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# Estimating the Efficacy of Mild Heating Processes taking into Account Microbial Non-linearities: a Case Study on the Thermisation of a Food Simulant

Vasilis Valdramidis

*Technological University Dublin, vvaldram@gmail.com*

Brijesh Tiwari

*Manchester Metropolitan University*

Patrick Cullen

*Dublin Institute of Technology, pjculen@dit.ie*

Alain Kondjoyan

*INRA, UR370 Qualité des Produits Animaux*

Jan Van Impe

*Katholieke Universiteit Leuven*

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Estimating the efficacy of mild heating  
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Vasilis Valdramidis\*      Brijesh Tiwari†      P. J. Cullen‡  
Alain Kondjoyan\*\*      Jan Van Impe††

\*Dublin Institute of Technology, vvaldram@gmail.com

†Manchester Metropolitan University

‡Dublin Institute of Technology, pjcullen@dit.ie

\*\*INRA, UR370 Qualité des Produits Animaux

††Katholieke Universiteit Leuven

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1 **Estimating the efficacy of mild heating processes taking into account microbial non-**  
2 **linearities: a case study on the thermisation of a food simulant**

3

4 V. P. Valdramidis<sup>1\*</sup>, A. H. Geeraerd<sup>2,3</sup>, B. K. Tiwari<sup>4</sup>, P.J. Cullen<sup>1</sup>, A. Kondjoyan<sup>5</sup> and J.  
5 F. Van Impe<sup>2,6</sup>

6

7 <sup>1</sup>School of Food Science & Environmental Health, Dublin Institute of Technology, Cathal  
8 Brugha Street, Dublin 1, Ireland.

9 <sup>2</sup>CPMF<sup>2</sup>-Flemish Cluster Predictive Microbiology in Foods

10 <sup>3</sup>Division of Mechatronics, Biostatistics and Sensors (MeBioS), Department of  
11 Biosystems (BIOSYST), Katholieke Universiteit Leuven, W. de Croylaan 42, B-3001  
12 Leuven, Belgium.

13 <sup>4</sup>Department of Food & Tourism Management, Manchester Metropolitan University,  
14 Manchester, M14 6HR, UK

15 <sup>5</sup>INRA, UR370 Qualité des Produits Animaux, F-63122 St Genès Champanelle, France.

16 <sup>6</sup>BioTeC – Bioprocess Technology and Control, Department of Chemical Engineering,  
17 Katholieke Universiteit Leuven, W. de Croylaan 46, B-3001 Leuven, Belgium

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27 **\*Corresponding author: [vvaldram@gmail.com](mailto:vvaldram@gmail.com), Tel : +353 876331281**

28 **Abstract**

29 Traditional and novel approaches for the calculation of the heat treatment efficiency are  
30 compared in this work. The Mild Heat value (*MH*-value), an alternative approach to the  
31 commonly used sterilisation, pasteurisation and cook value (*F*, *P*, *C*-value), is calculated  
32 to estimate the efficiency of a mild heat process. *MH*-value is the time needed to achieve  
33 a predefined microbial reduction at a reference temperature and a known thermal resistant  
34 constant, *z*, for log-linear or specific types of non log-linear microbial inactivation  
35 kinetics. An illustrative example is given in which microbial inactivation data of *Listeria*  
36 *innocua* CLIP 20-595 are used for estimating the inactivation parameters under  
37 isothermal conditions of 58, 60, 63 and 66°C by the use of the log-linear and the Geeraerd  
38 et al., (2000) model. Thereafter, dynamic temperature profiles (targeting at 54 and 57°C)  
39 representing milk thermisation are exploited for illustrating the application of *MH*-value.  
40 Finally, the equivalent holding times of different temperatures are calculated taking into  
41 account the observed non-linearity.

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58 **Keywords: Thermisation, *Listeria innocua*, non-linearity, modelling**

59 **Introduction**

60 When microbial inactivation processes are described as log-linear, a linear relationship  
61 between the logarithm of the microbial population level (in absolute value or relative to  
62 the initial value) and the treatment time are considered. If log-linearity is indeed being  
63 observed, the thermal death time, *F*-value is used as a basis for comparing heat  
64 sterilisation procedures. The *F*-value (Eq. (1)) (Ball, 1923) is defined as the time required  
65 in order to achieve a specific reduction in microbial numbers at a given temperature and it  
66 thus represents the total time-temperature combination received by a food. Similarly, the  
67 *P*-value and the *C*-value are the corresponding thermal death value under pasteurisation  
68 and cooking conditions, respectively (Pittia, Furlanetto, Maifreni, Mangina & Rosa,  
69 2008).

