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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

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Abstract

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

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

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 pasterurisation (Samelis et al., 2009). Thermisation is applied at temperatures that range between 52 to 67^{o}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

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

Materials and methods

Modelling approaches

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

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

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

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

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

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

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

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

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

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

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

Case study

Microbial essay

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

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

Parameter estimation

The microbial inactivation parameters under the isothermal conditions were estimated:

(i) by taking into account the appearing shoulder and tailing effects (Eqs (3), (4)),

(ii) by assuming first order inactivation kinetics for all the inactivation data (Eq. (3) when omitting the second and third factor of the right hand side)

(iii) by assuming first order inactivation kinetics only for the log-linear portion of the inactivation kinetics.

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

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

Thermisation temperature profile

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

Results & Discussion

Defining the MH-value

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

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

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

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

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

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).

Microbial parameter identification

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

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

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

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

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

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

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

The advantages of this equation, if compared with previous literature studies, are twofold: (i) it takes into account non-log-linearity and (ii) it can easily be extended with respect to other environmental conditions or/and adjusted according to the microbial physiological state. Although similar concepts have been discussed in the literature like the method of 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.
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Table 1. Parameter estimates (and their standard errors) derived from the non-log-linear regression of Eqs 3-5 on the microbial data performed in capillary tubes at temperatures 58-66°C.

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

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

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

Table 3. Parameter estimates (and their standard errors) derived from a linear regression of the microbial data when assuming first order inactivation kinetics only for the log-linear portion of the inactivation kinetics.

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

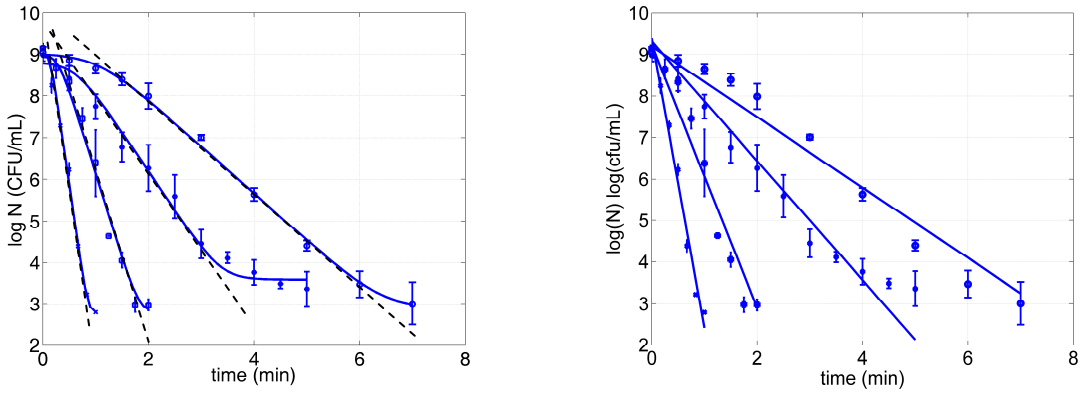

Figure 1. Regression analysis by taking into account shoulder and tailing effects (Left Figure, continuous line), by assuming first order inactivation kinetics only for the log-linear portion of inactivation (Left Figure, dashed line), by assuming first order inactivation kinetics for all inactivation data (Right Figure).

