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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.

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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 inactivate *Escherichia coli* O157:H7. Therefore, additional heating is required to obtain a 5D reduction of the pathogen (Beumer, 2001). Another example is the use of cook–chill systems where recontamination can play an important role. Therefore, the reheating step in this system should be sufficient to inactivate pathogens present. In this paper, we focus on 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:

$$D_{average} = (5D + \lambda)/5 \tag{1}$$

with:

> *D* is the amount of heating time needed to obtain a 1-log reduction (min)
> λ 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:

$$\log D = \log D_{ref} - (T - T_{ref})/z \tag{2}$$

with:

> log *D* is the logarithm of the *D*-value (log min)
> log D_{ref} is the log *D*-value at T_{ref} (log min)
> T_{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_{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:

$$\log D_{ref} = \text{intercept}(\log D, T) - T_{ref}/z \tag{3}$$

$$z = -1/\text{slope}(\log D, T) \tag{4}$$

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_{ref} as:

$$\log D_{ref} \pm t_{DF,1-0.5\alpha} \sqrt{\frac{RSS}{DF}} \tag{5}$$

with:

> t_{DF} is the student *t*-value with *DF* degrees of freedom (number of points—2) and confidence level α ($\alpha = 0.05$)
> *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_{ref} can be used as a conservative estimation of log D_{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

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

PI 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}; σ is the standard deviation of the dataset ($\sqrt{\frac{RSS}{DF}}$ with RSS is residual sum of squares and DF is degrees of freedom); n is the amount of data used for the linear regression.

[a] *B. cereus* is more heat resistant when present in olive oil, soybean oil or pumpkin pie or when strain 9818 was present in 7.7 M glycerol. One other heat resistant strain present in phosphate buffer also showed increased heat resistance comparable to these products.

[b] References:

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4. Baird-Parker et al., 1970
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9. Bradshaw et al., 1987
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11. Bradshaw et al., 1977
12. Breeuwer et al., 2003
13. Casadei et al., 1998
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15. Clavero et al., 1998
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21. Edelson-Mammel and Buchanan, 2004
22. Fernandez et al., 2001
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31. Holsinger et al., 1992
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33. Huang and Juneja, 2001
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37. Juneja and Eblen, 1995
38. Juneja and Eblen, 2000
39. Juneja et al., 1995
40. Juneja and Marmer, 1998
41. Juneja et al., 2001
42. Kornacki and Marth, 1989
43. Lee et al., 1989
44. Lovett et al., 1982
45. Lynt et al., 1979
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49. Mazzotta, 2001
50. Murphy et al., 2003
51. Nakamura and Converse, 1967
52. Nazarowec White and Farber, 1997
53. Novak et al., 2001
54. Patterson and Kilpatrick, 1998
55. Peck et al., 1993
56. Peterson et al., 1997
57. Rajkowski and Mikolajcik, 1987
58. Riordan et al., 2000
59. Roy et al., 1981
60. Scott and Bernard, 1985
61. Shah et al., 1991
62. Singh and Ranganathan, 1980
63. Smith et al., 2001
64. Stiles and Witter, 1965
65. Stringer et al., 2000
66. Thomas et al., 1966
67. Whiting and Golden, 2002
68. Yang et al., 2001

