A systematic approach to determine global thermal inactivation parameters for various food pathogens

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Abstract

Thermal inactivation of pathogens has been studied extensively, which has resulted in a wide range of D- and z-values. Estimating the inactivation rate for a specific condition based on these reported values is difficult, since one has to select representative conditions, and data obtained exactly at the required representative conditions are generally not available. Therefore, a first step could be to globally assess a heat treatment taking into account largest effects only. Once the most important parameters are known, a more precise study of inactivation can be performed. Therefore, in this study a large quantity of D-values \((n=4066)\) was collected from literature for various pathogens and linear regression was applied to obtain average D-values (together with the 95% upper prediction level) and z-values. When comparing these overall data, it can be seen that most factors reported to have an effect on the D-value are smaller than the variability of all published D-values. Even effects of shoulders disappear in the overall analysis. Only a limited number of factors that did have a significant effect \((p<0.05)\) on the D-value were identified: for Salmonella spp., the presence of chocolate ingredients gave protection to the cells, for Listeria monocytogenes the presence of 10% salt (or \(a_w < 0.92\)) resulted in a higher heat resistance, for Bacillus cereus there were significant differences for various strains and in oily products and for Clostridium botulinum there were significant differences in heat resistance between different types of C. botulinum. This does not mean that other effects do not occur, but it shows the main effects that have to be included for a first impression on the performance of a heating process. The obtained 95% upper prediction levels of the D-values can be used as a (conservative) estimate of inactivation and can be used to give order of magnitude values in overall process evaluations.

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1. Introduction

Many food-processing systems contain a heating step to reduce the number of bacteria in a product. This enhances food safety and increases the shelf-life of a product. To achieve these goals, the required time–temperature combinations are set, usually based on challenge tests, legislation and experience. In order to assess the adequacy of a heating step, one can estimate log reductions of bacteria based on the D/z-concept. This concept assumes a loglinear inactivation during time. Although literature sometimes reports a non-loglinear relationship, there is no general model available yet to describe these curves and the necessary parameters for these kind of models are also not readily available for all pathogens. Due to its broad applicability, the D/z-concept is most appropriate to obtain a first impression on the performance of an inactivation process. This is especially useful for the food industry where elaborate knowledge and the necessary tools for complicated models are not available. One can use the D/z-concept to globally determine what the most important pathogen is for a specific production process. Once this is known, one can decide to obtain more specific information for this pathogen in experiments with the required product. This may reveal a non-loglinear inactivation pattern, which can then be used to describe more precisely the inactivation of the pathogen in the desired product.
Over the years, various strains and a variety of products and laboratory media have been studied resulting in numerous D- and z-values for various circumstances (e.g. ICMSF, 1996; Doyle and Mazzotta, 2000; Doyle et al., 2001). Factors reported to have an influence on the heat resistance of a pathogen are amongst others: strain variations, presence of salt or acid, growth phase of the cells, experimental conditions, the products or laboratory media used etc. (Doyle et al., 2001). An overview of D/z-ranges for various pathogens in various products can be found for example in a report from the FDA/CFSAN (FDA/CFSAN, 2000).

When the effect of a heat inactivation has to be estimated for a given production process, it is difficult to choose the appropriate D-value from these lists of reported values. D-values are not always determined for the exact same conditions as are found in a production process. Moreover, the physiological state of the bacteria and the specific contaminating strain present is unknown. It is then unclear which D-value to use from the large datasets available and whether to include factors that may influence heat resistance. When there is an effect reported of a factor (e.g. food product), this is usually tested in one lab for a few conditions and a few strains. It is unclear whether such effects are relevant overall in comparison with other conditions.

In this study, general D- and z-values are estimated based on a large set of D-values and the importance of factors known to influence heat resistance is assessed. Heat inactivation is usually designed to improve the shelf-life of a product, but the inactivation of pathogens is important as well, since a product should also be safe. For example, in the fermentation of sausage the pH-decrease is insufficient to inactivation is usually designed to improve the shelf-life of known to influence heat resistance is assessed. Heat inactivation of pathogens only. The obtained parameters can be used to evaluate the performance of a heat treatment and can be applied in the exposure assessment part of a microbiological risk assessment, especially in a first screening phase.