$$F_{T_{ref}} = \int_0^t 10^{(T(t)-T_{ref})/z} dt \quad (1)$$

70

71 Eq. (1), as mentioned above, is valid if the survival curve obeys first order kinetics.  
72 Despite the world-wide use of this approach especially in the canning industry for the so-  
73 called '12D process' of the proteolytic strains (Group I) of *Clostridium botulinum* spores  
74 (Stumbo, 1965; ICMSF, 1996), a lot of deviations from log-linearity have been observed  
75 (e.g., Corradini, Normand and Peleg (2005)). As other authors acknowledge, the success  
76 of the canning industry in using the *F*-value as a measure of the heat processes efficacy  
77 could be attributed to over-processing and not to the calculation method's correctness  
78 (Corradini, Normand & Peleg, 2006). These deviations are evident particularly at lower  
79 temperatures than the sterilisation ones, and for vegetative cells (Valdramidis, Geeraerd,  
80 Bernaerts & Van Impe, 2006; Huang, 2009; Miller, Gil, Brandao, Teixeira & Silva,  
81 2009).

82 A mild heat treatment often applied in the dairy industry is thermisation of milk  
83 destined for cheese making. This process has milder effects on the raw milk flora and  
84 the functionality of milk caseins and salts than pasteurisation (Samelis et al., 2009).  
85 Thermisation is applied at temperatures that range between 52 to 67°C for a treatment  
86 time of few seconds, i.e., 20 s, to about half an hour (Zehetner, Bareuther, Henle &

87 Klostermeyer, 1996; McKellar & Piyasena, 2000; Christiansen, Nielsen, Vogensen,  
88 Brogren & Ardo, 2006; Leveux, Geneix & Leveux, 2007; Samelis et al., 2009) while  
89 according to the council directive 92/46/EEC of 16 June 1992, thermisation is the heating  
90 of raw milk for at least 15 s at a temperature between 57°C and 68°C such that after  
91 treatment the milk shows a positive reaction to the phosphate test.

92 When focusing on microbiological safety, the accurate description of the kinetics of the  
93 target pathogenic microorganism (or a surrogate of a pathogenic target microorganism) is  
94 essential. Milk designed for milk based products should receive a heat treatment process  
95 to reduce the probability of survival of *L. monocytogenes* by at least a factor of  $10^4$  (FIL-  
96 IDF, 1994). Taking a safety margin into account, usually a log reduction of 6 is  
97 considered (Claeys, Van Loey & Hendrickx, 2002). Estimation of the time to achieve this  
98 log reduction is a very critical issue for the design and application of an efficient heat  
99 treatment.

100 Over the last 30 years a number of inactivation models have been developed aiming at  
101 describing non-log-linear microbial inactivation kinetics. An overview of inactivation  
102 models portraying eight common type curves is given by Geeraerd, Valdramidis and Van  
103 Impe, (2005). The development of these models raises the need of redefining the thermal  
104 death time by a modelling approach that includes the possibility of non-log-linear  
105 microbial survival curves, especially in cases of mild heat treatments, like thermisation.  
106 Recent studies in the broader field of heat processing suggested alternative approaches  
107 for evaluating the efficacy of a process when assuming that microbial heat resistances  
108 follow a weibulian frequency distribution model (Mafart, Couvert, Gaillard & Leguerinel,  
109 2002; Corradini et al., 2006; Sant'Ana, Rosenthal & Massaguer, 2009). Nevertheless,  
110 these approaches take into account only two types of non-log-linearity (i.e., concave,  
111 convex) and are not retaining classical parameters (like the  $z$  - value) for evaluating the  
112 achieved microbial reduction.

113 The main objective of this study was to calculate a value for the efficacy of a mild  
114 heating process similar to thermisation in which inactivation kinetics are not log-linear  
115 and to test the approach for the mild heat treatment of thermisation for a (model) liquid  
116 food system. *L. innocua* is considered as the surrogate safety target attributed for the  
117 studied simulant liquid food. The final objective was to develop an alternative (to the

118 classical  $F$ ,  $P$ ,  $C$  values) mathematical expression in order to evaluate the efficiency of a  
119 thermal treatment if additional environmental or physiological factors are considered.

