https://doi.org/10.17221/83/2023-CJFS

Refined approach to the evaluation of heat resistance applied to *Enterobacteriaceae* in cheese stretching

Irena Němečková, Šárka Trešlová, Eliška Lešková*

Dairy Research Institute, Prague, Czech Republic

**Corresponding author: nemeckova@milcom-as.cz*

Citation: Němečková I., Trešlová Š., Lešková E. (2023): Refined approach to the evaluation of heat resistance applied to *Enterobacteriaceae* in cheese stretching. Czech J. Food Sci., 41: 358–366.

Abstract: Heat resistance of bacteria is a factor potentially limiting the production of safety foods. We focused on five *Enterobacteriaceae* strains related to cheese stretching and sub-pasteurisation experimental temperatures of 50–59 °C. Heat resistance was screened and obtained data were fitted to a classical log-linear model with *D*-values indicating highly heat-resistant strains used. For example in *Klebsiella oxytoca* S525, *D*(50)-value was 96.1 min and *D*(59)-value 5.1 min. In subsequent detailed measurements, the shape of inactivation curves was sigmoid with defined lag, log-linear and stationary phase. We suggest calculating refined *D*-values (D^r-values) using only data obtained in log-linear phases, namely D^r(temperature; lag phase). In *K. oxytoca* S525, the obtained results were: $D^r(50; 80.9) = 61.7$ min, $D^r(53; 12.4) = 36.8$ min, $D^r(56; < 10) = 10.6$ min, and $D^r(59; < 3) = 4.3$ min. The research of particular inactivation phases can provide interesting findings both in science and industrial practice, especially concerning the passage or persistence of hazardous strains in food processing plants.

Keywords: *D*-value; *Klebsiella*; non-linear model; *Pantoea*; sub-pasteurisation heating

Proper heating is crucial both to ensure food safety and to enable the technological processing of foods. In dairy plants, the optimisation of thermal procedures is of special interest. Except for some cheeses made of raw milk, pasteurisation is practically ever-present in dairy technologies to reduce the microbial contamination of milk and particularly to inactivate pathogens. Nevertheless, some other technological procedures include heating to sub-pasteurisation or higher temperatures, e.g. milk centrifugation, milk evaporation or drying, curd cooking and scalding, cheese stretching, unripe soft cheese thermisation, processed cheese melting, and others. Typically used heating regimes and other conditions of particular dairy processing procedures were described by Bylund (1995).

To compare the effect of various heating regimes on a given microbial strain, the parameters of inactivation models can be calculated. Assumedly, the best-known model was defined by Bigelow and Esty (1920). This log-linear model defines *D*-value and *z*-value in terms of first-order reactions. *D*-value at a given temperature is the time (in minutes) needed for the decimal reduction of microbial density, i.e. by 1 log CFU·mL^{-1} or 1 log CFU·g^{-1} (CFU – colony forming unit). The *z*-value (in °C) represents the increase in temperature needed to the decimal reduction

Supported by the National Agency for Agricultural Research, Czech Republic, Project No. QK1910121, Programme ZEMĚ.

https://doi.org/10.17221/83/2023-CJFS

of *D*-values. Both *D*-values and *z*-values are suitable for comparing the heat resistance of microbial strains within a given experiment and also among other research teams. Thus, this model is widely used as summarised e.g. by Lindsay et al. (2021) or Dash et al. (2022).

However, in many cases, experimental data fit better to a concave or convex shape of inactivation curves. One of the causes is the presence of subpopulations that differ in heat resistance from each other, as explained by Den Besten et al. (2018). This problem is reflected in non-linear models described e.g. using parameters δ (in hours, the time for the first decimal reduction) and *p* (dimensionless shape parameter) as summarised by Van Impe et al. (2018). The basic, more than a hundred years old, non-linear Weibull model and its modifications (Peleg model, Mafart model) were compared by Buzrul (2022). To simplify the use of models, software tools were developed, e.g. GInaFiT software (Geeraerd et al. 2005). In general, a disadvantage of non-linear models is their mathematical/statistical description, which can seem non-intuitive and quite awesome to practically oriented users.

To evade non-linearity at the beginning of inactivation curves, Lehotová et al. (2021) suggested using t_{4D}-value (fourth decimal reduction time) as the time needed to lower the microbial density by ten-thousandfold. This attempt resembles our point of view in an effort to use the advantages of both log-linear and non-linear models. Nevertheless, in some cases, t_{4D}-values can be longer than the operating period of the studied technological process, which complicates the optimisation of the heating regime. For example, in *Staphylococcus aureus*, Lehotová et al. (2021) determined t_{4D}-values at 55, 57, and 61 °C as long as 122.3, 71.9, and 37.0 min, respectively.

We want to suggest a refined approach to the evaluation of the heat resistance of bacteria. We aim at a model that is both simple in use and concisely describes the inactivation of bacteria during heating. In this work, we evaluated heat resistance in *Enterobacteriaceae* strains isolated from samples related to cheese stretching to give a practical example.

