Incorporating strain variability in the design of heat treatments: A stochastic approach and a kinetic approach

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A B S T R A C T

For the design of thermal processes, the decimal reduction times (D-values) of target organisms can be used. However, many factors influence the D-value, like inherent organism’s characteristics (strain variability), the effect of the history of the cells, as well as product factors and process factors. Strain variability is a very large contributor to the overall variation of the D-value. Hence, the overall reduction of microbial contaminants by a heat treatment is a combination of the occurrence of a strain with a certain heat resistance and its reduction given the prevailing conditions. This reduction can be determined using two approaches: a kinetic analysis based on integral equations or a stochastic approach based on Monte Carlo analysis. In this article, these two approaches are compared using as case studies the inactivation of two microorganisms: *Listeria monocytogenes* in a pasteurization process and the sporeformer *Geobacillus stearothermophilus* in a UHT process. Both approaches resulted in similar conclusions, highlighting that the strains with the highest heat resistance are determinant for the overall inactivation, even if the probability of cells having such extreme heat resistance is very low.

1. Introduction

The concepts of appropriate level of protection (ALOP) and food safety objective (FSO) have been set up to make food safety control transparent along the food chain. They enable the definition of quantitative targets for public health protection and for maximum tolerable contamination levels at different steps of the food production chain (ICMSF, 2018). To achieve an appropriate level of protection for a specific pathogen, this public health objective can be translated into a maximum tolerable concentration of the hazard in a food at the moment of consumption, the so-called FSO. When an FSO has been defined, the performance criteria (PC) can be set at the various food production steps using the ICMSF equation:

\[ H_0 + \Sigma I - \Sigma R \leq FSO \]  

(1)

with \( H_0 \) the initial level of the pathogenic contaminant, \( \Sigma I \) the sum of all increases of the hazard in the chain (due to growth and recontamination) and \( \Sigma R \) the sum of all reductions of the hazard. FSO, \( H_0 \), \( R \) and \( I \) are all expressed in log\(_{10}\) units.

If we have a process without growth and recontamination (i.e. \( \Sigma I = 0 \)) this results in:

\[ H_0 - \Sigma R \leq FSO \]  

(2)

with the FSO determined by the initial level, \( H_0 \), and the sum of the reductions. The reduction of the pathogen by, for example, a heat treatment can be determined using quantitative microbiology models (McMeekin et al., 2002). Users of quantitative microbiology request accurate models to predict behavior of microorganisms in food products. It should however be realized that exact models do not exist, since reality is very variable. Also, both the initial contamination level and the reduction will not be fixed values but be variable due to many different aspects (Zwietering, 2015).

For the design of heat treatments often the \( D \)-value is used to determine a target inactivation. The \( D \)-value is the decimal reduction time, which is the time it takes for 1 log\(_{10}\) (i.e. factor 10) reduction during an isothermal treatment. The \( z \)-value is the temperature increase needed to decrease the \( D \)-value by a factor of 10. In this paper we will focus on the variability in microbial reduction during a heat treatment. This variability in reduction is partly determined by the processing variables temperature and time, but will also depend on the product characteristic (e.g. pH and \( a_w \)) and variability therein, matrix effects, the specific strain of the organism, and the physiological state of the cells to be inactivated. In meta-analyses carried out for several pathogenic and spoilage organisms (Den Besten, Wells-Bennik, & Zwietering, 2018) it was found that the difference in the \( D \)-value can span up to two log\(_{10}\)
difference due to various effects, including but not limited to bacterial strain, product and history effects. It was concluded that variability between different strains is a major contributor to the overall variation and can result in more than a 1 log₁₀ difference in the D-value of different strains. This implies that when some strains of the organism are inactivated by a heat treatment for a specific time and temperature regime by 10 log₁₀ (i.e. factor 10⁵, so virtually fully inactivated), others are only inactivated by 1 log₁₀ (resulting in 10% survival). Although the chance of finding an extremely heat resistant strain is very low, these strains do have a much higher survival and determine the overall survival of a mixed population of strains. Therefore, strain variability can potentially be very relevant for the efficacy of the inactivation treatment. This was demonstrated using a kinetic approach for describing the effect of pasteurization on reduction of *Listeria monocytogenes* (Den Besten et al., 2018). This approach is further explored in this paper by comparing this kinetic analysis with a stochastic calculation to demonstrate how these different approaches can complement each other. Furthermore, sensitivity analyses are carried out to compare the impact of different influencing factors (time, temperature, the z-value, the mean of the log₁₀ D-value and its standard deviation) on various outcomes like the log₂ inactivation but also the arithmetic number of the survivors (and the reduction then based on this arithmetic number of survivors). The impact of strain variability on achieved reduction of *Listeria monocytogenes* when performing a pasteurization treatment is also compared for an ultra-high temperature treatment that aimed to inactivate spores of *Geobacillus stearothermophilus*.