120

121

## 122 **Materials and methods**

123

### 124 *Modelling approaches*

125

126 In this study a general expression of the microbial inactivation kinetics (Van Impe,  
127 Poschet, Geeraerd & Vereecken, 2005), was considered. This expression is described as  
128 follows.

$$\frac{dN}{dt} = -k(N, \langle env \rangle, \langle phys \rangle) \cdot N \quad (2)$$

129

130  $N$  is the cell density of the microbial species (cfu/mL),  $\langle env \rangle$  denotes the actual  
131 (micro)environmental conditions (not or only slightly influenced by the microbial  
132 evolution) such as temperature, high pressure, salt concentration, water activity, etc and  
133  $\langle phys \rangle$  is the physiological state of the species, for instance, as influenced by the  
134 temperature history. This expression can then be coupled with differential equations that  
135 describe the dynamics of the physiological state parameters e.g., Geeraerd, Herremans &  
136 Van Impe, (2000).

137 A sound set of differential equations, which is a sub-case of Eq. (2), and describes the  
138 microbial inactivation kinetics by incorporating physiological adjustments during the  
139 microbial inactivation experiments is the dynamic, non-log-linear model of (Geeraerd et  
140 al., 2000). This model is constructed for microbial inactivation by mild heating.

141



$$\frac{dN}{dt} = -k_{\max} \cdot \left( \frac{1}{1 + C_c} \right) \cdot \left( 1 - \frac{N_{res}}{N} \right) \cdot N \quad (3)$$

$$\frac{dC_c}{dt} = -k_{\max} \cdot C_c \quad (4)$$

142

143 Herein,  $N$  represents the microbial cell density [cfu/mL],  $C_c$  is related to the physiological  
 144 state of cells [-],  $k_{\max}$  denotes the specific inactivation rate [1/min] and  $N_{res}$  the residual  
 145 population density [cfu/mL]. This is a model for describing non-linearities that  
 146 incorporate shoulder and/or tailing effects and it automatically reduces to log-linear  
 147 inactivation kinetics if the data do not include these effects. Although there are more  
 148 known survivor curve shapes for vegetative bacterial cells (Geeraerd et al., 2005) the  
 149 current modelling approach is built based on the features of the microbial data of the case  
 150 study presented hereunder.

151 Thermal inactivation parameters, i.e., the asymptotic decimal reduction time (*AsymD*-  
 152 value) (Juneja, Eblen & Marks, 2001) and the thermal resistance constant (*z*-value), i.e.,  
 153 the temperature change required to achieve a tenfold change in *AsymD*-value, integrated  
 154 into the Bigelow model (Eq. (5)) yields predictions for the specific inactivation rate at a  
 155 given temperature.

156

$$k_{\max}(T) = \frac{\ln 10}{AsymD_{ref}} \cdot \exp\left(\frac{\ln 10}{z} \cdot (T - T_{ref})\right) \quad (5)$$

157

158 Herein,  $AsymD_{ref}$  [min] is the asymptotic decimal reduction time at the reference  
 159 temperature  $T_{ref}$  [°C]. Observe that  $D_{ref}$  of the original Bigelow equation is replaced by  
 160  $AsymD_{ref}$  as it describes the negative inverse of the slope of the log linear part of the  
 161 inactivation curve.

162

163 Eqs (2)-(5) are the main set of equations for calculating the mild heat pasteurisation  
164 value, *MH*-value (see Results).

165 The explicit version of Eqs (3)-(5) is as follows (Eq. (5) is inserted in the explicit version  
166 of Eqs (3), (4)).

167

$$\log(N(t)) = \log\left(\left(10^{\log(N(0))} - 10^{\log(N_{res})}\right) \cdot \exp\left(-\frac{\ln 10}{AsymD_{ref}} \cdot \exp\left(\frac{\ln 10}{z} \cdot (T - T_{ref})\right) \cdot t\right)\right) \\ \cdot \frac{1 + 10^{\log(C_c(0))}}{1 + 10^{\log(C_c(0))} \cdot \exp\left(-\frac{\ln 10}{AsymD_{ref}} \cdot \exp\left(\frac{\ln 10}{z} \cdot (T - T_{ref})\right) \cdot t\right)} + 10^{\log(N_{res})} \quad (6)$$

168

169 In order to demonstrate the validity of the modelling approach that evaluates the *MH*-  
170 value some illustrative examples are given. Firstly, a dynamic profile representing  
171 thermisation was generated and secondly, the static temperatures of different time  
172 treatments for achieving the same microbial reduction were evaluated. Both studies were  
173 performed for a given microbial inactivation kinetics (see hereunder).