2. Materials and methods

2.1. Data collection

D-values were collected from literature for different temperatures for various strains and in various products or media tested. Data were organised in Excel spreadsheets including all relevant details: strain used, product tested, addition of components (salts, sugars) etc. Pathogens studied were: Bacillus cereus (n=484), Campylobacter spp. (n=46), Clostridium botulinum (n=375), Clostridium perfringens (n=210), Enterobacter sakazakii (n=79), Escherichia coli (n=382), Listeria monocytogenes (n=967), Salmonella spp. (n=1161), Staphylococcus aureus (n=243), Streptococcus pyogenes (n=11), Vibrio spp. (n=43) and Yersinia enterocolitica (n=63).

In case shoulders were mentioned, this was incorporated in the D-value by assuming a 5D reduction. The average D-value for a certain temperature was then estimated as:

\[ D_{\text{average}} = \frac{(5D + \lambda)}{5} \]  
with:

\[ D \] is the amount of heating time needed to obtain a 1-log reduction (min)
\[ \lambda \] is the shoulder, time before a micro-organism starts to be inactivated (min)

2.2. Inactivation model

Once the D-value at a certain reference temperature is known, D-values can be estimated for every desired temperature:

\[ \log D = \log D_{\text{ref}} - \frac{(T - T_{\text{ref}})}{z} \]  
with:

\[ \log D \] is the logarithm of the D-value (log min)
\[ \log D_{\text{ref}} \] is the log D-value at \( T_{\text{ref}} \) (log min)
\[ T_{\text{ref}} \] is the reference temperature (°C)
\[ z \] is the temperature increase (°C) needed to reduce the D-value with a factor of 10

In order to determine an overall \( \log D_{\text{ref}} \) for each pathogen, the obtained D-values from literature were log transformed to obtain a linear relationship with temperature. These log D-values were then plotted versus temperature and linear regression was applied to obtain the z-value and the log D-value at a reference temperature as follows:

\[ \log D_{\text{ref}} = \text{intercept}(\log D, T) - T_{\text{ref}}/z \]  
\[ z = -1/\text{slope}(\log D, T) \]  

For spores, a reference temperature of 120 °C was used and for vegetative cells a temperature of 70 °C, since these temperatures are in the range used in heating processes.

A 95% prediction interval (PI) was calculated for \( \log D_{\text{ref}} \) as:

\[ \log D_{\text{ref}} \pm t_{DF,1-0.05} \sqrt{RSS/DF} \]  
with:

\[ t_{DF} \] is the student t-value with DF degrees of freedom (number of points—2) and confidence level \( \alpha \) (\( \alpha=0.05 \))
\[ RSS \] is the residual sum of squares, calculated from the deviation of the data from the regression line

The upper 95% value for \( \log D_{\text{ref}} \) can be used as a conservative estimation of \( D_{\text{ref}} \).
### 2.3. Statistical analysis

Log $D$-values were plotted versus the temperature to visually determine whether there were differences between pathogenic strains, food products or other factors. If this was the case, an $F$-test was performed on the slope and intercept of log $D$ and temperature to determine whether the differences were significant. A significance level of 5% was used.

#### Table 1
Mean log $D$-values at reference temperature $T_{ref}$, upper 95% PI for log $D_{ref}$ and $z$-values for various pathogens