The presence of *Enterobacteriaceae* in a cooker-stretcher seems appealing because cheese stretching can be the only heat treatment used. Typically, cooking water is kept at a temperature of 82–85 °C (Bylund 1995), while the temperature of stretched curd rises to about 60–70 °C (Lehotová et al. 2021). These conditions are close to thermisation (about 57–68 °C for 5 s to 30 min) or pasteurisation (minimally 63 °C for 30 min or 72 °C for 15 s) (Dash et al. 2022).

MATERIAL AND METHODS

Microorganisms. *Enterobacteriaceae* isolates were obtained during a phase control in a stretched cheese semi-continual production line. The isolates were identified using matrix-assisted laser desorption/ionisation time of flight mass spectrometry (MALDI-TOF MS) in an accredited laboratory (State Veterinary Institute Jihlava, Czech Republic) and preserved in Brain Heart Infusion (BHI) broth (Merck, Germany) with 20% glycerol at –43 °C. In chronological order, *Klebsiella oxytoca* S525 originated from curd in a trolley waiting for further processing, *Klebsiella oxytoca* S810-1A and *Pantoea agglomerans* S810-1B from a mixture of curd and cooking water in the middle of the cooking process, *Klebsiella variicola* S540 from stretched curd just leaving the cooker-stretcher for a forming cylinder, and *Enterobacter xiangfangensis* S544 from a formed cheese just leaving the forming cylinder for a transport trolley with cooling tap water. For the measurement of heat resistance, the strains were freshly cultivated on the surface of BHI agar at 37 °C for 24 h.

Measurement of heat resistance. At first, the heat resistance of strains at 50, 53, 56, and 59 °C was screened to estimate classical *D*-values and *z*-values. Samples were taken 4–6 times every 10–30 min. Afterwards, detailed measurements were done, and the obtained data were evaluated statistically. Samples were taken every 2–15 min to figure inactivation curves well and preferably until a stable microbial density was reached. Principally, the experimental procedure was described previously by Němečková et al. (2020). The inactivation experiments were done in tubes with 50 mL of tempered and stirred milk (0.5% fat). The density of survived bacteria was determined by plating and colony counting on BHI agar after cultivation at 37 °C for 24 h.

Statistical evaluation. The screening was performed once. Afterwards, the entire inactivation experiments were repeated twice and the results were expressed as arithmetic means. The inactivation curves were figured as bacterial density (log CFU·mL^{-1}) depending on heating time (min).

For the screening, the linear regression analysis was performed and the *D*-value was calculated as the absolute reciprocal value of the slope. Similarly, the logarithms of *D*-values (log min) depending on heating temperature (°C) were subjected to the linear regression analysis to calculate *z*-value as the absolute reciprocal value of the slope.

For the detailed evaluation, the linear regression analysis was applied separately to data for the lag,

https://doi.org/10.17221/83/2023-CJFS

log-linear and stationary phases. Refined D^r-value was calculated only from the linearly decreasing part of the inactivation curve (log-linear phase). The intersections of the line for the log-linear phase with lines for lag phase and for the stationary phase gave the time to the end of lag phase (t_{lag}) and the time to the end of the log-linear phase (t_{ll}). Bacterial densities at times 0 and t_{ll} were converted to CFU·mL^{-1} and inactivation effect (*IE*) was calculated as the percentage of bacteria inactivated by the end of log-linear phase. Moreover, the time in minutes for the first decimal reduction (δ^{min}) was calculated using the lag phase line, t_{lag} and D^r-value. If a lag phase was not detected (t_{lag} was shorter than the shortest sampling time), δ^{min} was estimated graphically. Generally, if any phase was not detected, the parameters were estimated using data in the shortest or longest measured time and a 'lower than' or 'higher than' sign. Refined z^r-value was calculated in the same way as *z*-value but using the logarithms of D^r-values.

MS Excel (version 2013) was used as a software tool.

RESULTS AND DISCCUSION

Screening for heat resistance. The heat resistance of five *Enterobacteriaceae* strains was screened at four temperatures. As seen in Figures 1–5, the obtained data fitted to the log-linear model quite well. The calculated results are shown in Table 1. For nineteen calculated inactivation lines, the coefficients of determination were higher than 0.85 in seventeen cases and even higher than 0.95 in six cases. However, the coefficient of determination as low as 0.5858 occurred in *K. varicola* S540 at 50 °C. The reason was in a relatively high *D*(50)-value that was several-fold higher than the measured period, which caused particular uncertainty in the determined slope of the inactivation line.

E. xiangfangensis S544, which was as heat-sensitive as its inactivation line at 59 °C, could not be determined. After 10-min heating, the density of this strain was below the limit of detection (< 1.0 log CFU·mL^{-1}). Thus, *E. xiangfangensis* S544 was excluded from further testing. This strain originated from a sample at the end of production line where tap water is applied. The heat sensitivity of this strain suggests that it could be a post-process contaminant (maybe from water) rather than it could pass through the cooker-stretcher.