174

## 175 **Case study**

### 176 *Microbial essay*

177

178 The studied methodological approach is illustrated based on data of microbial kinetics of  
179 *Listeria innocua* CLIP 20-595 originating from Peroval, Portanguen and Kondjoyan,  
180 (2004). Summarising, the heat resistance of *Listeria innocua* was studied by the use of  
181 100 µL of the cell suspension sealed in sterile glass capillary tubes. The tubes were  
182 immersed in a thermostat controlled circulating water bath at temperatures of 58, 60, 63,  
183 66, 68 and 70°C. Come-up times, which were in any case very small, were included as  
184 part of the total heating time used to calculate the inactivation parameters. Decimal serial  
185 dilutions of the samples were made in TS medium and surface plated in duplicate on

186 PALCAM agar (Merck). Plates were incubated for 24 -48h at 37°C and colony-forming  
187 units (cfu) were enumerated. Heat resistance experiments were carried out in triplicate.

188

### 189 *Parameter estimation*

190

191 The microbial inactivation parameters under the isothermal conditions were estimated:

- 192 (i) by taking into account the appearing shoulder and tailing effects (Eqs (3), (4)),
- 193 (ii) by assuming first order inactivation kinetics for all the inactivation data (Eq.  
194 (3) when omitting the second and third factor of the right hand side)
- 195 (iii) by assuming first order inactivation kinetics only for the log-linear portion of  
196 the inactivation kinetics.

197 The last two approaches are the classical approaches for parameter estimation, but in  
198 case (iii) there is some subjectivity on the choice of the microbial data that belong to  
199 the log-linear portion. In this study the choice was made taking into account the  
200 information coming from case (i) (i.e., initiation of the log-linear portion at the end of  
201 the shoulder up to the beginning of the tailing). Consequently, the parameters of the  
202 last procedure will be similar to the parameters of the log-linear portion of case (i).  
203 Hereupon, these three different case studies have been used in order to evaluate the  
204 equivalent isothermal temperature of a dynamic temperature profile.

205

206 In order to identify the model parameters a so called global identification making use of  
207 all static experiments in one step was implemented. So for example in the case (i)  
208  $AsymD_{ref}$ ,  $z$ ,  $\log C_c(0)$ , and  $\log N(0)$  (one for each temperature),  $\log N_{res}$  (one for each  
209 temperature) (Eq. (6)) were estimated. The selected reference temperature was chosen to  
210 be equal to 62°C, as the optimal choice to minimize the uncertainty on  $AsymD_{ref}$  was in  
211 the middle of the studied temperature range as commonly chosen (Poschet, Geeraerd,  
212 Van Loey, Hendrickx & Van Impe, 2005). Parameters were estimated based on the  
213 minimisation of the Sum of Squared Errors (SSE).

214

215

### 216 *Thermisation temperature profile*

217

218 Thermisation experiments were conducted in a pilot scale retort following the same  
219 procedure described in (Patras, Tiwari, Brunton & Butler, 2009). The prepared cans (75 ×  
220 110 mm, WEI/WEISS03, Germany) were filled with the same suspension used for the  
221 microbial studies, i.e., Tryptone-Salt (TS) medium 0.1% w/v, 0.85% w/v NaCl) and were  
222 loaded into the pilot scale retort (Barriquand Steriflow, Roanne, France). Sample core  
223 temperature profiles were recorded during the process, using an Ellab E-Val TM TM9608  
224 data module (Ellab [UK] Ltd., Norfolk, England) connected to a laptop. A standard Ellab  
225 SSA-12080-G700-TS temperature probe was inserted through an Ellab GKM-13009-  
226 C020 packing gland (20 mm) into the a can to record the temperature cycle. Temperature  
227 was monitored every 10 s. The samples were heated targeting at a final temperature of 54  
228 and 57°C. Prior to any canning experiment, all Ellab unit probes were calibrated against a  
229 JOFRA (ATC-155B) calibration unit.

230

## 231 **Results & Discussion**

### 232 *Defining the MH-value*

233

234 *MH*-value was defined as the time needed to achieve a predefined microbial reduction at  
235 a reference temperature and a known thermal resistant constant,  $z$ , when microbial  
236 inactivation kinetics is not linear. Mathematically, this expression can be calculated by  
237 deriving the achieved microbial reduction at given temperature conditions. So if the non-  
238 log-linear microbial kinetics are described by the (Geeraerd et al., 2000) Eqs (3)-(5) then  
239 the achieved microbial reduction is given as follows.

