2011-01-01

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
Technological University Dublin, pj.cullen@tudublin.ie

See next page for additional authors

Authors

Vasilis Valdramidis, Brijesh Tiwari, Patrick Cullen, Alain Kondjoyan, and Jan Van Impe

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[*]   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

— Use Licence —

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[1*], A. H. Geeraerd[2,3], B. K. Tiwari[4], P.J. Cullen[1], A. Kondjoyan[5] and J.

5 F. Van Impe[2,6]

6

7 [1]School of Food Science & Environmental Health, Dublin Institute of Technology, Cathal

8 Brugha Street, Dublin 1, Ireland.

9 [2]CPMF[2]-Flemish Cluster Predictive Microbiology in Foods

10 [3]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 [4]Department of Food & Tourism Management, Manchester Metropolitan University,

14 Manchester, M14 6HR, UK

15 [5]INRA, UR370 Qualité des Produits Animaux, F-63122 St Genès Champanelle, France.

16 [6]BioTeC – Bioprocess Technology and Control, Department of Chemical Engineering,

17 Katholieke Universiteit Leuven, W. de Croylaan 46, B-3001 Leuven, Belgium

18

19

20

21

22

23

24

25

26

27 ***Corresponding author:** 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.

42

43

44

45

46

47

48

49

50

51

52

53

54

55

56

57

Introduction

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

$$F_{T_{ref}} = \int_{0}^{t} 10^{(T(t)-T_{ref})/z} dt \quad (1)$$

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

A mild heat treatment often applied in the dairy industry is thermisation of milk destinated for cheese making. This process has milder effects on the raw milk flora and the functionality of milk caseins and salts than pasteururisation (Samelis et al., 2009). Thermisation is applied at temperatures that range between 52 to 67°C for a treatment 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; Levieux, Geneix & Levieux, 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 $57^{o}C$ and $68^{o}C$ 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

4

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.

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

129

130 *N* is the cell density of the microbial species (cfu/mL), < *env* > 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 < *phys* > 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

$$\log(N(t)) = \log((10^{\log(N(0))} - 10^{\log(Nres)}) \cdot \exp\left(-\frac{\ln 10}{AsymD_{ref}} \cdot \exp\left(\frac{\ln 10}{z} \cdot (T - T_{ref})\right) \cdot t\right)$$

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

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, logC_c(0), and log$N$(0) (one for each temperature), logN_{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.

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

240

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

241

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

$$MH = \ln 10 \cdot \exp\left(\frac{\ln 10}{z} \cdot (T_{eq} - T_{ref})\right) \cdot$$

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

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

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 ($z = 7^{o}$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)).

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

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 δ 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.