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 ($p \ll 0.0001$) 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
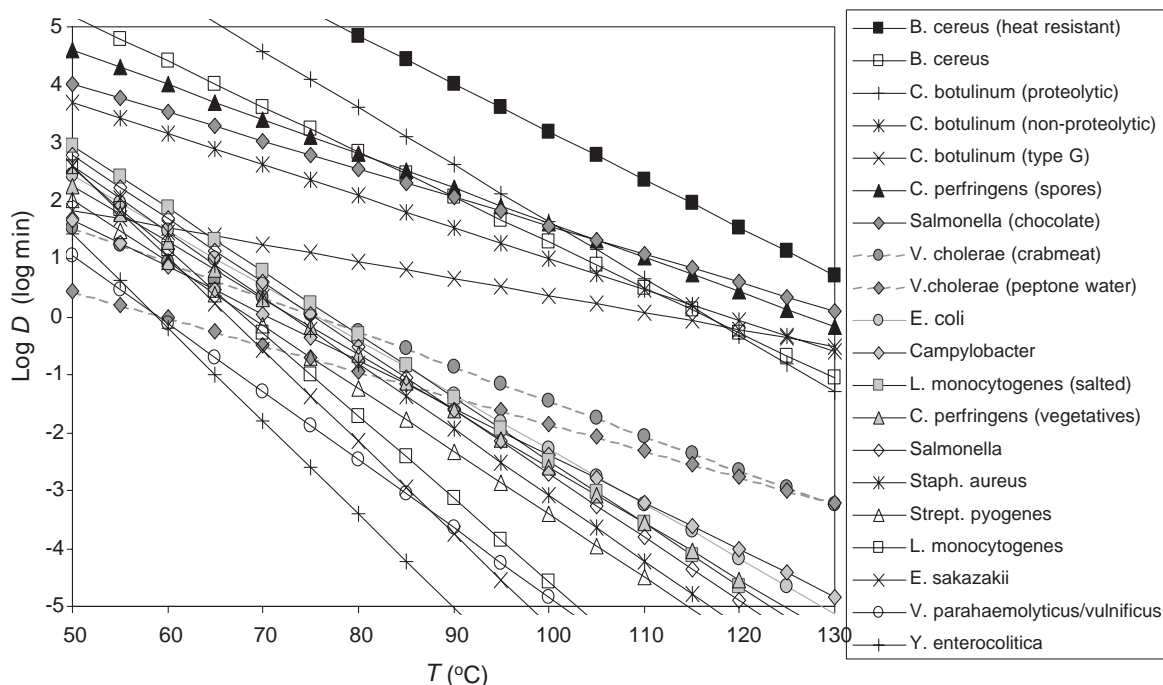

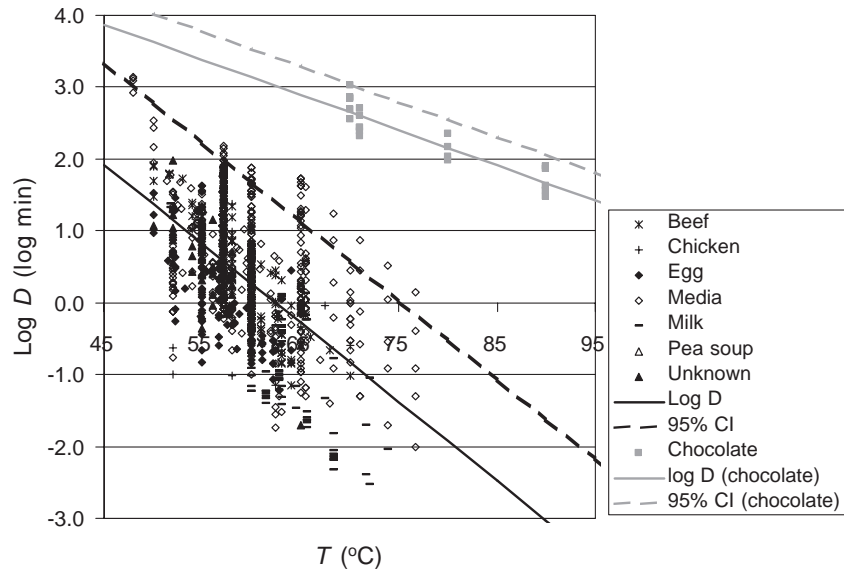Fig. 1. Heat resistance of all pathogens using the 95% prediction upper level as given in Table 1.

Fig. 2. Heat resistance of several *Salmonella* spp. in various food products and laboratory media. The solid line reflects the linear regression of log *D* on the temperature and the dotted line is the upper 95% prediction level. The grey lines represent chocolate and the black lines represent all other products.