240

$$\int_0^t \frac{dN(t)}{N(t)} = -\int_0^t k_{\max} \cdot \left( \frac{1}{1 + C_c} \right) \cdot \left( 1 - \frac{N_{res}}{N} \right) \cdot dt$$

241

$$\ln \left( \frac{N(t) - N_{res}}{N(0) - N_{res}} \right) = -\frac{\ln 10}{\text{Asym } D_{ref}} \cdot \int_0^t \exp \left( \frac{\ln 10}{z} \cdot (T - T_{ref}) \right) \cdot \left( \frac{1}{1 + C_c(t)} \right) \cdot dt$$

242 with  $C_c(t)$  described by Eq (4)

$$\frac{dC_c}{dt} = -k_{\max} \cdot C_c$$

243

244 Similar to the pasteurisation/sterilisation/cooling principles (*P/F/C*-values) the mild heat  
 245 value (*MH*-value) can be calculated in the following form when considering non-  
 246 isothermal conditions and microbial parameter identification originates from microbial  
 247 data that incorporate shoulder and/or tailing effects.

$$MH = \int_0^t \ln 10 \cdot \exp\left(\frac{\ln 10}{z} \cdot (T(t) - T_{ref})\right) \cdot \left(\frac{1}{1 + C_c(t)}\right) \cdot dt \quad (7)$$

248

$$\frac{dC_c}{dt} = -k_{\max}(T(t)) \cdot C_c(t) \quad (8)$$

249

250 Observe that the *MH*-value is given by a set of two equations in which the second  
 251 describes the evolution of the microbial physiological state of the cells. Under isothermal  
 252 conditions for an equivalent temperature  $T_{eq}$  Eqs (7), (8) will look as follows (the explicit  
 253 version is given).

254

255

$$MH = \ln 10 \cdot \exp\left(\frac{\ln 10}{z} \cdot (T_{eq} - T_{ref})\right) \cdot \left( t + \frac{1}{\frac{\ln 10}{AsymD_{ref}} \cdot \exp\left(\frac{\ln 10}{z} \cdot (T_{eq} - T_{ref})\right)} \cdot \ln \left( \frac{1 + C_c(0) \cdot \exp\left(-\frac{\ln 10}{AsymD_{ref}} \cdot \exp\left(\frac{\ln 10}{z} \cdot (T_{eq} - T_{ref})\right) \cdot t\right)}{C_c(0) + 1} \right) \right) \quad (9)$$

256

257

258 where instead of  $T(t)$  the equivalent temperature  $T_{eq}$  is chosen, in which the same  
 259 microbial inactivation is achieved for the same time treatment (see Welt, Teixeira,  
 260 Balaban, Smerage and Sage (1997) for a similar example when considering first order  
 261 inactivation kinetics).

262

263 *Microbial parameter identification*

264

265 A so called global identification making use of all static experiments (presented in Figure  
266 1) in one step was implemented. Parameters estimated taking into account the non-  
267 linearities are presented in Table 1. In the case that shoulder and tailing effects were not  
268 considered, Eq. (3) reduces to the classical first order inactivation model. Similarly the  
269 inactivation parameters were estimated for that case study and the results are illustrated in  
270 Table 2. As expected, when assuming first order inactivation kinetics only for the log-  
271 linear portion of the inactivation kinetics the estimated microbial parameters i.e.,  
272  $AsymD_{62}$ ,  $z$ , coincide with those estimated during the non-log-linear regression analysis  
273 (Table 3). The obtained  $z$ -values appear to be rather high but they seem to be on the range  
274 of the estimated  $z$ -value ( $z = 7^{\circ}C$ ) of *L. monocytogenes* obtained for various food  
275 products (van Asselt & Zwietering, 2006).

276 The conducted thermisation experiments resulted on the temperature profiles given in  
277 Figure 2. The targeting final temperature of 54 and 57°C was achieved in less than 20  
278 minutes. The effect of non-log-linearity on describing the microbial inactivation kinetics  
279 was evaluated for both tested temperature profiles (by coupling these profiles with the  
280 microbial modelling models) (see Figure 2). These predictions are performed considering  
281 that inactivation of *L. innocua* is initiated at temperatures higher than 47°C (Valdramidis  
282 et al., 2008).

283 Depending on the severity of the temperature treatment the curves are diverging (see  
284 Figure 2). Considering that non-log-linearity described better the microbial data at hand it  
285 is evident that at temperature profiles targeting at 57°C an overestimation of the achieved  
286 microbial reductions is predicted from the classical log-linear modelling approaches  
287 (Figure 2). However for a target temperature of 54°C only when considering the  
288 microbial parameters estimated from a log-linear-regression of all the data results in the  
289 less conservative predictions for the achieved microbial reduction (Figure 2).