347

348

349

350

351

352

353

354

References

356 Ball, C. O. (1923). Thermal Process Time for Canned Food. *National Research Council*
357 *Bulletin*, 7(37).
358 Christiansen, P., Nielsen, E. W., Vogensen, F. K., Brogren, C. H., & Ardo, Y. (2006).
359 Heat resistance of Lactobacillus paracasei isolated from semi-hard cheese made of
360 pasteurised milk. *International Dairy Journal*, 16(10), 1196-1204.
361 Claeys, W. L., Van Loey, A. M., & Hendrickx, M. E. (2002). Intrinsic time temperature
362 integrators for heat treatment of milk. *Trends in Food Science & Technology*, 13(9-10),
363 293-311.
364 Corradini, M. G., Normand, M. D., & Peleg, M. (2005). Calculating the efficacy of heat
365 sterilization processes. *Journal of Food Engineering*, 67(1-2), 59-69.
366 Corradini, M. G., Normand, M. D., & Peleg, M. (2006). Expressing the equivalence of
367 non-isothermal and isothermal heat sterilization processes. *Journal of the Science of Food*
368 *and Agriculture*, 86(5), 785-792.
369 FIL-IDF (1994). Recommendations for the hygienic manufacture of milk and milk based
370 products. Brussels, International Dairy Federation.
371 Geeraerd, A. H., Herremans, C. H., & Van Impe, J. F. (2000). Structural model
372 requirements to describe microbial inactivation during a mild heat treatment.
373 *International Journal of Food Microbiology*, 59(3), 185-209.
374 Geeraerd, A. H., Valdramidis, V., & Van Impe, J. F. (2005). GInaFiT, a freeware tool to
375 assess non-log-linear microbial survivor curves. *International Journal of Food*
376 *Microbiology*, 102(1), 95-105.
377 Huang, L. H. (2009). Thermal inactivation of Listeria monocytogenes in ground beef
378 under isothermal and dynamic temperature conditions. *Journal of Food Engineering*,
379 90(3), 380-387.
380 ICMSF (1996). ICMSF: Microorganisms in Food 5, Characteristics of microbial
381 pathogens London, Blackie Acedmic and professional.
382 Juneja, V. K., Eblen, B. S., & Marks, H. M. (2001). Modeling non-linear survival curves
383 to calculate thermal inactivation of *Salmonella* in poultry of different fat levels.
384 *International Journal of Food Microbiology*, 70(1-2), 37-51.
385 Levieux, D., Geneix, N., & Levieux, A. (2007). Inactivation-denaturation kinetics of
386 bovine milk alkaline phosphatase during mild heating as determined by using a
387 monoclonal antibody-based immunoassay. *Journal of Dairy Research*, 74(3), 296-301.
388 Mafart, P., Couvert, O., Gaillard, S., & Leguerinel, I. (2002). On calculating sterility in
389 thermal preservation methods: application of the Weibull frequency distribution model.
390 *International Journal of Food Microbiology*, 72(1-2), 107-113.
391 McKellar, R. C., & Piyasena, P. (2000). Predictive modelling of inactivation of bovine
392 milk alpha-L-fucosidase in a high-temperature short-time pasteurizer. *International Dairy*
393 *Journal*, 10(1-2), 1-6.
394 Miller, F. A., Gil, M. M., Brandao, T. R. S., Teixeira, P., & Silva, C. L. M. (2009).
395 Sigmoidal thermal inactivation kinetics of Listeria innocua in broth: Influence of strain
396 and growth phase. *Food Control*, 20(12), 1151-1157.

397 Patras, A., Tiwari, B. K., Brunton, N. P., & Butler, F. (2009). Modelling the effect of
398 different sterilisation treatments on antioxidant activity and colour of carrot slices during
399 storage. *Food Chemistry*, 114(2), 484-491.
400 Peroval, C., Portanguen, S., & Kondjoyan, A. (2004). Thermal inactivation of *Listeria*
401 *innocua* CLIP 20-595 in broth or attached to Teflon surfaces. Food Factory of the Future,
402 Laval, France.
403 Pittia, P., Furlanetto, R., Maifreni, M., Mangina, F. T., & Rosa, M. D. (2008). Safe
404 cooking optimisation by F-value computation in a semi-automatic oven. *Food Control*,
405 19(7), 688-697.
406 Poschet, F., Geeraerd, A. H., Van Loey, A. M., Hendrickx, M. E., & Van Impe, J. F.
407 (2005). Assessing the optimal experiment setup for first order kinetic studies by Monte
408 Carlo analysis. *Food Control*, 16(10), 873-882.
409 Samelis, J., Lianou, A., Kakouri, A., Delbes, C., Rogelj, I., Bogovic-Matijasic, B., &
410 Montel, M. C. (2009). Changes in the Microbial Composition of Raw Milk Induced by
411 Thermization Treatments Applied Prior to Traditional Greek Hard Cheese Processing.
412 *Journal of Food Protection*, 72(4), 783-790.
413 Sant'Ana, A. S., Rosenthal, A., & Massaguer, P. R. (2009). Heat resistance and the
414 effects of continuous pasteurization on the inactivation of *Byssochlamys fulva* ascospores
415 in clarified apple juice
416 *Journal of Applied Microbiology*, 107(1), 197-209.
417 Stumbo, C. R. (1965). Thermobacteriology in food processing. New York, Academic
418 Press.
419 Valdramidis, V. P., Geeraerd, A. H., Bernaerts, K., & Van Impe, J. F. (2006). Microbial
420 dynamics versus mathematical model dynamics: The case of microbial heat resistance
421 induction. *Innovative Food Science & Emerging Technologies*, 7(1-2), 80-87.
422 Valdramidis, V. P., Peroval, C., Portanguen, S., Verhulst, A. J., Van Impe, J. F. M.,
423 Geeraerd, A. H., & Kondjoyan, A. (2008). Quantitative evaluation of thermal inactivation
424 kinetics of free-floating versus surface-attached *Listeria innocua* cells. *Food and*
425 *Bioprocess Technology*, 1(3), 285-296.
426 van Asselt, E. D., & Zwietering, M. H. (2006). A systematic approach to determine
427 global thermal inactivation parameters for various food pathogens. *International Journal*
428 *of Food Microbiology*, 107(1), 73-82.
429 Van Impe, J. F., Poschet, F., Geeraerd, A. H., & Vereecken, K. M. (2005). Towards a
430 novel class of predictive microbial growth models. *International Journal of Food*
431 *Microbiology*, 97-105.
432 Welt, B. A., Teixeira, A. A., Balaban, M. O., Smerage, G. H., & Sage, D. S. (1997). An
433 hypothesis paper - Iterative method for kinetic parameter estimation from dynamic
434 thermal treatments. *Journal of Food Science*, 62(1), 8-14.
435 Zehetner, G., Bareuther, C., Henle, T., & Klostermeyer, H. (1996). Inactivation of
436 endogenous enzymes during heat treatment of milk. *Netherlands Milk and Dairy Journal*,
437 50(2), 215-226.
438
439
440
441
442