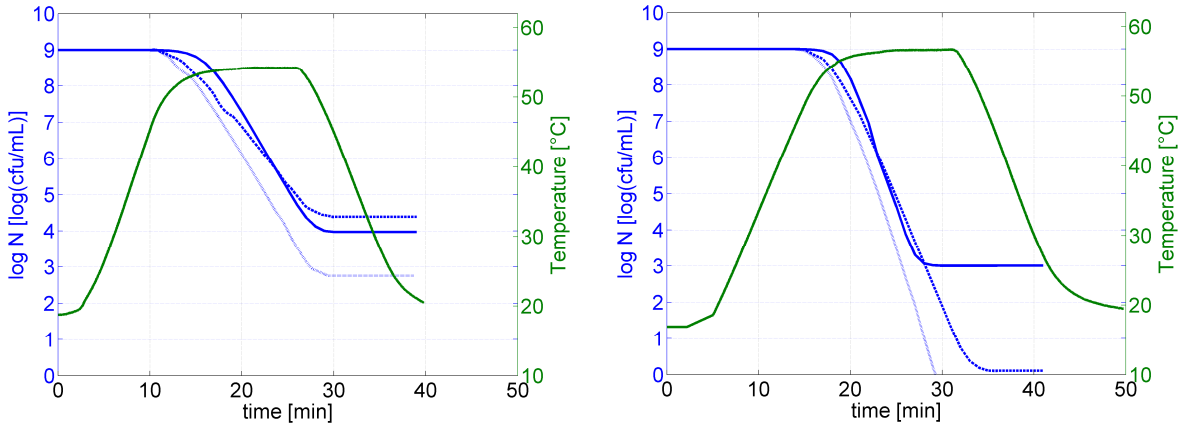

Figure 2. Dynamic temperature profile of a mild heat process representing thermisation with a target temperature of 54°C (Left), 57°C (Right) and associated microbial simulations (i) (considering non-log-linearity), continuous line, case (ii) (considering log-linearity when taking the log-linear portion of the data), dotted line, case (iii) (considering log-linearity for all data), dashed line,

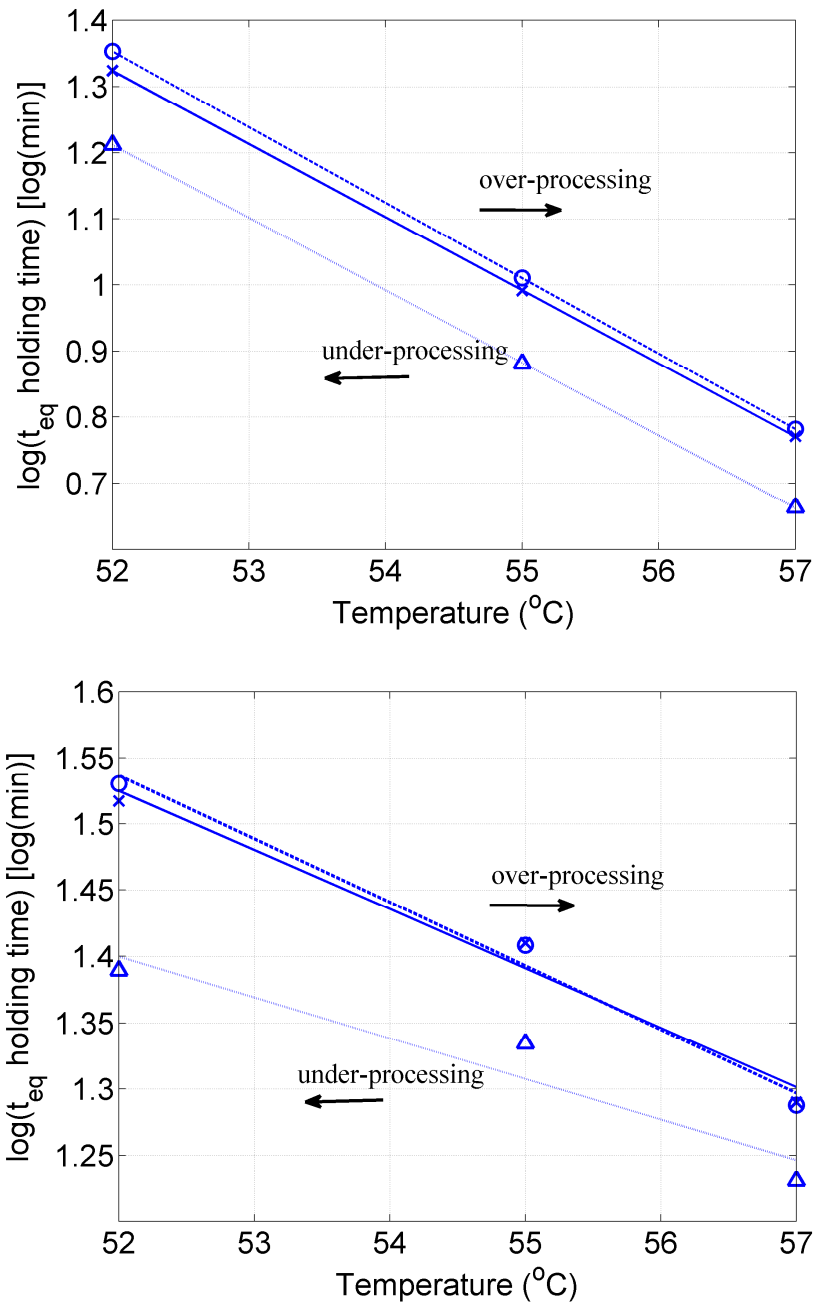

Figure 3. Derivation of equivalent time and temperature values (o: considering log-linearity for all data), (Δ: considering log-linearity when taking the log-linear portion of the data), (x: considering non-log-linearity) when targeting at 4 log (top Figure) and 6 log reduction (bottom Figure) of *L. 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.