290 Further on, the developed methodological approach of the *MH*-value is tested for  
291 evaluating the efficiency of the dynamic temperature treatments when targeting at a  
292 specific microbial log reduction. Therefore, for a set of chosen equivalent temperatures,

293 i.e., 52, 55, 57°C, the *MH* -values are estimated by the use of Eq. (9) when considering  
294 the three approaches of linearity and non-linearity. The desired microbial log reduction of  
295 *L. innocua* was set to 4 logs (FIL-IDF, 1994) and 6 logs (safety margin (Claeys et al.,  
296 2002)). Observe that the derivation of *MH*-value (or the so called equivalent holding  
297 time,  $t_{eq}$ ) when considering log-linear inactivation were calculated by omitting the factor  
298 which describes the physiological state of the cells in Eq. (9). Given that non-log-linearity  
299 describes more accurately than log-linearity the static microbial inactivation data and  
300 considering that the  $t_{eq}$  from the non-log-linear inactivation kinetics is the true one some  
301 observations can be drawn from the results presented in Figure 3. On one hand when  
302 assuming log-linear kinetics at the examined temperature range, i.e. case (iii), an over-  
303 processing treatment seems to happen which is more evident when targeting a 4 log  
304 reduction. On the other hand when considering log-linear inactivation kinetics only for  
305 the log-linear portion of the data, an under-processing effect is quite pronounced. These  
306 results highlight that process efficiency can be wrongly calculated if assuming log-  
307 linearity for non-log-linear inactivation data. It should be noted that similar over-  
308 processing effect in case of log-linear inactivation kinetics at sterilisation temperatures  
309 for microbial spores were observed by Corradini et al., (2006). In this study it appears  
310 that the different types of log-linearity as well as the temperature range tested are both  
311 influencing the estimated *MH* values. Inactivation temperature levels higher than 57°C  
312 are expected to result in an interchange of the over- and under- processing regions (as the  
313 lines depicted in Figure 3 are not parallel) while at much higher temperatures the  
314 shoulder and tailing effects are less evident.

315 The *MH*-value can be written in a more general form as follows (from Eqs (2), (7)).

$$MH = AsymD_{ref} \cdot \int_0^t k(N, \langle env \rangle, \langle phys \rangle) dt \quad (11)$$

316

317 The advantages of this equation, if compared with previous literature studies, are twofold:  
318 (i) it takes into account non-log-linearity and (ii) it can easily be extended with respect to  
319 other environmental conditions or/and adjusted according to the microbial physiological  
320 state. Although similar concepts have been discussed in the literature like the method of  
321 Paired Equivalent Isothermal Exposures (PEIE) (Welt et al., 1997), they considered

322 irreversible first-order inactivation. Thus, in the case of microbial kinetics exhibiting a  
323 shoulder effect, PEIE method would assume a straight line between two points on the  
324 actual survivor curve. Nevertheless, there are recent studies that evaluate the non-log-  
325 linearity effects by assuming convex, concave or log-linear kinetics (Mafart et al., 2002;  
326 Corradini et al., 2006; Sant'Ana et al., 2009). If these approaches are compared with the  
327 developed *MH*-value then it can be seen that the parameters of the classical Bigelow  
328 approach are retained in the current approach while the observed non-log-linearity, i.e.,  
329 shoulder, tailing effects are described by additional factors. Particularly, the non-log-  
330 linearity effect in those studies is described by the parameters  $\delta$  and  $p$  and  $b$  and  $n$ ,  
331 respectively, which do not discriminate between log-linear and non-log-linear parts of the  
332 inactivation kinetics. The advantage of the current approach is that the additional  
333 parameters can be interpreted independently and Eq. (11) can be further specified  
334 depending on the case-study at hand, for example, when more (environmental or  
335 physiological) factors are considered.

336

### 337 **Future work**

338

339 Further studies on coupling microbial inactivation kinetics with heat transfer phenomena  
340 especially for non-homogeneous products (including solid foods) are of interest for  
341 designing similar thermal process. Investigation with other microorganisms and cases that  
342 result in high non-log-linearity and comparison with the classical acceptable approaches  
343 will work as additional validation of the developed modelling approach. For industrial  
344 application purposes a comparative economical impact of the calculation of different  
345 thermal death values is also of interest as it can avoid over and under-processing  
346 schemes.

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355 **References**

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443 Table 1. Parameter estimates (and their standard errors) derived from the non-log-linear  
 444 regression of Eqs 3-5 on the microbial data performed in capillary tubes at temperatures  
 445 58-66°C.  
 446

| Asym $D_{62}$ [min]  | $z$ [°C]             | $\log(C_c(0))$ [-]   |                      |
|----------------------|----------------------|----------------------|----------------------|
| $0.32 \pm 0.01$      | $9.02 \pm 0.24$      | $1.14 \pm 0.23$      |                      |
| $\log N(0)_{58}$ [-] | $\log N(0)_{60}$ [-] | $\log N(0)_{63}$ [-] | $\log N(0)_{66}$ [-] |
| $8.99 \pm 0.14$      | $8.77 \pm 0.14$      | $9.01 \pm 0.14$      | $9.05 \pm 0.16$      |
| $\log N_{res58}$ [-] | $\log N_{res60}$ [-] | $\log N_{res63}$ [-] | $\log N_{res66}$ [-] |
| $2.89 \pm 0.25$      | $3.59 \pm 0.14$      | $2.77 \pm 0.29$      | $2.77 \pm 0.25$      |