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

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

447
448
449
450
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

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

454
455
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.
459

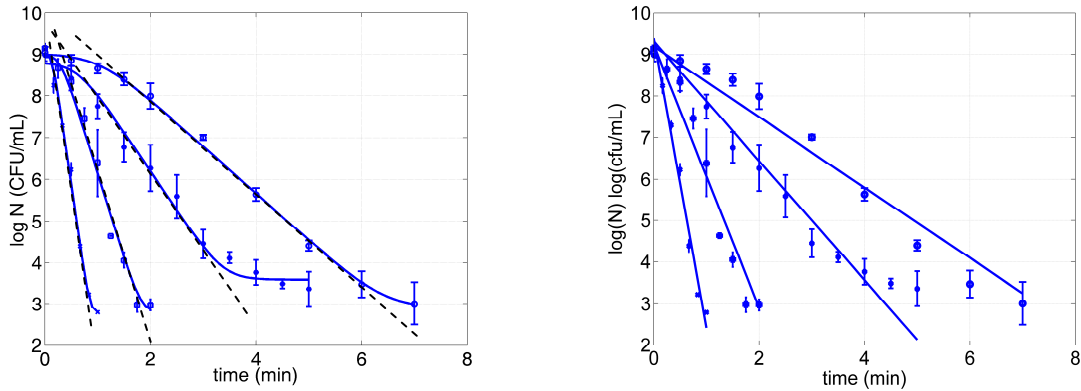| AsymD_{62} [min]	z [°C]
0.32 ± 0.01	9.02 ± 0.24

460
461

462

463

464

465

474

475 Figure 1. Regression analysis by taking into account shoulder and tailing effects (Left
476 Figure, continuous line), by assuming first order inactivation kinetics only 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).
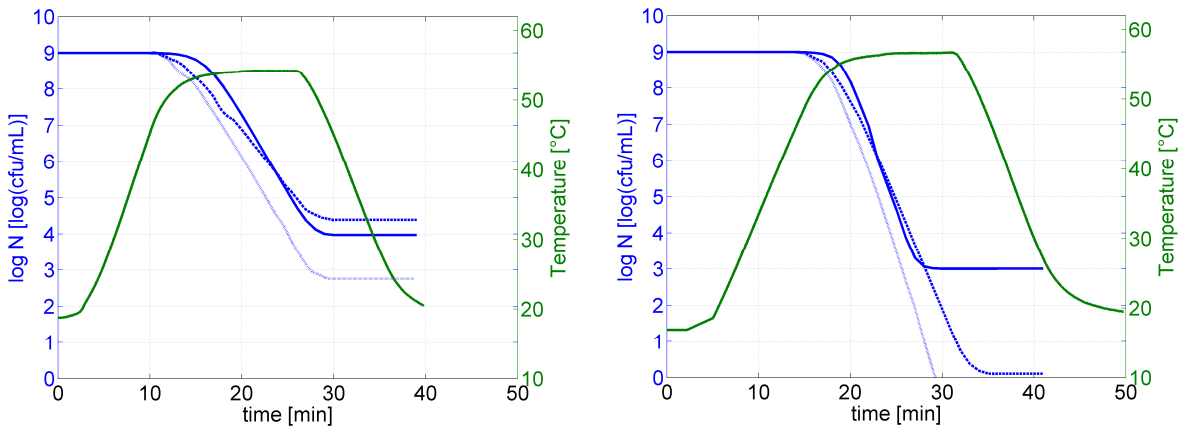
479