2. Materials and methods

2.1. Heat resistance of *Listeria monocytogenes* and *Geobacillus stearothermophilus*

Aryani et al. (2015) quantified the strain variability in heat resistance of *L. monocytogenes* by determining the heat resistance of 20 strains that were isolated from various sources. The overall z-value based on the D-values determined at 55 °C, 60 °C, 65 °C and 70 °C was 5.22 °C (Den Besten et al., 2018). Den Besten et al. (2018) reported the mean and standard deviation of log₁₀ D₂₀ based on these 20 strains assuming that the log₁₀ D₂₀ is normally distributed with mean of -0.343 log₁₀(s) and standard deviation of 0.226 log₁₀(s). Wells-Bennik et al. (2019) followed a similar approach as Aryani et al. (2015) to quantify strain variability in heat resistance of *G. stearothermophilus* using spores of 18 different strains. The D-values were determined at 125 °C and 130 °C and resulted in an overall z-value of 11.1 °C. The mean heat resistance expressed in log₁₀ D₁₄₀ (log₁₀(s)) was 0.00385 with a standard deviation of 0.171.

2.2. Determination of overall inactivation using a kinetic approach

The reduction or log₁₀ inactivation, $R = \log₁₀(N / N₀)$, of the log₁₀(N/N₀) in a deterministic case in an isothermal case following log-linear inactivation, can be described by:

$$R = \log₁₀(N / N₀) = \frac{t}{D} = \frac{t}{10^{0.343}}$$  \hspace{1cm} (3)

With $N$, the number and $N₀$ the arithmetic fraction of survivors. The D-value is however not a constant value but varies because not all strains have the same thermal resistance. It can be assumed that the log₁₀ D-value can be described by a normal distribution, meaning that D is log-normally distributed (Aryani et al., 2015). In Den Besten et al. (2018) an integral equation was derived to determine the integrated lethal effect for a population of strains of which the D-value is log-normally distributed:

$$N / N₀ = \int_{-\infty}^{\infty} f(u)10^{-\frac{u^2}{2}} du$$  \hspace{1cm} (5)

With $f$ the probability density function of a normal distribution with expected value $\mu$ and standard deviation $\sigma$. The variable $u$ is a dummy variable for integration.

The overall inactivation was also determined using Monte Carlo simulations that were also performed in MS Excel 2013® and verified using @RISK®. The number of iterations was 1,000,000 to come to a stable outcome.

2.4. Sensitivity analyses

For a given fixed D-value the reduction $R$ as function of heating time would be expected to be linear:

$$R = \log₁₀(N / N₀) = t / 10^{D} = t \cdot 10^{-0.343}$$  \hspace{1cm} (8)

The effect of temperature on the log₁₀ of the reduction would also be expected to be linear:

$$\log₁₀R = \log₁₀t - \log₁₀D = \log₁₀t - \log₁₀Dref + \frac{T - Tref}{\xi}$$  \hspace{1cm} (9)

With Dref the D-value at the reference temperature ($Tref = 72 \degree C$), and $\xi$ the z-value.