<table>
<thead>
<tr>
<th>Micro-organism</th>
<th>Product</th>
<th>$T_{ref}$ (°C)</th>
<th>$z$ (°C)</th>
<th>Log $D_{ref}$ (mean) (min)</th>
<th>Log $D_{ref}$ (95% PI) (min)</th>
<th>$\sigma$</th>
<th>$n$</th>
<th>References$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus cereus</td>
<td>Various</td>
<td>120</td>
<td>12.8</td>
<td>$-1.38$</td>
<td>$-0.28$</td>
<td>0.56</td>
<td>465</td>
<td>10, 22–26, 29, 34, 36, 48, 57</td>
</tr>
<tr>
<td>Bacillus cereus (heat resistant)$^a$</td>
<td>Oily products$^a$</td>
<td>120</td>
<td>12.1</td>
<td>$0.53$</td>
<td>$1.54$</td>
<td>0.48</td>
<td>48</td>
<td>19, 10, 29, 34, 48</td>
</tr>
<tr>
<td>Campylobacter spp.</td>
<td>Various</td>
<td>70</td>
<td>12.3</td>
<td>$-0.96$</td>
<td>$0.05$</td>
<td>0.50</td>
<td>46</td>
<td>1, 25, 34, 68</td>
</tr>
<tr>
<td>Clostridium botulinum proteolytic</td>
<td></td>
<td>Various</td>
<td>120</td>
<td>10.2</td>
<td>$-0.78$</td>
<td>$-0.32$</td>
<td>0.23</td>
<td>176, 1, 25, 34</td>
</tr>
<tr>
<td>Clostridium botulinum non-proteolytic types (ABF)</td>
<td></td>
<td>Various</td>
<td>120</td>
<td>18.6</td>
<td>$-1.47$</td>
<td>$-0.07$</td>
<td>0.71</td>
<td>175, 1, 25, 34, 37, 39, 45, 55, 56, 60</td>
</tr>
<tr>
<td>Clostridium botulinum proteolytic type G</td>
<td></td>
<td>Various</td>
<td>120</td>
<td>34.0</td>
<td>$-0.60$</td>
<td>$-0.22$</td>
<td>0.18</td>
<td>24, 34</td>
</tr>
<tr>
<td>Clostridium perfringens spores</td>
<td>Various</td>
<td>120</td>
<td>16.8</td>
<td>$-0.52$</td>
<td>$0.43$</td>
<td>0.48</td>
<td>64</td>
<td>11, 25, 34, 51</td>
</tr>
<tr>
<td>Clostridium perfringens vegetative cells</td>
<td></td>
<td>Various</td>
<td>70</td>
<td>10.3</td>
<td>$-0.42$</td>
<td>$0.32$</td>
<td>0.37</td>
<td>146, 34, 40, 41, 53, 59</td>
</tr>
<tr>
<td>Enterobacter sakazakii</td>
<td>Various</td>
<td>70</td>
<td>6.3</td>
<td>$-1.51$</td>
<td>$-0.57$</td>
<td>0.47</td>
<td>79</td>
<td>12, 21, 35, 52</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>Various</td>
<td>70</td>
<td>10.6</td>
<td>$-0.67$</td>
<td>$0.54$</td>
<td>0.62</td>
<td>382</td>
<td>1, 7, 12, 15, 17, 25, 28, 32–34, 49, 58, 62, 63, 65, 67</td>
</tr>
<tr>
<td>Listeria monocytogenes</td>
<td>Various</td>
<td>70</td>
<td>7.0</td>
<td>$-1.06$</td>
<td>$-0.28$</td>
<td>0.40</td>
<td>940</td>
<td>1, 8, 9, 13, 14, 18, 20, 25, 30, 31, 34, 46, 49, 50</td>
</tr>
<tr>
<td>Listeria monocytogenes</td>
<td></td>
<td>Salted (10%)</td>
<td>70</td>
<td>9.2</td>
<td>$0.18$</td>
<td>0.78</td>
<td>0.29</td>
<td>27, 20</td>
</tr>
<tr>
<td>Salmonella spp.</td>
<td>Various</td>
<td>70</td>
<td>9.1</td>
<td>$-0.83$</td>
<td>$0.59$</td>
<td>0.72</td>
<td>1141</td>
<td>1, 2, 4, 7, 12, 16, 19, 25, 28, 34, 38, 43, 47, 49, 50, 61, 63, 66</td>
</tr>
<tr>
<td>Salmonella spp.</td>
<td></td>
<td>Chocolate</td>
<td>70</td>
<td>20.4</td>
<td>$2.65$</td>
<td>3.04</td>
<td>0.19</td>
<td>20, 19, 34</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>Various</td>
<td>70</td>
<td>8.8</td>
<td>$-0.59$</td>
<td>$0.33$</td>
<td>0.47</td>
<td>204</td>
<td>6, 25, 27, 34, 42, 54, 64, 66</td>
</tr>
<tr>
<td>Streptococcus pyogenes</td>
<td>Various</td>
<td>70</td>
<td>9.2</td>
<td>$-1.45$</td>
<td>$-0.15$</td>
<td>0.57</td>
<td>11</td>
<td>34</td>
</tr>
<tr>
<td>Vibrio cholerae</td>
<td>Crableat</td>
<td>70</td>
<td>16.7</td>
<td>$-0.25$</td>
<td>$0.34$</td>
<td>0.19</td>
<td>5</td>
<td>34</td>
</tr>
<tr>
<td>Vibrio cholerae</td>
<td>Pepton water</td>
<td>70</td>
<td>21.8</td>
<td>$-0.72$</td>
<td>$-0.48$</td>
<td>0.05</td>
<td>4</td>
<td>34</td>
</tr>
<tr>
<td>Vibrio parahaemolyticus,</td>
<td>Various</td>
<td>70</td>
<td>8.5</td>
<td>$-2.24$</td>
<td>$-1.30$</td>
<td>0.46</td>
<td>34</td>
<td>3, 5, 34</td>
</tr>
<tr>
<td>Vibrio vulnificus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yersinia enterocolitica</td>
<td>Various</td>
<td>70</td>
<td>6.2</td>
<td>$-1.80$</td>
<td>$-0.91$</td>
<td>0.44</td>
<td>63</td>
<td>1, 8, 25, 34, 44</td>
</tr>
</tbody>
</table>