When focused on *Escherichia coli* and *Klebsiella* spp., Mozzarella cheeses, and *D*-values altogether, previous works are unavailable and only a partial comparison is possible. Already in 1992, Massa et al. referred to *Klebsiella* (*K. pneumoniae, K. oxytoca*) together with *Enterobacter aerogenes* and *E. coli* as major contaminants of stretched Mozzarella cheeses. Comparably to our findings, only 1.15% of *K. pneumoniae* colony forming units survived after heating at 60 °C for 15 min. On the contrary, for a clinical *K. pneumoniae* isolate, Morgan et al. (1988) reported considerably lower heat resistance: *D*(52) = 16.09 min, *D*(58) = 0.13 min, and *z* = 2.8 °C. Based on actual results on *E. coli* dairy isolates, Němečková et al. (2020) accentuated the need to revise previous knowledge of heat resistance in hazardous bacteria due to the spread of highly heat-resistant strains. They determined exceedingly high *D*(50)-values from 182 to 417 min and *D*(59)-values from 20 to 32 min, which are comparable with *D*(50) = 500.0 min and *D*(59) = 11.1 min determined in our most heat-resistant strain *K. variicola* S540.

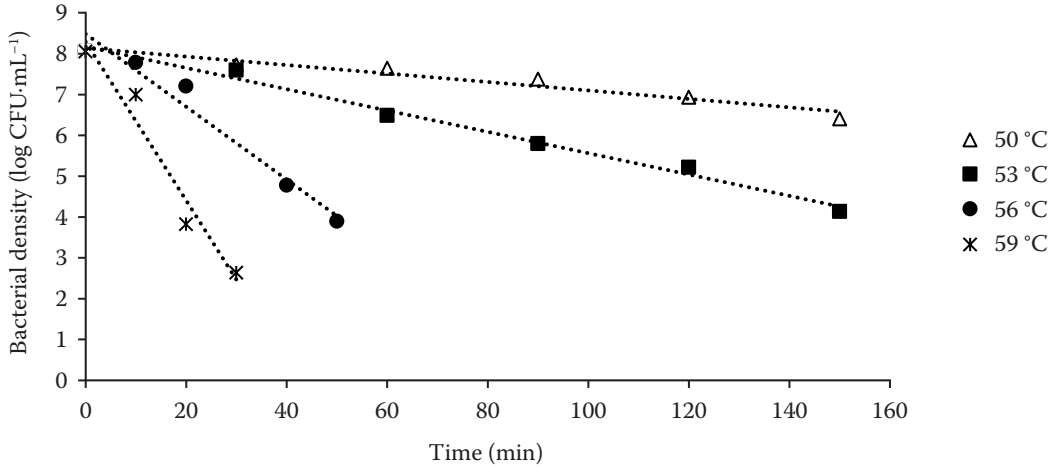

Figure 1. Screening for the heat resistance of *Klebsiella oxytoca* S525 (*n* = 1)

CFU – colony forming unit

https://doi.org/10.17221/83/2023-CJFS

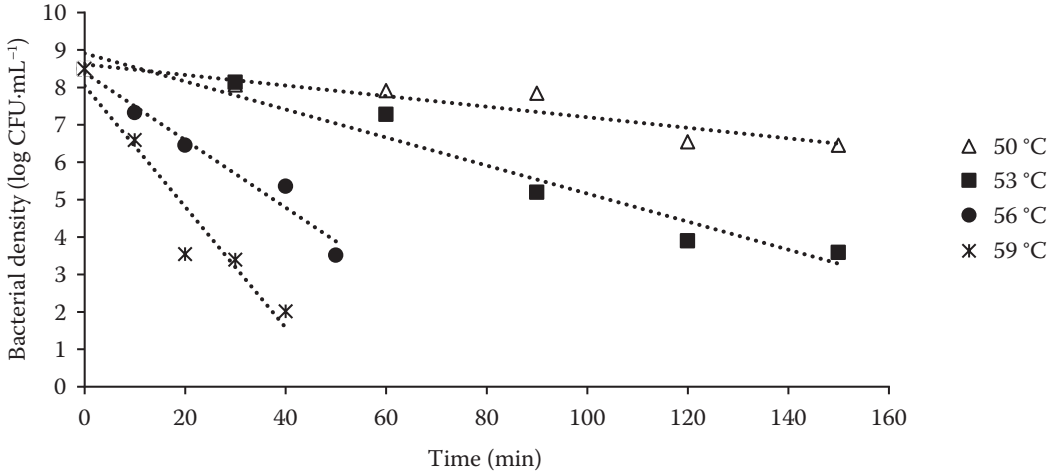

Figure 2. Screening for the heat resistance of *Klebsiella oxytoca* S810-1A (n = 1)