The sensitivity for $R$ has been calculated analytically in Appendix A for several variables, taking into account that the log₁₀ D-value is not fixed but has a variability. The sensitivity analysis was also done with the overall number of survivors on arithmetic scale as output, taking the variability in log₁₀ D-value into account, and then the log₁₀ fraction of the survivors was calculated, log₁₀ $\left( \frac{N}{N₀} \right)$. Note, that this latter value is not equal to the average log₁₀ inactivation, since first the average is determined and afterwards the logarithm, and this gives another outcome than averaging the log₁₀-values.

Sensitivity analyses were performed in MS Excel 2013® and calculations were verified in R. The impact of variation in different variables on achieved reduction was tested, namely, variation in heating time, temperature, the z-value and the standard deviation of log₁₀ D. When testing one variable, the other variables were fixed at the reference condition, which was heat treatment for 15 s at 72 °C, z-value of 5.22 °C, and standard deviation of the log₁₀ D equals 0.226 log₁₀(s).
sufficient reduction of *L. monocytogenes* in milk (Bean et al., 2012). Strain variability in heat resistance is an important factor that determines the efficacy of a heat treatment to reduce all contaminants to an acceptable level. Aryani et al. (2015) quantified the strain variability in heat resistance and expressed the strain variability in the standard deviation of the log10*D*-value, assuming a normal distribution of the log10*D*-values, with a mean log10*D*-2*value and standard deviation of –0.343 log10(s) and 0.226 log10(s), respectively (Fig. 1, blue line). The following up study of Den Besten et al. (2018) calculated the achieved overall reduction taking into account this strain variability. This calculation was performed by a numerical integration (Equation (5)), combining the probability of a certain log10*D*-value and the achieved reduction, taking into account the whole probability distribution of log10*D*-values (Fig. 1, yellow line).

Based on an average log10*D*-value and corresponding D-value (10^{-0.343} s), 33.1 log10 inactivation would be achieved with a heat treatment for 15 s at 72 °C. However, when taking into account the strain variability in log10*D*-values and the probability to have these cells with this heat resistance, and thus integrating the effect of strain variability, a 7.8 log10 inactivation would be achieved. This value of 7.8 log10 inactivation is much lower than expected based on the average log10*D*-value, and is equivalent to the number of reductions calculated when assuming that every heat-treated cell had a D-value corresponding to the 99.7th percentile of the log10*D*-value distribution. Likewise, the 97.5th percentile would give 11.9 log10 inactivation and the 99th percentile 9.8 log10.

### 3.2. Overall inactivation using a Monte Carlo approach

Alternatively, the analysis to determine the overall reduction can be performed by a Monte Carlo analysis (Balsa-Canto, Alonso, & Banga, 2008). In this approach multiple random samples or draws are taken from the normal distribution of the log10*D*-value (Fig. 2). Subsequently, the distribution of D-values can be estimated by taking the exponent with base 10 of the sampled log10*D*-values [Fig. 3](#). The D-values that are calculated for the two extreme points that were highlighted in Fig. 2 (colored green: log10*D2 = 0.313 log10(s); and colored orange: log10*D2 = 0.325 log10(s)) are also shown in Fig. 3. It is obvious from these two data points that the inverse log10 transformation makes these points even more extreme.

For each of the simulated D2*values the reduction can be determined for a heat treatment of 15 s at 72 °C with 15/D [Fig. 4](#). The extreme points are again indicated using the same color code as for the previous figures. This figure illustrates that the majority (more than 80%) of the draws or iterations give 20–100 log10 inactivation, which can be considered as absolute inactivation, and these draws contribute only very limited to the overall achieved level of reduction. However, some random draws result in much lower reductions. It should be realized that a treatment causing 4 log10 inactivation differs from a treatment with 10 log10 inactivation by a factor of one million cells, so these few draws have a very relevant impact on the overall achieved reduction. For all random draws, the number of survivors can be