$D$ is the prediction interval; $z$ is the temperature increase needed to reduce the $D$-value with a factor of 10 (°C); $T_{ref}$ is the reference temperature (°C); Log $D_{ref}$ is the logarithm of the $D$-value (the amount of heating time needed to obtain a 1-log reduction (min)) at $T_{ref}$; 95% log $D_{ref}$ is the upper 95% prediction level of log $D_{ref}$; $\sigma$ is the standard deviation of the dataset ($\sqrt{RSS/DF}$ with RSS is residual sum of squares and $DF$ is degrees of freedom); $n$ is the amount of data used for the linear regression.

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3. Results

3.1. Data analysis

A summary of all estimated log $D_{ref}$ and $z$-values together with the 95% upper prediction level for log $D_{ref}$ is given in Table 1. Fig. 1 gives an overview of the relation between the log $D_{ref}$-values (using the 95% upper prediction level) and the temperature for all micro-organisms. As can be seen in this graph, spore formers clearly have a higher heat resistance than vegetative cells, as is expected. The same effect can be seen for C. perfringens spores and vegetative cells (Table 1). Statistical analysis showed that the log $D$-values for these spores and vegetative cells were significantly different ($p=0.0007$). Fig. 1 also shows that the slope of the lines for spore formers is usually smaller than for vegetatives indicating that the $z$-values between these two groups are different. Exceptions are for Salmonella spp. present in chocolate with a $z$-value of 20.4 °C and V. cholerae with $z$-values of 16.7 and 21.9 °C. All other vegetatives have lower $z$-values between 6.2 and 10.6 °C.

For most pathogens, there were no significant differences between various strains, products or laboratory media tested. Furthermore, there was no significant effect of pH and $a_w$ on the inactivation of the pathogens. This is mainly due to the large variability found. Some pathogens did show differences. A visual interpretation of the $D$-values of Salmonella spp., for example, showed that chocolate has a protective effect on Salmonella (Fig. 2). The Log $D$-value at $T_{ref}=70$ °C is more than 3 logs higher in chocolate than in other food products. Statistical analysis showed that this difference was significant ($p<0.0001$) even though there was a large variability in the dataset. Apparently, the products in which pathogens are present can sometimes largely influence the heat resistance of these micro-organisms. Fig. 3 shows that L. monocytogenes is more heat resistant in the presence of 10% salt or when the water activity ($a_w$) is below 0.92. The log $D_{ref}$ for these products with low $a_w$ is 0.18 compared to a log $D_{ref}$ of $-1.06$ for all other products. V. cholerae also showed significant differences in heat resistance depending on the product in which the pathogen was present. However, only data for two products (crabmeat and peptone water) were available and the number of data was limited (total of 9 samples). S. aureus showed increased heat resistance when present in a combination of mackerel and oil (Gaze, 1985). Although the authors designate this to the protective effect of oil, this effect was only seen for one of the two strains tested. This strain, strain NCTC 10625, therefore appeared to be significantly different from all other S. aureus strains with a log $D_{ref}$ of 0.48 (96% upper level at 1.02) and a $z$-value of 31.4 °C ($n=39$). However, data for strain 196E (which according to Bean and Roberts (1975) is the same strain as strain NCTC 10652) and additional data for strain NCTC 10652 (Thomas et al., 1966; Bean and Roberts, 1975; Patterson and Kilpatrick, 1998) did not show significant differences with other S. aureus strains. Therefore, since the $D$-values for strain NCTC 10652 from Gaze (1985) could not be linked to a strain nor to a product effect, these data were not used in the overall linear regression on reported $D$-values.