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451 Table 2. Parameter estimates (and their standard errors) derived from a linear regression  
 452 of the microbial data performed in capillary tubes at temperatures 58-66°C.  
 453

| Asym $D_{62}$ [min]  | $z$ [°C]             |                      |                      |
|----------------------|----------------------|----------------------|----------------------|
| $0.41 \pm 0.13$      | $8.75 \pm 0.29$      |                      |                      |
| $\log N(0)_{58}$ [-] | $\log N(0)_{60}$ [-] | $\log N(0)_{63}$ [-] | $\log N(0)_{66}$ [-] |
| $9.19 \pm 0.19$      | $9.31 \pm 0.24$      | $9.23 \pm 0.19$      | $9.38 \pm 0.26$      |

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456 Table 3. Parameter estimates (and their standard errors) derived from a linear regression  
 457 of the microbial data when assuming first order inactivation kinetics only for the log-  
 458 linear portion of the inactivation kinetics.  
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| Asym $D_{62}$ [min] | $z$ [°C]        |
|---------------------|-----------------|
| $0.32 \pm 0.01$     | $9.02 \pm 0.24$ |

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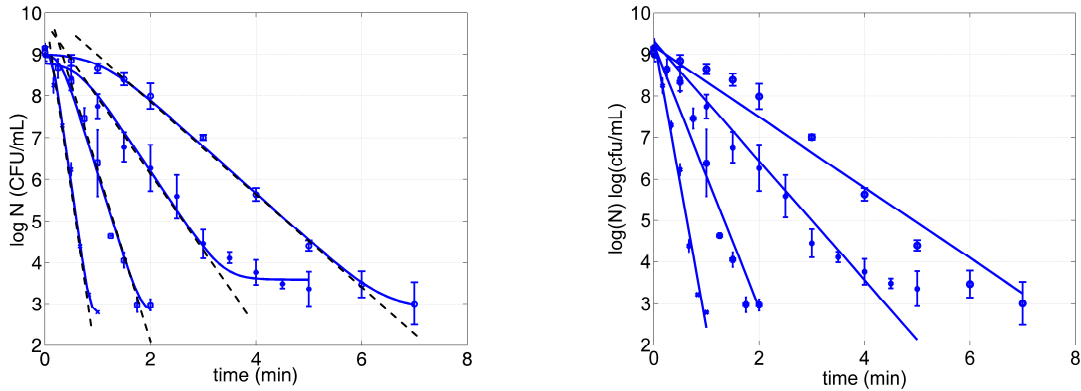
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475 Figure 1. Regression analysis by taking into account shoulder and tailing effects (Left

476 Figure, continuous line), by assuming first order inactivation kinetics for the log-

477 linear portion of inactivation (Left Figure, dashed line), by assuming first order

478 inactivation kinetics for all inactivation data (Right Figure).

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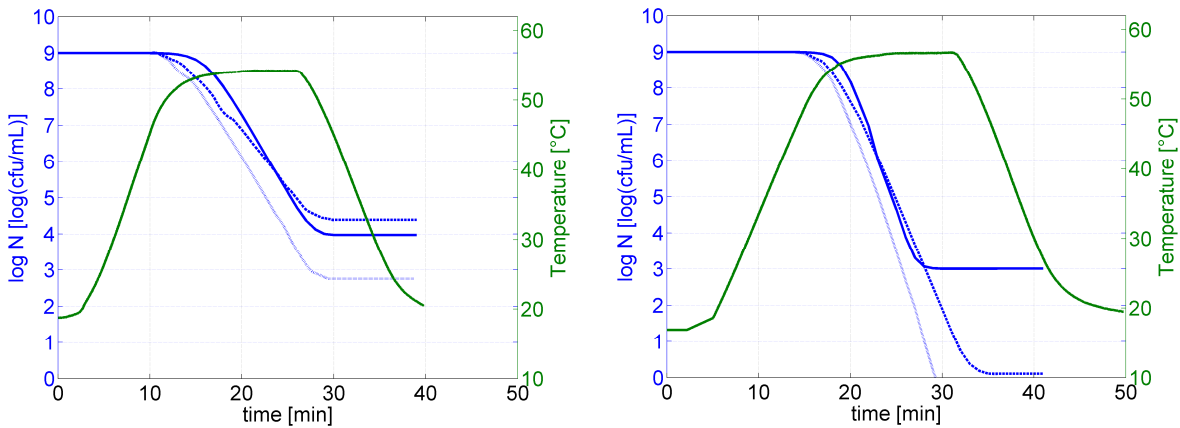
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494 Figure 2. Dynamic temperature profile of a mild heat process representing thermisation