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**Fig. 1.** Probability density distribution of log10*D2*-values following a normal distribution (linear scale) (blue line), the number of cells having these log10*D2*-values assuming $N_0 = 10^8$ cells (log10 scale, right) (brown line, long-size dashes, this is the blue line (linear scale, left) multiplied with $10^8$), the number of cells from $10^8$ cells that would survive a 15 s heat treatment given such a D2*-value (log10 scale, right) (grey line, medium-size dashes, this is $10^{-10^{-15.09}}$), and the integrated number of surviving cells based on the log-normal distribution of D2*-values (log10 scale, right) (yellow line, small-size dashes, the combination of the brown long-size dashed line and the grey small-dashed line), thus combining the probability and the inactivation effect (adapted from Den Besten et al., 2018). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

**Fig. 2.** 1000 random draws from a normal distribution of log10*D2*-values (in log10(s)), with a distribution mean log10*D2 = –0.343 log10(s) and standard deviation 0.226 log10(s), and the density distribution of log10*D2* (grey line) of the random draws. Two high log10*D2*-values are indicated in orange and green. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

**Fig. 3.** 1000 random draws for the D2*-value from a log-normal distribution of D2*-values (in s), having as mean log10*D2 = –0.343 log10(s) and standard deviation 0.226 log10(s). The same high values in green and orange are indicated, but now on linear scale. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

**Fig. 4.** 1000 values for the reduction (R) of cells (-log10(Nf/Ni)) when heat treated at 72 °C for 15 s, based on random draws from a log-normal distribution of D2*-values (in s), having as mean log10*D2 = –0.343 log10(s) and standard deviation 0.226 log10(s), and the density function of R (grey line) of the random draws. This density function of R is skewed, but when the R-values are log10* transformed, then the density function becomes normal-shaped. The lower R-values of the distribution are most relevant and determine the number of survivors. The green and orange points are now on the low site of the graph (small reduction). The median is at 33 log10 inactivation. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
calculated assuming an initial population of $10^9$ cells (chosen as illustration for a population in a large quantity of food for example a bulk tank of milk) for each of the draws. To show more extreme values 10,000 random draws are represented in Fig. 5, with again the two extremes from the first 1000 random draws indicated using the same color code as above. The sum of the survivors of all draws is then the number of survivors from $10^5$ cells, being $10^2$ cells in each of the 10,000 random draws. This means that there are 10,000 draws of D-values, simulating variability in D-values, and that for each typical D-value there are initially $10^5$ cells present with this characteristic. By summing the surviving cells from the 10,000 random draws, the total number of survivors from $10^5$ cells can be determined. This resulted in about 25 cells surviving from 100,000 cells in 10,000 iterations, which is 25 cells from $10^5$ cells and this represents a fraction of surviving cells of 2.5·$10^{-5}$, indicating $7.6 \log_{10}$ inactivation. When these 10,000 iterations were repeated the outcome differed from 25 cells, e.g. the outcome was between 0.6 and 335 cells. The reason for that is the use of a numerical method whose truncation error depends on the number of iterations. Using 10,000 iterations the relative prediction error is rather high (25 and also values between 0.6 and 335 were output values), so 10,000 iterations was clearly not yet sufficient to give a stable estimate. Therefore, the number of iterations were increased to 1,000,000 draws, calculating an average reduction of $7.8 \log_{10}$ that remained stable when the calculations were repeated (meaning that 15 cells survived from $10^9$ cells).

This overall reduction based on this MC analysis is equal to the previously performed kinetic numerical analysis that also showed a $7.8 \log_{10}$ reduction (Den Besten et al., 2018) and confirms that both approaches - a kinetic analysis and a Monte Carlo simulation - result in an equal outcome.

These simulations were performed using the native functions included in MS Excel 2013®, but can also be reproduced in @RISK. Excel has as advantage that intermediate results and all specific simulation results are visible and can be investigated, but @RISK can more easily perform large number of random draws, i.e. iterations, and statistical analysis of the output is more easily represented. Therefore, the calculations were also reproduced in @RISK using the same simulation settings (parameter values and number of iterations).

In Table 1 it can be seen that, on average, there is a $37.8 \log_{10}$ reduction (Den Besten et al., 2018) and confirms that both approaches - a kinetic analysis and a Monte Carlo simulation - result in an equal outcome.