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.
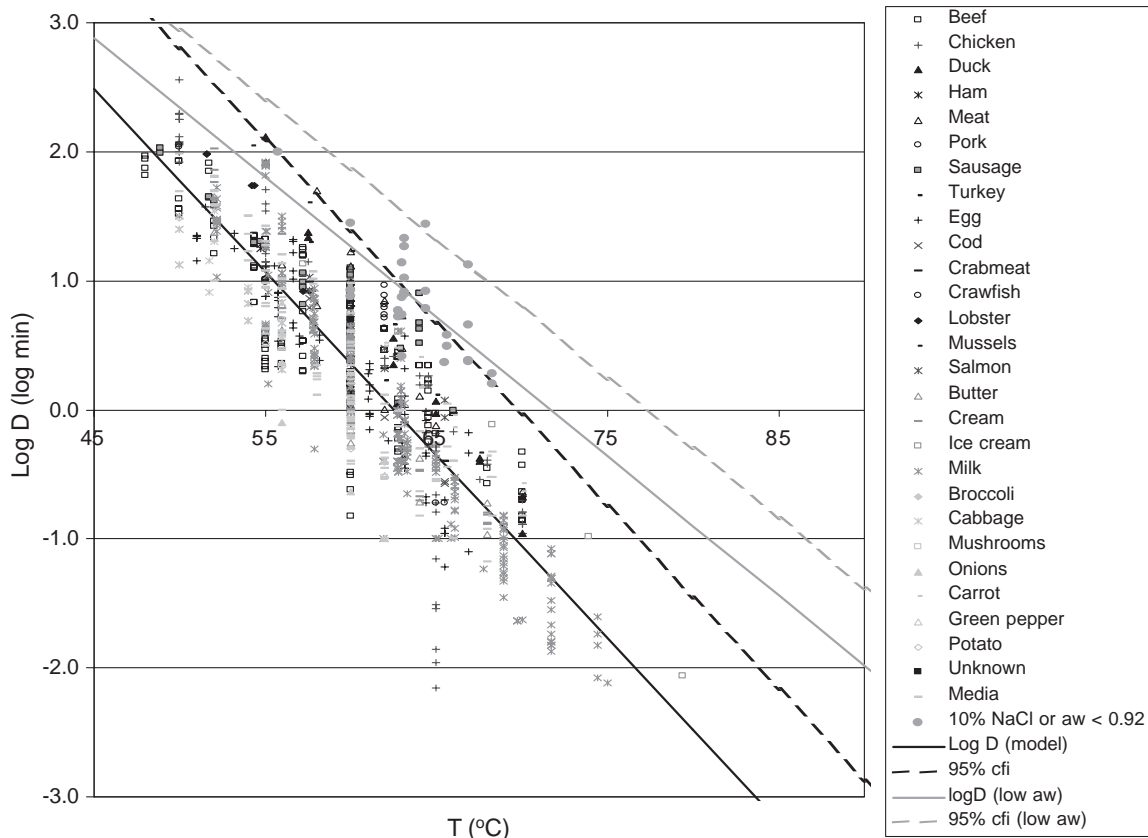

Fig. 3. Heat resistance of *Listeria monocytogenes* for various food products and laboratory media. The solid line reflects the linear regression of log *D* on the temperature and is for and the dotted line is the upper 95% prediction level. The grey lines represent products with low a_w (more than 10% NaCl or $a_w < 0.92$) and the black lines represent all other products.
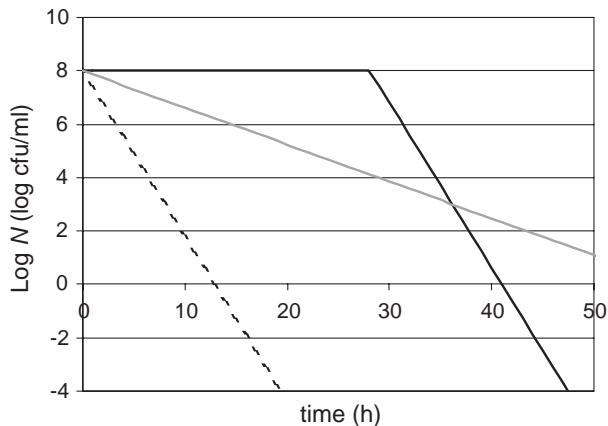
Fig. 4. Example of linear inactivation using $\log N = \log N_0 - t/D$, with N_0 as the initial number of micro-organisms (cfu/g), D is the D-value (min) and t is the time (min). In this example the lag time was 28.1 min and the D-value was 1.61 min at 58 °C (Juneja and Eblen, 2000). The black solid 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)).

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
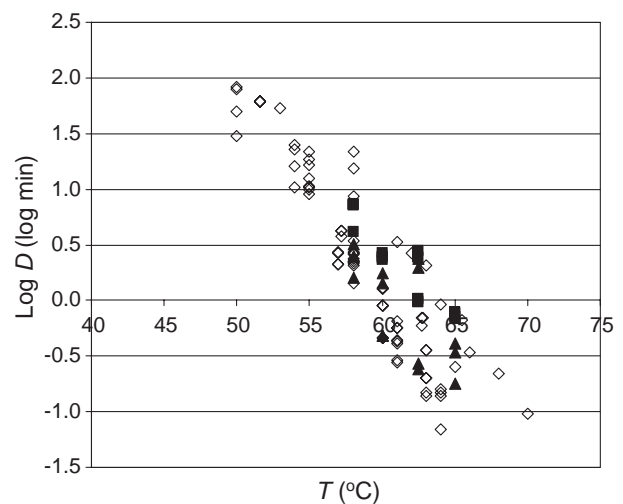