For B. cereus there was indeed a protective effect of oil resulting in increased heat resistance in oily products. Apart from this product effect, specific strains also had an effect on the heat resistance. Such effects are reported more often. For example, S. senftenberg shows higher $D$-values than other Salmonella strains (Doyle and Mazzotta, 2000). However, this factor appeared to be irrelevant compared to the large variation in $D$-values found for Salmonella spp. Even within the same product (tested for beef products and eggs), there were no

![Fig. 1. Heat resistance of all pathogens using the 95% prediction upper level as given in Table 1.](image-url)
significant differences ($p > 0.05$). For *C. botulinum*, significant differences were found between proteolytic types (ABF), non-proteolytic types (BCEF) and the proteolytic type G. Some papers also mention an effect of lysozyme in the recovery medium. This lysozyme is assumed to improve the germination of spores resulting in a higher recovery of *C. botulinum* (Scott and Bernard, 1985; Peck et al., 1993). Although in some cases we did find differences for treatment with and without lysozyme, the differences were not significant ($p = 0.15$) compared to the variability of the data.
3.2. Effect of shoulders

Some papers mentioned a shoulder when heating a product (Holsinger et al., 1992; Juneja and Eblen, 2000). During such a lag phase, no inactivation takes place. Fig. 4 gives an example of Salmonella spp. in beef with a lag phase of 28.1 min (highest lag phase found) and a D-value of 1.61 min at 58 °C (values from Juneja and Eblen (2000)). The lag times reported by Juneja and Eblen (2000) included the time needed to heat the beef samples, which was negligible. As can be seen in this figure, ignoring such a lag phase results in an overestimation of the inactivation of the pathogen (dotted line). The D-value can be adjusted for such a lag phase as given in Eq. (1), which results in slower inactivation (given by the grey line). The difference in inactivation with and without lag times seems to be quite large in one dataset. However, when evaluating a large dataset as is shown in Fig. 5 for Salmonella spp. in beef (Thomas et al., 1966; ICMSF, 1996; Blackburn et al., 1997; Doyle and Mazzotta, 2000; Forsythe, 2000; Smith et al., 2001), including D-values with and without lag times (data from Juneja and Eblen (2000)), the overall effect of lag times is negligible compared to the variation in reported D-values in beef.

3.3. Practical implications

The D-values obtained in the data analysis and described in Table 1 can be used to globally assess the performance of a heating step in a production process. As an example, log reductions of pathogens are estimated based on common heating procedures. For this purpose, a pasteurisation process (15 s 72 °C), a sterilisation process (3 min 120 °C) and a UHT process (2 s 140 °C) were used to estimate survival of pathogens (Fig. 6). These graphs clearly show which pathogens are the least heat resistant (Vibrio spp.) and which pathogen is the most heat resistant (heat resistant spores from B. cereus). It can also be seen that sterilisation is more effective in reducing spore formers and heat resistant pathogens than UHT. This is due to the fact that the z-value of spores is larger than 10 °C. If z = 10 °C, then heating at 120 °C for 180 s gives the same reduction as heating at 140 °C for 2 s (see C. botulinum proteolytic types). If z = 20 °C, then to obtain an equal reduction for heating at 120 °C for 180 s, a heating time of 18 s at 140 °C is necessary. Therefore, the larger the z-value, the larger the difference between sterilisation (3 min 120 °C) and UHT (2 s at 140 °C).

Pasteurisation gives a 6-log reduction for L. monocytogenes using the average D-values (Fig. 6a) and only a 1-log reduction using the 95% upper prediction level (Fig. 6b). Spore formers in both cases are not inactivated, which is to be expected. The 95% upper prediction levels can be seen as worst-case estimations to design a fail-safe process. For example, in the production of acid based spread, the first heating step is 1 min at 92 °C and S. aureus is the most important pathogen for this product (van Gerwen, 2000). Using the 95% upper prediction level, a reduction of −152 log units is obtained at this temperature. Calculations based on linear inactivation show that this heating temperature can be reduced to 85 °C giving a reduction of 24 log units, which is largely sufficient to inactivate all S. aureus present. However, the present heating step is probably required to achieve the desired shelf-life of the product. It is demonstrated that such calculations can show if a pathogen is sufficiently reduced and thus that the production process can be regarded as safe. On the other hand, if calculations show that reduction is not sufficient more specific data may be necessary.