<table>
<thead>
<tr>
<th>$R (-\log_{10}N_t/ N_0)$</th>
<th>fraction survivors ($N_t/N_0$)</th>
<th>$\log_{10}$ fraction survivors ($\log_{10}(N_t/N_0)$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mode</td>
<td>25.34</td>
<td>9.039 $10^{-34}$</td>
</tr>
<tr>
<td>Median</td>
<td>33.04</td>
<td>-33.04</td>
</tr>
<tr>
<td>Average</td>
<td>37.84</td>
<td>1.562 $10^{-8}$</td>
</tr>
<tr>
<td>5th percentile</td>
<td>14.04</td>
<td>-7.80</td>
</tr>
<tr>
<td>1st percentile</td>
<td>9.85</td>
<td></td>
</tr>
</tbody>
</table>

The effect of the standard deviation of the $\log_{10}$D-value some curvature is visible and the effects are not fully linear.

The effect of temperature is very large, and therefore the numeric approach was used. First, the effect of heating time (Fig. 6A) and temperature (Fig. 6B) was investigated. Note that for a heat treatment time of 15 s at 72 °C the expected reduction is $7.8 \log_{10}$. It can be seen in Fig. 6A and 6B that due to the variability in the D-value some curvature is visible and the effects are not fully linear.

The results also depend on the assumed z-value (Fig. 6D). Aryani et al. (2015) determined the D-values of the 20 L. monocytogenes strains at 55 °C, 60 °C and 65 °C, and for the most heat resistant strain also at 70 °C, and we estimated the D-values based on extrapolation to 72 °C because inactivation at 72 °C goes too fast to be experimentally determined.

The effect of temperature is very large, and therefore the y-axis in Fig. 6B had to be $\log_{10}$-transformed. Clearly, the temperature is the

Fig. 5. Number of survivors from an initial total population of $10^9$ cells that are heat treated at 72 °C for 15 s. Survivors are calculated for each of the 10,000 random draws, randomly taken from the log-normal distribution of D2-values (in s), having as mean $\log_{10}(D_{25}) - 0.343 \log_{10}(s)$ and standard deviation 0.226 $\log_{10}(s)$, with each draw representing $10^2$ cells. Only those iterations with a number of survivors higher than 0.000001 cells is shown (most of the 10,000 points were lower; in this simulation 153 of the 10,000 points were above 0.000001, on average this was 172 out of 10,000).
factor that has the largest effect on control – only slight temperature deviations have a huge impact on achieved reduction, and tight temperature control is crucial.

3.4. Efficacy of UHT processing on inactivation of *Geobacillus stearothermophilus* spores

As a second example to illustrate how strain variability affects the achieved microbial reduction, we used the D-values of spores of 18 strains of *Geobacillus stearothermophilus*, determined at 125 °C and 130 °C (Wells-Bennik et al., 2019). Based on these 18 strains the strain variability in log$_{10}$D-values was determined and estimation of the D-values at different temperatures was done assuming a z-value of 11.1 °C (Wells-Bennik et al., 2019). The log$_{10}$ inactivation was determined for the average log$_{10}$D-value based on the 18 strains and on the 97.5th percentile using a numeric approach, and also using the Monte Carlo approach (Table 2).

When calculating the overall reduction taken into account the strain variability (i.e. $R_{\text{numeric}}$), the achieved reduction is about 3 log$_{10}$ lower than when using the average log$_{10}$D-value (i.e. $R_{\text{avgD}}$) when the heat treatment is performed at 120 °C for 1200 s, and the difference between these values becomes higher when the heat treatment becomes more severe. However, the inactivation of strains at the 97.5th percentile ($R_{97.5}$) show even lower reductions. In these simulations (both numeric and MC) the inactivation determined taking the whole distribution into account corresponds with the D-value at around the 90-95th percentile. The differences between $R_{\text{numeric}}$ and $R_{\text{avgD}}$ are less extreme than for *Listeria* presented above. This can be explained by two reasons. Firstly, the standard deviation for *Geobacillus* is lower (0.171 log$_{10}$D(s)) versus 0.226 log$_{10}$D(s) for *Geobacillus* and *Listeria*, respectively. We showed that especially the right-hand tail of the distribution of the log$_{10}$D-values determines the overall achieved reduction and when the standard deviation is lower, then also the prevalence of extremely highly heat resistant strains is lower. And, secondly, the targeted reduction in this second example is lower, since *Geobacillus* is a spoiler and not a pathogen. When for *Geobacillus* higher reductions are targeted (see for example heat treatment for 20 s at 145 °C), then also the more extreme parts of the log$_{10}$D-value distribution become more relevant and the values becomes higher when the heat treatment becomes more severe.