480

481

482

483

484

485

493

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,

499

500

501

502

503
504
505
506
507
508
509
510
511
512
513
514
515
516
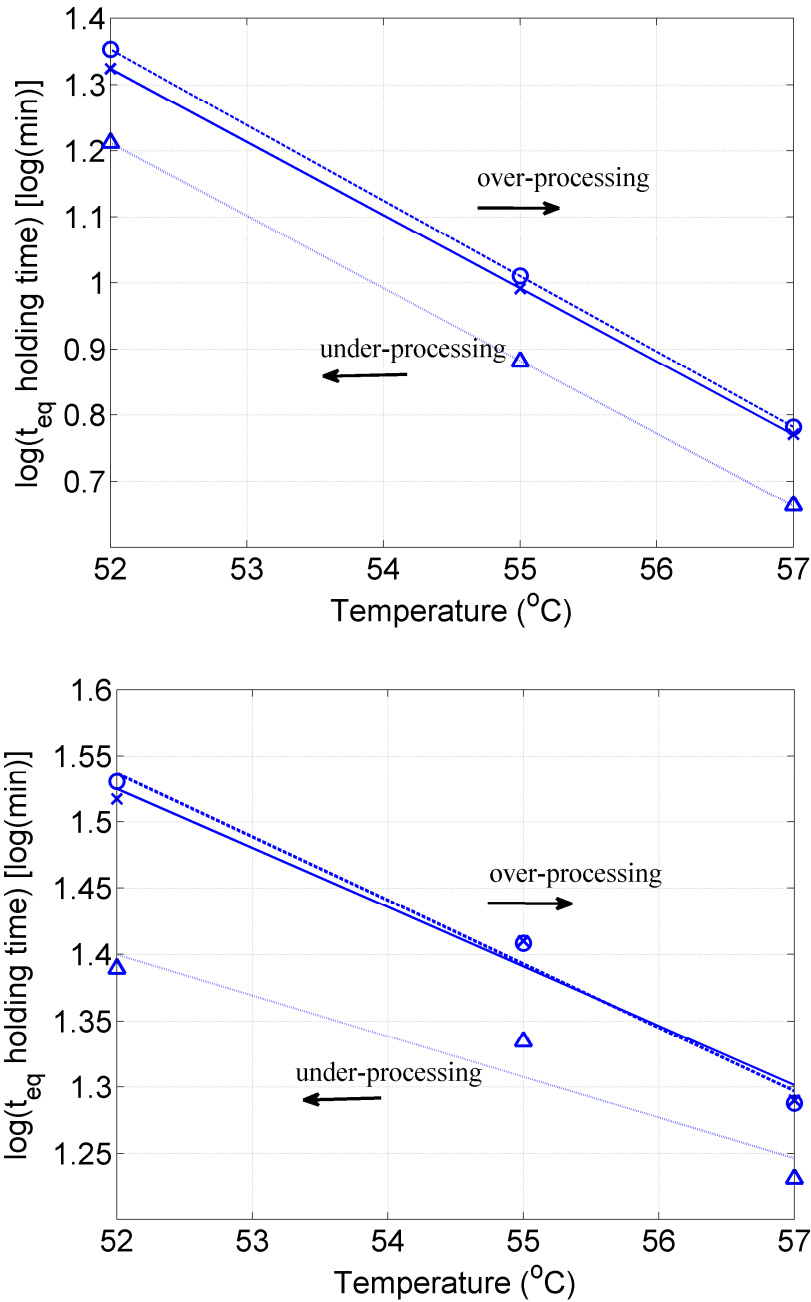517
518
519
520
521
522
523
524
525
526
527
528
529
530

Figure 3. Derivation of equivalent time and temperature values (o: considering log-linearity for all data), (Δ: 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.