CFU – colony forming unit

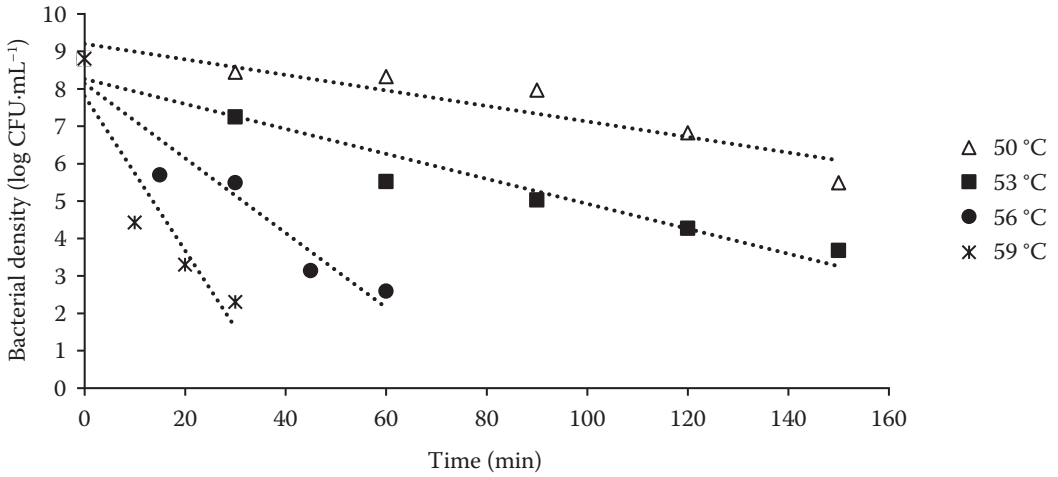

Figure 3. Screening for the heat resistance of *Pantoea agglomerans* S810-1B (n = 1)

CFU – colony forming unit

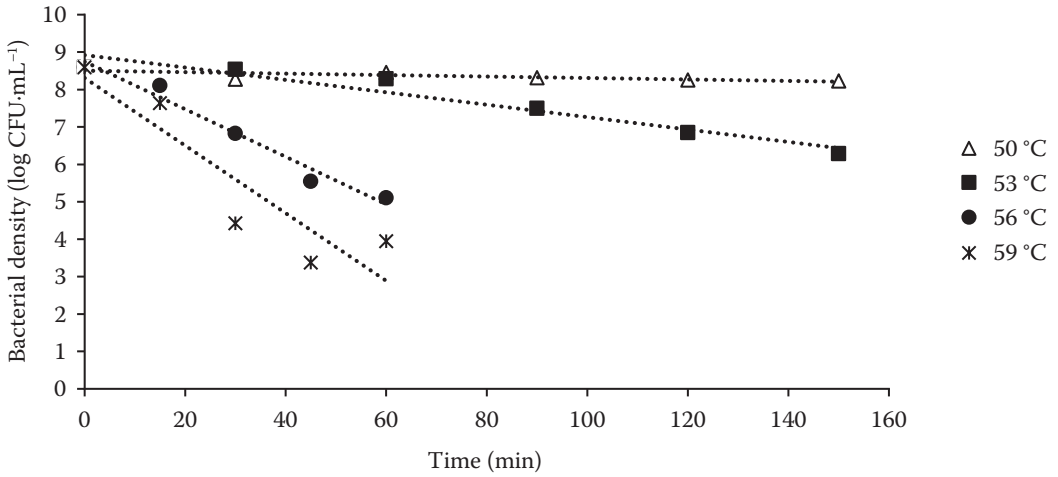

Figure 4. Screening for the heat resistance of *Klebsiella variicola* S540 (n = 1)

CFU – colony forming unit

https://doi.org/10.17221/83/2023-CJFS

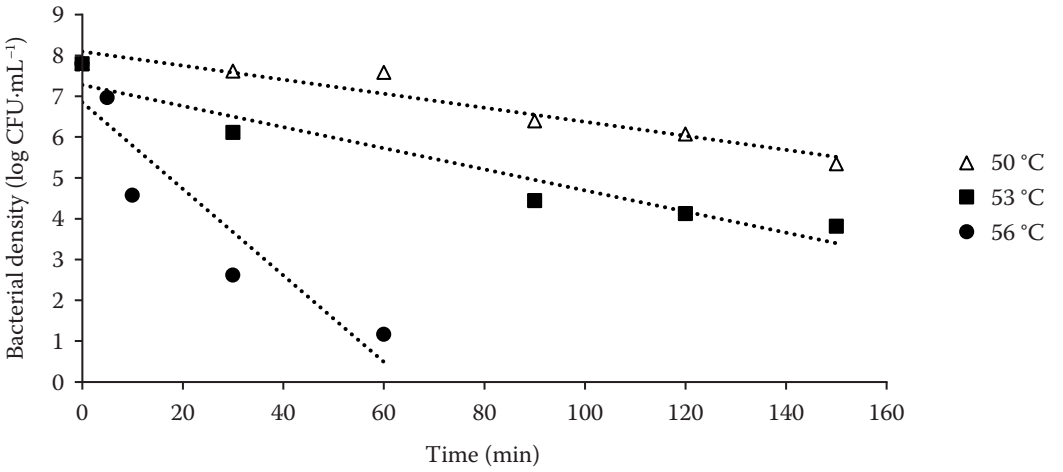

Figure 5. Screening for the heat resistance of *Enterobacter xiangfangensis* S544 (n = 1)

CFU – colony forming unit

Detailed evaluation of heat resistance. Detailed inactivation curves for four *Enterobacteriaceae* strains are shown in Figures 6–9. When compared with the screened inactivation curves in Figures 1–4, the linear shape has changed to a sigmoid form similar to the reversed growth curve with defined lag, log-linear and stationary phase. These inactivation curves are characterised by originally designed parameters summarised in Table 2.