### Table 2

<table>
<thead>
<tr>
<th>$T$ (°C)</th>
<th>$t$ (s)</th>
<th>$R_{\text{average}}$ (s)</th>
<th>$R_{\text{avgD}}$ (log$_{10}$($N_t/N_0$))</th>
<th>$R_{\text{97.5 upper}}$</th>
<th>$R_{\text{97.5}}$ (log$_{10}$($N_t/N_0$))</th>
<th>$R_{\text{numeric}}$ (log$_{10}$($N_t/N_0$))</th>
<th>$R_{\text{MC}}$ (log$_{10}$($N_t/N_0$))</th>
</tr>
</thead>
<tbody>
<tr>
<td>120</td>
<td>1200</td>
<td>178</td>
<td>6.76</td>
<td>390</td>
<td>3.07</td>
<td>4.02</td>
<td>4.03</td>
</tr>
<tr>
<td>121.1</td>
<td>1000</td>
<td>141</td>
<td>7.07</td>
<td>311</td>
<td>3.22</td>
<td>4.14</td>
<td>4.15</td>
</tr>
<tr>
<td>125</td>
<td>500</td>
<td>63.1</td>
<td>7.92</td>
<td>139</td>
<td>3.60</td>
<td>4.46</td>
<td>4.46</td>
</tr>
<tr>
<td>130</td>
<td>180</td>
<td>22.4</td>
<td>8.02</td>
<td>49.4</td>
<td>3.65</td>
<td>4.50</td>
<td>4.50</td>
</tr>
<tr>
<td>135</td>
<td>60</td>
<td>7.98</td>
<td>7.52</td>
<td>17.6</td>
<td>3.42</td>
<td>4.31</td>
<td>4.32</td>
</tr>
<tr>
<td>140</td>
<td>20</td>
<td>2.84</td>
<td>7.05</td>
<td>6.24</td>
<td>3.20</td>
<td>4.13</td>
<td>4.14</td>
</tr>
<tr>
<td>145</td>
<td>10</td>
<td>1.01</td>
<td>9.91</td>
<td>2.22</td>
<td>4.51</td>
<td>5.15</td>
<td>5.16</td>
</tr>
<tr>
<td>145</td>
<td>20</td>
<td>1.01</td>
<td>19.8</td>
<td>2.22</td>
<td>9.01</td>
<td>7.77</td>
<td>7.76</td>
</tr>
</tbody>
</table>

For the log$_{10}$D (log$_{10}$D(s)) at 140 °C a Normal distribution (mean 0.00385, standard deviation 0.171) and a z-value of 11.1 °C was used.
differences between \( R_{\text{numeric}} \) and \( R_{\text{avgD}} \) is large again and the \( R_{\text{numeric}} \) gives a lower reduction than the 97.5th percentile of the log\(_2\)D-values. The values for the numeric approach and the Monte Carlo calculation are virtually equal, showing again that both approaches result in equal outcomes and can both be used to calculate the overall reduction.