4. Discussion

4.1. Effect of low \( a_w \)

L. monocytogenes can be as much as eightfold more heat resistant than Salmonella spp. when tested in eggs under the

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**Fig. 4.** Example of linear inactivation using \( \log N = \log N_0 - t/D \), with \( N_0 \) as the initial number of micro-organisms (cfu/g), \( D \) is the D-value (min) and \( t \) is the time (min). In this example the lag time was 28.1 min and the D-value was 1.61 min at 58 °C (Juneja and Eblen, 2000). The black solid line gives inactivation with a lag phase. The dotted line gives inactivation when the lag phase is neglected and the solid grey line gives inactivation when the lag time is included in the D-value resulting in an average D-value of 7.23 min (using Eq. (1)).

**Fig. 5.** Log D-values for Salmonella spp. in beef. Diamonds are D-values from papers where no lag time was reported. Squares are data from Juneja and Eblen (2000), who reported lag times. These lag times were incorporated in the D-values using Eq. (1). Triangles are the same data as the squares, only in this case the lag time was ignored.
same experimental conditions (Doyle et al., 2001). However, in the current study, *L. monocytogenes* (without added salt in the product) is found to be less heat resistant than *Salmonella* spp. Apparently, when data of various products and conditions are combined, *L. monocytogenes* is overall less heat resistant than other pathogens. Fig. 6 and Table 1 on the other hand show that when *L. monocytogenes* is present in a low $a_w$ environment, it becomes the most heat resistant vegetative pathogen. Therefore, there is a significant effect of $a_w$ for *L. monocytogenes*. This effect can be caused by cross-protection, i.e. pre-adaptation to one environmental stress (a low $a_w$) can lead to enhanced resistance to another stress, like heat (Lou and Yousef, 1999). Various studies on *Salmonella* spp. also showed that there is an effect of $a_w$ on heat resistance and that this effect depends on the solutes used to decrease $a_w$ (Goepfert et al., 1970; Corry, 1974; Mattick et al., 2000). However, the reported effects of solutes and $a_w$ on $D$-values of *Salmonella* are conflicting and it is therefore difficult to determine a direct relationship between $a_w$ and heat resistance (Baird-Parker et al., 1970; Goepfert et al., 1970; Corry, 1974). This may be the
reason why there was no significant effect of $a_w$ on heat resistance for *Salmonella* spp. in the overall dataset. The fact that *Salmonella* is more heat resistant in chocolate than in other products can, however, partly be attributed to its low $a_w$. Another important factor in this case is the fat content in chocolate that may protect the cells from high temperatures. This effect was also seen for *B. cereus* in oily products. It can be assumed that other products with high fat content and low $a_w$ also have a protective effect on the heat inactivation of bacteria.

4.2. Shoulders

In most cases, inactivation follows a linear decline with time. However, sometimes shoulder or tails are found. These may be due to various reasons: artefacts caused by limitations of the experimental procedure, heterogeneity within the population or heat adaptation during a heat treatment (Stringer et al., 2000). In cases where shoulders or tails are found a loglinear relation does not give a good fit of the inactivation data and other models will give better results, like a loglogistic (Stringer et al., 2000) or a Weibull model (van Boekel, 2002). However, overall for all inactivation data, such effects are negligible given the large variations due to for example strain or lab differences as was seen in Fig. 5. Approximation with a straight line is then sufficient to obtain order of magnitude values, which gives valuable information for the food industry in order to adjust their heating processes.

4.3. Strain variability

Based on their characteristics, *C. botulinum* is divided in 4 subgroups: group I containing proteolytic types (ABF), group II containing non-proteolytic types (BEF), group III containing non-proteolytic types (CD) and group IV containing proteolytic type G (ICMSF, 1996). Statistical analysis on the heat resistance of all types (except for type D for which there were no data) showed that non-proteolytic type C could be combined with the other non-proteolytic types B, E and F. These non-proteolytic types had a significantly lower heat resistance than the proteolytic types. This effect is described in literature more often and is the reason why proteolytic strains were used for the development of the botulinum cook or 12D process (ICMSF, 1996). Type G showed a different heat resistant pattern, but this strain has not been isolated from foods (ICMSF, 1996), so is less important to incorporate in a process evaluation.