According to our knowledge, time parameters t_{lag} and t_{ll}, as well as parameters D^r and z^r refined only for the

Table 1. Screening for the heat resistance of *Enterobacteriaceae* strains (n = 1)

Strain	T (°C)	D (min)	R^2 (for D)	z (°C)	R^2 (for z)
Klebsiella oxytoca S525	50	96.1	0.9463	6.9	0.9935
	53	38.3	0.9892		
	56	11.2	0.9639		
	59	5.1	0.9574		
Klebsiella oxytoca S810-1A	50	70.9	0.8758	8.4	0.9880
	53	26.7	0.9508		
	56	11.1	0.9644		
	59	6.2	0.9267		
Pantoea agglomerans S810-1B	50	48.1	0.8622	8.6	0.9802
	53	29.9	0.9421		
	56	10.0	0.9219		
	59	4.8	0.8646		
Klebsiella variicola S540	50	500.0	0.5858	5.4	0.9120
	53	60.2	0.9390		
	56	15.7	0.9716		
	59	11.1	0.8290		
Enterobacter xiangfangensis S544	50	58.1	0.9177	7.6	0.9081
	53	38.6	0.9242		
	56	6.4	0.8619		
	59	ND	ND		

T – temperature; D – time needed for the decimal reduction of bacterial density; z – increase in temperature needed for the decimal reduction of D-values; R^2 – coefficient of determination; ND – not determined

https://doi.org/10.17221/83/2023-CJFS

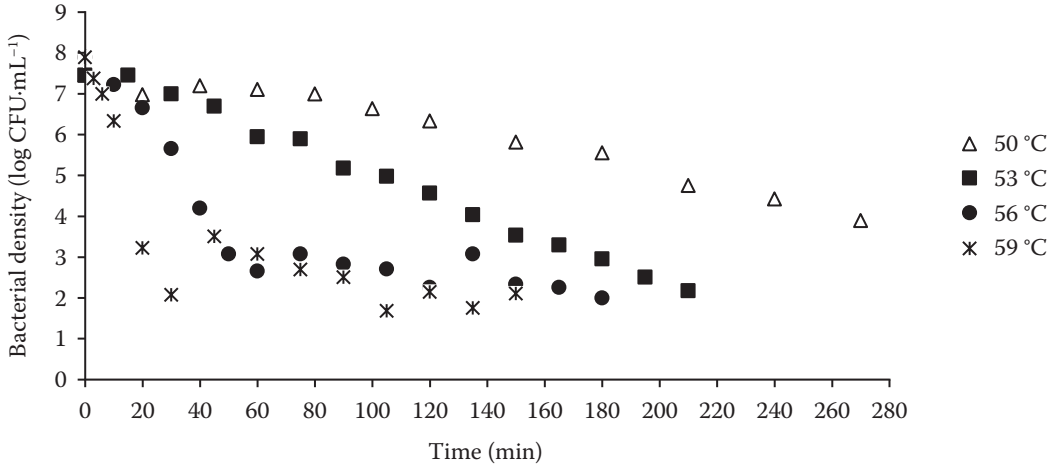

Figure 6. Heat inactivation curves of *Klebsiella oxytoca* S525 (n = 2)

CFU – colony forming unit

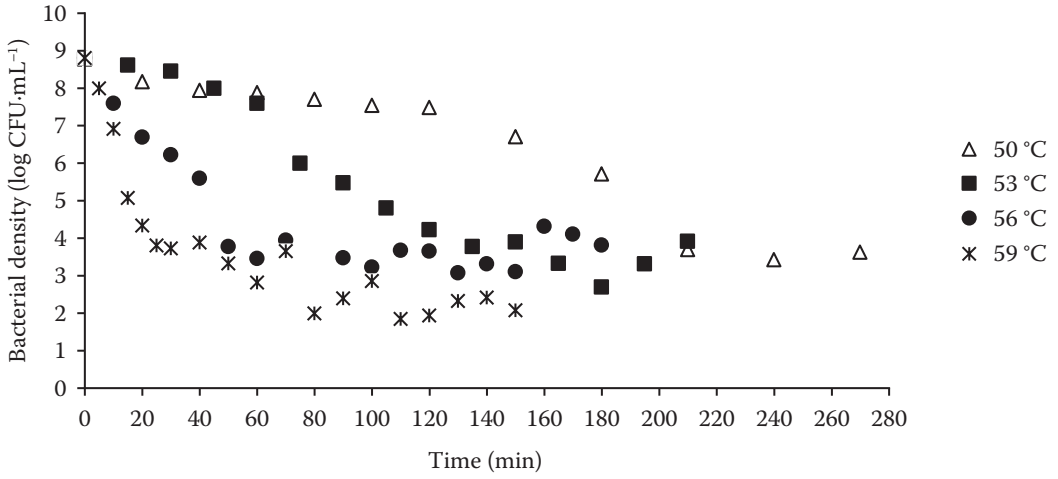

Figure 7. Heat inactivation curves of *Klebsiella oxytoca* S810-1A (n = 2)