4. Discussion

Food processors are producing very large volumes of foods, and then looking at efficacy of critical control steps at a large scale is of importance. As discussed in this paper, a factor that is very relevant for the efficacy of an inactivation treatment is strain-to-strain variation. However, this is a parameter inherent to microorganisms and cannot be controlled by the producer. Additionally, the physiological state of the organism (related to its history) can have a large impact (Den Besten et al., 2017; Richter et al., 2010; Smelt & Brul, 2014; Li & Ganzle, 2016), and this latter factor cannot be easily controlled either. Instead, producers must manipulate other factors that also influence the level of inactivation during a heat treatment. For instance, process characteristics such as temperature and duration, or properties of the product like water activity or pH. Additional matrix effects, like the fat content, might also be relevant (Verheyen et al., 2019a, Verheyen et al., 2019b, Verheyen et al., 2020).

In this analysis it is assumed that inactivation is log-linear, thus, not taking account of a curvature. This choice was made since other effects on inactivation, like \( T \), matrix and strain variability are much more affecting the inactivation efficacy for the microorganisms analyzed (Aryani et al., 2016; Van Asselt & Zwietiering, 2006; Welle-Bennik et al., 2019). Including a curvature parameter, for example using the Weibull model (Van Boekel, 2002), increases the complexity of the calculations and is hard to generalize for other microorganisms and conditions, due to the large variability of this value between different conditions.

Both the numeric-kinetic and the Monte Carlo approach give valuable insight and can be done in parallel. These approaches highlighted the relevance of the tails of the log\(_2\)D-value distribution, and the effect of introduced asymmetry when calculating the reduction. The kinetic approach shows in which part of the distributions most of the survivors are found (Fig. 1). The stochastic approach shows actual iterations of resulting survivors (Fig. 5). In the analysis performed in this study, the kinetic approach and the Monte Carlo approach gave similar outcomes, which is not surprising, but gives reassurance that the calculations are correctly implemented and that the outcomes are stable (sufficient number of iterations). The assessment of food safety is a complex topic, where calculation or methodological errors are possible (Zwietering, 2009). It is, thus, sensible to verify calculations, ideally applying a different approach, as done in this paper. Due to the skewness and asymmetry of the distributions and the application of non-linear mathematical transformations, it is relevant to investigate factors on different scales as we did with log\(_2\)D, reduction, and number of survivors (Tables 1 and 2). The average on one scale will not be at the same place as the average on the other scale (see Appendix B). For average consumer exposure, the arithmetic number of survivors is most relevant.

In this study we showed that a kinetic approach and a stochastic approach can be applied to determine the effect of strain variability on achieved microbial reduction. For the kinetic approach an integral equation (Eq. (5)) has to be solved numerically, making use of many small integration steps. For the numeric approach the integration steps need to be small enough to get a stable outcome, while for the stochastic approach the number of iterations needs to be large enough to get a stable outcome. Therefore, the truncation error is a source of uncertainty that impacts the outcome of the investigation. Its relevance should be investigated by decreasing the integration step-size and by increasing the number of iterations to check if the variable of interest converges within a reasonable tolerance. In both approaches, due to the relevance of the right-hand tail, many iteration (Monte Carlo) and many small time steps (numeric approach) were needed to come to stable outcomes.

Since both analyses give insight and also give a confirmation of the magnitude of the effect, it is advised to do both analyses instead of only one.

It should be realized that the prevalence of the extreme D-values based on the normal distribution determined with 20 strains, might be not fully accurate and representative. The estimation of the standard deviation based on many more strains might slightly differ and, as presented in this paper, small differences in standard deviation significantly affect the overall reduction. Furthermore, the distribution as quantified for the 20 strains will not be fully representative for the variability that will be found in practice. So, the ‘real’ distribution of log\(_2\)D-values can be different from the distribution based on the specific selection of 20 strains. Also, some strains can be more common in a specific food commodity and this also determines the expected microbial variability in the food of interest (Painset et al., 2019). On the other hand, a recent review comparing the strain variabilities among different species, including sporeforming and vegetative cells, concluded that the order of magnitude among species is rather comparable (Den Besten et al., 2018), though the exact values really differ.

It can be concluded from this paper that including the effect of strain-to-strain variability in heat resistance has a large impact on the obtained reduction, especially for harsh processes.

CRediT authorship contribution statement


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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2020.109973.

References