*B. cereus* has been known to have considerable strain variability (ICMSF, 1996; González et al., 1999; Mazas et al., 1999). Indeed, in our dataset we saw significant differences for various strains, with one unidentified strain, which was isolated from food (Bradshaw et al., 1975) and strain 9818 as being the most heat resistant. Since in a production process the strain type is usually unknown, values for the heat resistant spores can be used in worst-case estimations. For *C. perfringens* there were significant differences between spores and vegetative cells, which is logical since spores are known to be more heat resistant than vegetative cells. Therefore, in order to estimate the heat inactivation of *C. perfringens* one needs to know in which state they are present in a food product. Since this is usually unknown and often both spores and vegetative cells are present, the values for spores can be used as a worst-case approach, as is also done for *B. cereus* and *C. botulinum*.

*S. senftenberg* 775W is usually reported as being more heat resistant than other *Salmonella* strains (Doyle and Mazzotta, 2000). This effect is mainly reported for moist foods (ICMSF, 1996), but by lowering the water activity other strains become more heat resistant (Goepfert et al., 1970). In the established dataset all sorts of food products are represented (with a range of $a_w$-values). This may be the reason why, overall for all data ($n=1161$), the heat resistance of *S. senftenberg* ($n=220$) appeared to be irrelevant compared to the variability in $D$-values reported for all *Salmonella* spp.

Data from Gaze (1985) showed that *S. aureus* strain NCTC 10652 had significantly higher $D$- and $z$-values than other *S. aureus* strains. Other papers using the same strain did not confirm these results (Thomas et al., 1966; Bean and Roberts, 1975; Patterson and Kilpatrick, 1998). The $D$-values from Gaze (1985) were calculated from the tail of the survival curve, indicating a heat resistant subpopulation. However, the same procedure was followed for strain 68-7895 in the same product resulting in $D$-values comparable to all other *S. aureus* $D$-values (Gaze, 1985). It is, therefore, unclear what caused the higher $D$-values for strain NCTC 10652 as reported by Gaze (1985).

4.4. Practical implications

In some cases, very few data were available (for *V. cholera* only 9 samples were found and for *S. pyogenes* only 11 samples), which makes it difficult to draw general conclusions regarding the heat resistance of these pathogens. More data are needed to obtain reliable results for these pathogens. For the other pathogens, it is shown that it is possible to obtain general $D$-values that can be used in a process evaluation to globally assess the inactivation of the pathogen. Although numerous factors can affect $D$-values, overall, these effects appear to be negligible compared to the large variations in $D$-values for various sources (see Figs. 2 and 3). In order to obtain order of magnitude values for inactivation of pathogens, one should only incorporate those factors that have a substantial effect on $D$-values. For example, the protective effect of chocolate on survival of *Salmonella* is much more important than the strain variability or the role of shoulders in inactivation. For specific conditions and strains, various effects can be of relevance and for example non-linear behaviour can be important to include. For an initial overview however, general inactivation parameters are of large practical relevance. For example, one can determine whether a specific measurement is within the overall range for a pathogen. If for a *Bacillus* spp. a log $D_{121}$ of 0 is found (at 120 °C), it is clearly out of the normal *Bacillus* range (between −2.48 and −0.28; see Table 1), but can be defined as in the high *Bacillus* range (between −0.48 and 1.54). Furthermore, the data given in Table 1 can be used to assess heat reduction in a production process using linear inactivation

### Table 1

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>$D_{121}$ (min)</th>
<th>$D_{121}$ (max)</th>
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<tr>
<td><em>Salmonella</em></td>
<td></td>
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<tr>
<td><em>Bacillus</em></td>
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kinetics as is shown in Fig. 6. This estimation can be used to generally assess the performance of a heating step. When more specific information is available (for example specific D-values for the product one is interested in), this can be used to obtain a more accurate prediction of heat inactivation. It is clear that many factors can have an effect on D-values in specific studies and thus can be of relevance for specific processes. For an initial global analysis, however, first the main effects have to be taken into account as have been identified in this study.

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References