CFU – colony forming unit

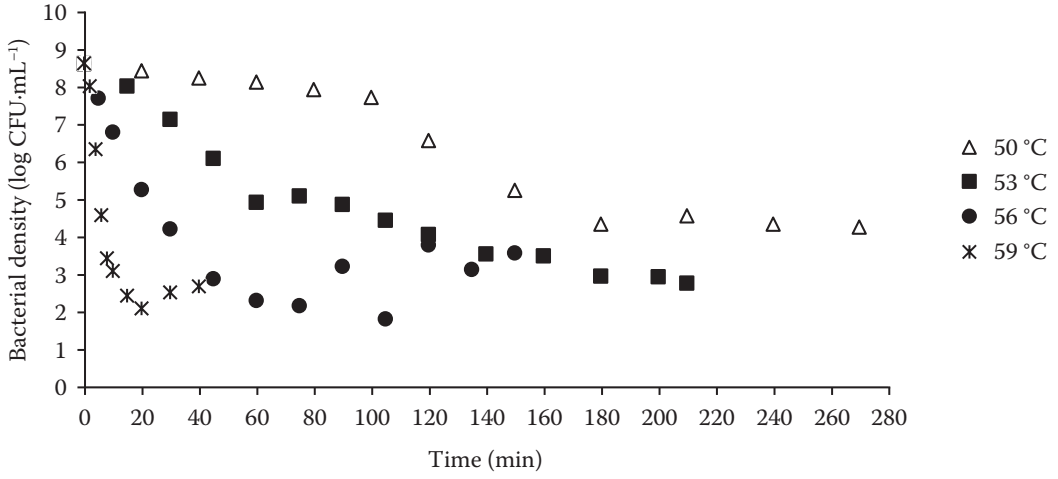

Figure 8. Heat inactivation curves of *Pantoea agglomerans* S810-1B (n = 2)

CFU – colony forming unit

https://doi.org/10.17221/83/2023-CJFS

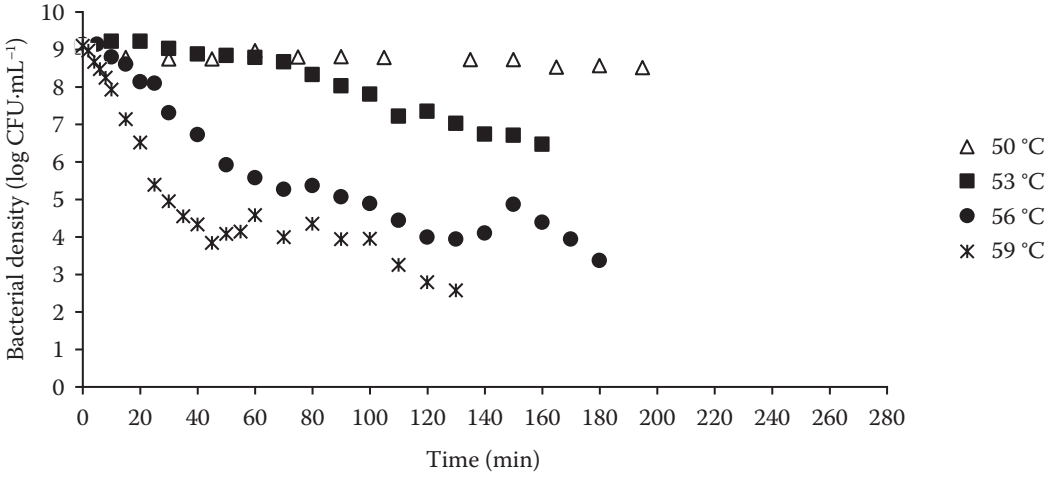

Figure 9. Heat inactivation curves of *Klebsiella variicola* S540 (n = 2)

CFU – colony forming unit

log-linear phase were used for the first time in our work. Moreover, the generally known parameter δ^{min} was customised to complete our data. The time for the first decimal reduction δ is usually given in hours (Van Impe et al. 2018), but for better comparability with other parameters, it was converted to minutes.

When compared with the data in Tables 1 and 2, lag phases involved in the calculation of D-values caused that the classical D-values to be higher than the refined D^r-values – the lower the temperature, the higher the difference. On the contrary, the classical z-values were slightly lower than the refined z^r-values. For fif-

Table 2. The heat resistance of *Enterobacteriaceae* strains (n = 2)

