td>56.23±10.69</td>
<td>16.04±8.00</td>
<td></td>
</tr>
<tr>
<td>4.5</td>
<td>0.3</td>
<td>1576.51±214.54</td>
<td>248.04±40.25</td>
<td>72.63±20.60</td>
<td>33.63±4.08</td>
<td></td>
</tr>
<tr>
<td>3.5</td>
<td>0.6</td>
<td>523.07±43.05</td>
<td>-</td>
<td>-</td>
<td>13.73±2.26</td>
<td></td>
</tr>
<tr>
<td>4.0</td>
<td>0.6</td>
<td>903.92±200.30</td>
<td>-</td>
<td>-</td>
<td>21.06±5.48</td>
<td></td>
</tr>
<tr>
<td>4.5</td>
<td>0.6</td>
<td>1144.36±245.68</td>
<td>301.23±23.06</td>
<td>63.58±9.81</td>
<td>33.80±10.24</td>
<td></td>
</tr>
</tbody>
</table>
Table 3: Model parameter values of the deterministic and Monte Carlo fit along with the probability distribution functions used in the analysis.

<table>
<thead>
<tr>
<th>Input parameter</th>
<th>Units</th>
<th>Deterministic value</th>
<th>Distribution function and its coefficients</th>
<th>Monte Carlo simulation value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary kinetic parameter ($a_{\text{ref (E, pH, %)}}$)</td>
<td>-</td>
<td>248.03±40.25</td>
<td>Beta general$^1$ (0.20; 0.19; 206.40; 292.12)</td>
<td>250.36± 36.27</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>at reference conditions</td>
<td></td>
</tr>
<tr>
<td>Initial number of microorganisms ($N_0$)</td>
<td>CFU/mL</td>
<td>3920225±1393483</td>
<td>Logistic$^2$ (3913586; 757909)</td>
<td>3913586±1374695</td>
</tr>
<tr>
<td>Rate change of $a$ with E ($z_E$)</td>
<td>kV/cm</td>
<td>15.27±0.46</td>
<td>Beta general (0.22; 0.19; 14.60; 15.72)</td>
<td>15.20± 0.47</td>
</tr>
<tr>
<td>Rate change of $a$ with pH ($z_{\text{pH}}$)</td>
<td>-</td>
<td>1.77±0.17</td>
<td>Beta general (0.29; 0.24; 1.47; 1.98)</td>
<td>1.75± 0.20</td>
</tr>
<tr>
<td>Rate change of $a$ with % pectin ($z_%$)</td>
<td>%</td>
<td>1.70±0.33</td>
<td>Beta general (0.23; 0.20; 1.09; 1.89)</td>
<td>1.51± 0.33</td>
</tr>
</tbody>
</table>

$^1$: Beta general ($\alpha_1$, $\alpha_2$, min, max)

$^2$: Logistic (a, b)
Table 4: Effect of process conditions on the final number of microorganisms generated by the Monte Carlo simulation

<table>
<thead>
<tr>
<th>pH</th>
<th>% pectin</th>
<th>E (kV/cm)</th>
<th>Time (µs)</th>
<th>Nx10³</th>
<th>Af</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(output parameter)</td>
<td></td>
</tr>
<tr>
<td>4.5</td>
<td>0.1</td>
<td>15</td>
<td>200</td>
<td>1110.10 ± 390.76</td>
<td>1.09</td>
</tr>
<tr>
<td>4.5</td>
<td>0.1</td>
<td>35</td>
<td>60</td>
<td>0.18 ± 0.02</td>
<td>1.13</td>
</tr>
<tr>
<td>3.5</td>
<td>0.1</td>
<td>15</td>
<td>100</td>
<td>40.31 ± 39.44</td>
<td>1.10</td>
</tr>
<tr>
<td>3.5</td>
<td>0.1</td>
<td>35</td>
<td>80</td>
<td>0.0033 ± 0.0022</td>
<td>1.26</td>
</tr>
<tr>
<td>4.5</td>
<td>0.3</td>
<td>15</td>
<td>150</td>
<td>1569.67 ± 531.99</td>
<td>1.06</td>
</tr>
<tr>
<td>4.5</td>
<td>0.3</td>
<td>35</td>
<td>40</td>
<td>2.34 ± 1.68</td>
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