495 with a target temperature of 54°C (Left), 57°C (Right) and associated microbial

496 simulations (i) (considering non-log-linearity), continuous line, case (ii) (considering log-

497 linearity when taking the log-linear portion of the data), dotted line, case (iii) (considering

498 log-linearity for all data), dashed line,

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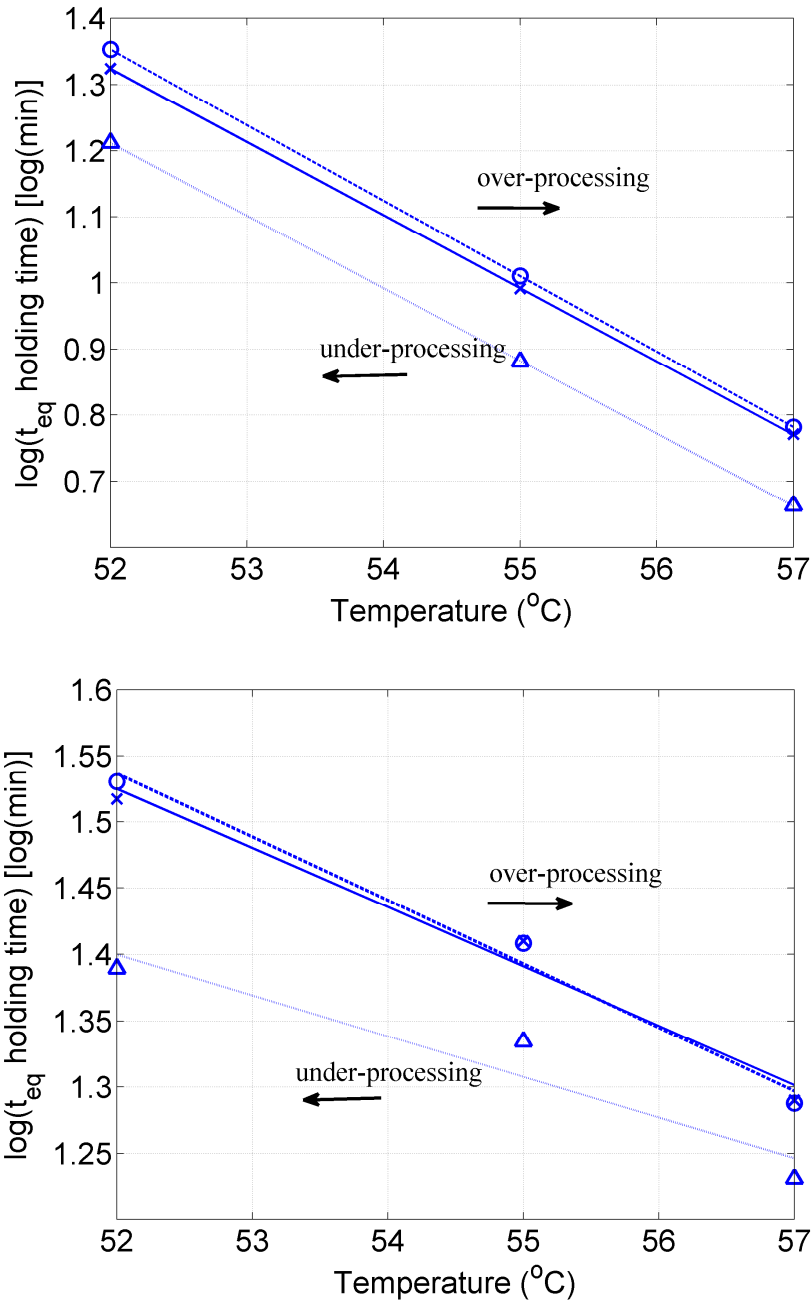


Figure 3. Derivation of equivalent time and temperature values (o: considering log-linearity for all data), ( $\Delta$ : considering log-linearity when taking the log-linear portion of the data), (x: considering non-log-linearity) when targeting at 4 log (top Figure) and 6 log reduction (bottom Figure) of *L. innocua*. Lines represent linear regression of the obtained data points (i) (considering non-log-linearity), continuous line, case (ii) (considering log-linearity when taking the log-linear portion of the data), dotted line, case (iii) (considering log-linearity for all data), dashed line, respectively.