Strain	T (°C)	t_{lag} (min)	δ^{min} (min)	D^r (min)	R^2 (for D^r)	t_{ll} (min)	IE (%)	z^r (°C)	R^2 (for z^r)
Klebsiella oxytoca S525	50	80.9	122.1	61.7	0.9936	> 270	> 99.9712	7.5	0.9783
	53	12.4	49.2	36.8	0.9955	> 210	> 99.9996		
	56	< 10	15*	10.6	0.9798	52.6	99.9980		
	59	< 3	6*	4.3	0.9662	22.2	99.9987		
Klebsiella oxytoca S810-1A	50	137.3	90.1	24.3	0.9481	227.0	99.9996	12.3	0.9257
	53	34.8	47.6	20.4	0.9658	136.5	99.9996		
	56	< 10	16*	11.1	0.9616	58.5	99.9996		
	59	< 5	7*	4.6	0.9736	24.0	99.9992		
Pantoea agglomerans S810-1B	50	93.5	98.6	23.8	0.9826	166.5	99.9772	8.8	0.9412
	53	< 15	23.6	16.1	0.9862	56.8	99.9532		
	56	< 5	3*	7.8	0.9765	49.0	99.9999		
	59	< 2	2*	2.2	0.8857	13.6	99.9999		
Klebsiella variicola S540	50	> 195	526.3	ND	ND	> 195	ND	8.0	0.9315
	53	52.9	79.5	43.7	0.9561	> 160	> 99.7958		
	56	22.7	19.8	12.2	0.9704	54.7	99.9771		
	59	1.2	7.9	7.7	0.9691	34.7	99.9969		

* graphically estimated values; T – temperature; t_{lag} – time to the end of lag phase; δ^{min} – time in minutes for the first decimal reduction, D^r – time needed for the decimal reduction of bacterial density refined for the log-linear phase; t_{ll} – time to the end of log-linear phase; IE – inactivation effect reached by the end of log-linear phase; z^r – increase in temperature needed for the decimal reduction of D^r-values; R^2 – coefficient of determination; ND – not determined

https://doi.org/10.17221/83/2023-CJFS

teen calculated refined inactivation lines, the coefficients of determination have improved (higher than 0.85 in all cases and higher than 0.95 in thirteen cases).

D-values suppose an equable decrease in bacterial density during heating. Thus for the classical log-linear model, the first decimal reduction of bacterial density occurs after *D*-minutes. More realistically, the time for the first decimal reduction δ^{min} reflects the time of lag phase t_{lag} and the slopes of the lines for lag and log-linear phase, which means that δ^{min} can be higher than *D*-value. This effect can be important, particularly for technological processes under moderate heating. For example, at 50 °C, δ^{min} in the tested strains were higher than *D*-values on average by about 30.5 min.

Another interesting comparison is between parameters δ^{min} and t_{lag}. During a lag phase, the response of bacterial cells to applied heating is only initiated, namely as an alteration of one or more cellular structures or functions generally known as cellular targets (Cebrián et al. 2017). Thus, bacterial density during the lag phase was stable or decreased very slowly. The end of lag phase was indicated as a rapid decrease in bacterial density. In most cases, δ^{min} took the whole t_{lag} and some additional time from the subsequent log-linear phase.

Nevertheless, in the case of *K. oxytoca* S810-1A at 50 °C, the transition from the lag phase to the log-linear phase occurred later, namely after more than 1.5-fold of δ^{min}. In *K. variicola* S540 at 50 °C, the end of the lag phase was not detected even after 195 min heating. Strains S810-1A and S540 resisted to the heating to 50 °C for more than two hours without serious cell damage indicated by the beginning of the log-linear phase. Such heat resistance predestined these strains to pass through various technological processes using such moderate heating and was really manifested in their occurrence in the cooker-stretcher. It is to be noted that the cooker-stretcher typically operated about 1.5–2.5 h for the processing of the whole batch. When they contaminate the cooker-stretcher in some suitable place protected from direct contact with cooking water, these strains could hypothetically survive there in the long term. This finding made the producer of stretched cheeses to elevate the temperature of cooking water from used 77 to actual 84 °C, which subsequently came right.

While the presence of lag phase indicates a delay until the heating induces cell damage, the presence of the stationary phase could reflect the induced protective mechanisms of cells against thermal stress. For example, an enhanced synthesis and accumulation of heat-shock proteins (HSPs) can be stimulated. HSPs are ubiquitous and highly conserved proteins whose expression is induced in response to a wide variety of physiological and environmental insults. Various mechanisms of HSPs protective functions allow the cells to survive otherwise lethal conditions (Parcellier et al. 2003).

The processes of heat inactivation and protection of bacterial cells progress and compete together till the end of log-linear phase at t_{ll} minutes. At a sub-pasteurisation temperature of 59 °C, it is to note that t_{ll} was about 15–35 min. Theoretically, during pasteurisation at 63 °C for 30 min, the tested strains can be supposed to reach the stationary phase and the prolongation of pasteurisation would not increase the inactivation effect.

In the stationary phase, the survived bacterial cells seem to be adapted to heating and able to resist for a relatively long time. For example, at a temperature of 56 °C, t_{ll} was about 50–60 minutes while the end of the stationary phase (assumedly second log-linear phase) was not detected even during heating for 150–180 min. Such a long stationary phase is quite surprising because, in many cases, only short tailing was observed and attributed to methodological causes (Cebrián et al. 2017), which led to the untimely termination of inactivation experiments. However, longer inactivation experiments could bring interesting results.