Fig. 5. Log D-values for *Salmonella* spp. in beef. Diamonds are D-values from papers where no lag time was reported. Squares are data from Juneja and Eblen (2000), who reported lag times. These lag times were incorporated in the D-values using Eq. (1). Triangles are the same data as the squares, only in this case the lag time was ignored.
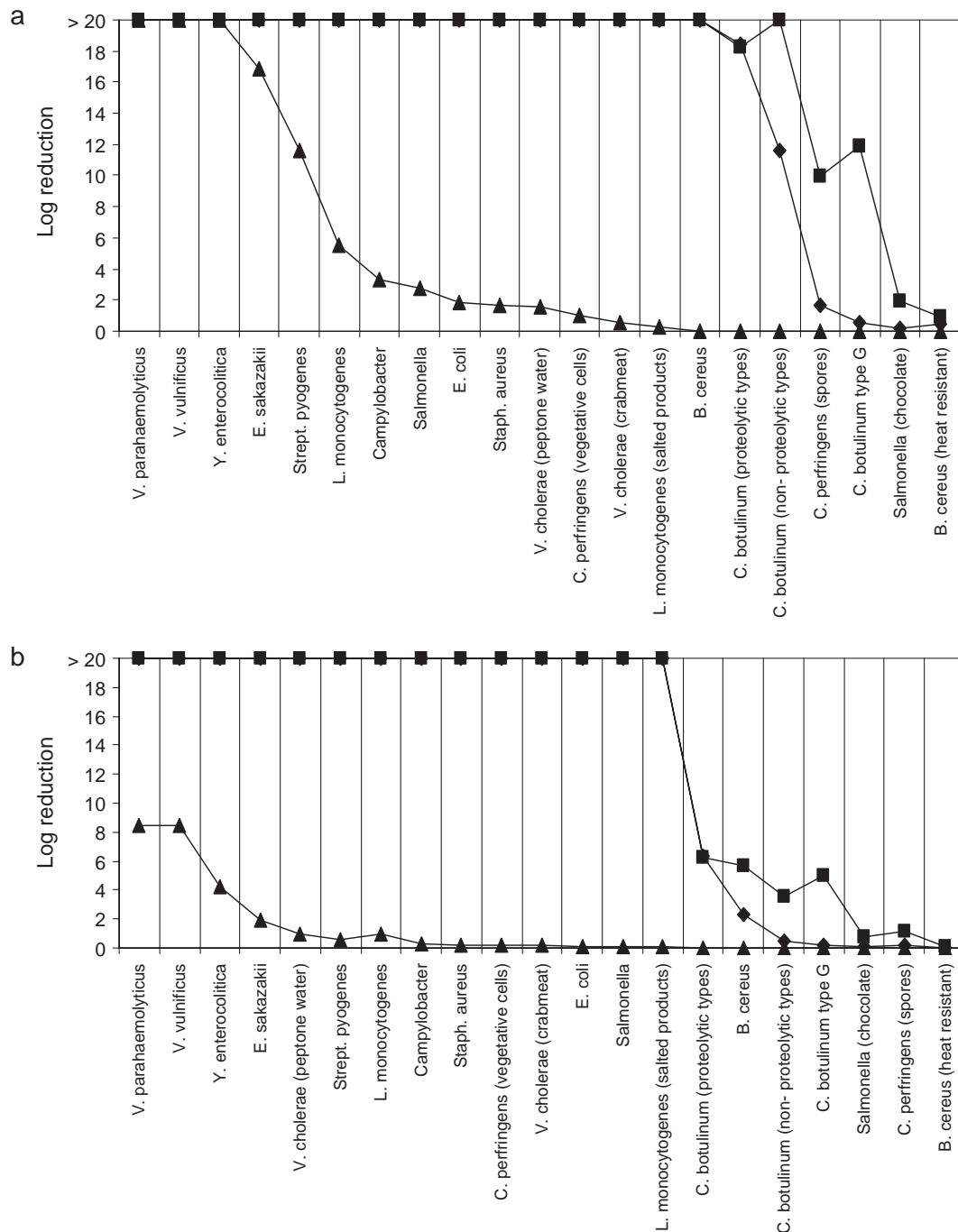
Fig. 6. Log reduction of pathogens estimated with the mean log D_{ref}-values (a) and the 95% upper prediction levels (b) as mentioned in Table 1, for a pasteurisation process (15 s 72 °C given in triangles), a sterilisation process (3 min 120 °C given in squares) and a UHT treatment (2 s 140 °C given in diamonds).

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_{ref} 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

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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