The ratio of bacterial cells inactivated in t_{ll} minutes (*IE*) slightly increased with increasing temperature in *P. agglomerans* S810-1B and *K. variicola* S540 while in *K. oxytoca* S525 and S810-1A the effect of temperature on *IE* was not observed. Factors affecting *IE*, e.g. the cultivation conditions of bacteria before heating or physical and chemical parameters of the heated medium, could be studied in future.

Another question is how such factors affect t_{lag}. To answer arisen questions, further detailed inactivation experiments and focus on particular inactivation phases are needed. A higher initial density of bacteria, longer applied heating, and more frequent sampling especially at the beginning of inactivation experiments, would be useful.

To express the results, we suggest using at least a refined D^r-value and z^r-value and to complete D^r-value with both the experimental temperature and the beginning and end of log-linear phase, namely $D^r(T; t_{lag}; t_{ll})$. For example in *K. oxytoca* S525, the obtained results were: $D^r(50; 80.9; > 270)$ = 61.7 min, $D^r(53; 12.4; > 210)$ = 36.8 min, $D^r(56; < 10; 52.6)$ = 10.6 min, and $D^r(59; < 3; 22.2)$ = 4.3 min. Such a form of recording provides time intervals when D^r-values are appli-

https://doi.org/10.17221/83/2023-CJFS

cable and enables to estimate the whole inactivation curves as constant bacterial density in lag phase, linear decrease with defined slope in log-linear phase and constant one in stationary phase. It is both illustrative and more precise than the classical log-linear model. Nevertheless, if desired, other parameters like δ^{min} or *IE* can be added to maximise the knowledge.

CONCLUSION

The heat resistance of bacteria can be approximately estimated using the log-linear model, *D*-values, and *z*-values. However, when measured in detail, the shape of inactivation curves can change to a sigmoid one similar to reversed growth curves with defined lag, log-linear and stationary phase. Thus, we suggest using refined values $D^r(T; t_{lag}; t_{ll})$ and z^r. The research of these phases could bring interesting findings both in science and industrial practice, e.g. in food processing.

REFERENCES

Bigelow W.D., Esty J.R. (1920): Thermal death point in relation to the time of typical thermophilic organisms. The Journal of Infection Diseases, 27: 602–617.

Buzrul S. (2022): The Weibull model for microbial inactivation. Food Engineering Reviews, 14: 45–61.

Bylund G. (1995): Dairy Processing Handbook. Lund, Sweden, Tetra Pak Processing Systems AB.

Cebrián G., Condón S., Mañas P. (2017): Physiology of the inactivation of vegetative bacteria by thermal treatments: Mode of action, influence of environmental factors and inactivation kinetics. Foods, 107: 6120107.

Dash K.K., Fayaz U., Dar A.H., Shams R., Manzoor S., Sundarsingh A., Deka P., Khan S.A. (2022): A comprehensive review on heat treatments and related impact on the quality and microbial safety of milk and milk-based products. Food Chemistry Advances, 1: 100041.

Den Besten H.M.W., Wells-Bennik M.H.J., Zwietering M.H. (2018): Natural diversity in heat resistance of bacteria and bacterial spores: Impact on food safety and quality. Annual Review of Food Science and Technology, 9: 383–410.

Geeraerd A.H., Valdramidis V.P., Van Impe J.F. (2005): GInaFiT, a freeware tool to assess non-log-linear microbial survivor curves. International Journal of Food Microbiology, 102: 95–105.

Lehotová V., Miháliková K., Medveďová A., Valík L. (2021): Modelling the inactivation of *Staphylococcus aureus* at moderate heating temperatures. Czech Journal of Food Sciences, 39: 42–48.

Lindsay D., Robertson R., Fraser R., Engstrom S., Jordan K. (2021): Heat inactivation of microorganisms in milk and dairy products. International Dairy Journal, 121: 105096.

Massa S., Gardini F., Sinigaglia M., Guerzoni M.E. (1992): *Klebsiella pneumoniae* as a spoilage organism in Mozzarella cheese. Journal of Dairy Science, 75: 1411–1414.

Morgan J.N., Lin F.J., Eitenmiller R.R., Barnhart H.M., Toledo R.T. (1988): Thermal destruction of *Escherichia coli* and *Klebsiella pneumoniae* in human milk. Journal of Food Protection, 51: 132–136.

Němečková I., Havlíková Š., Gelbíčová T., Pospíšilová L., Hromádková E., Lindauerová J., Baráková A., Karpíšková R. (2020): Heat-resistance of suspect persistent strains of *Escherichia coli* from cheesemaking plants. Czech Journal of Food Sciences, 38: 323–329.

Parcellier A., Gurbuxani S., Schmitt E., Solary E., Garrido C. (2003): Heat-shock proteins, cellular chaperones that modulate mitochondrial cell death pathways. Biochemical and Biophysical Research Communications, 304: 505–512.

Van Impe J., Smet C., Tiwari B., Greiner R., Ojha S., Stulić V., Vukušić T., Režek Jambrak A. (2018): State of the art of non-thermal and thermal processing for inactivation of micro-organisms. Journal of Applied Microbiology, 125: 16–35.

Received: May 31, 2023
Accepted: October 12, 2023
Published online: October 25, 2023