

A new predictive dynamic model describing the effect of the ambient temperature and the convective heat transfer coefficient on bacterial growth

Hana Ben Yaghlene, Ivan Leguérinel, Moktar Hamdi, Pierre Mafart

► **To cite this version:**

Hana Ben Yaghlene, Ivan Leguérinel, Moktar Hamdi, Pierre Mafart. A new predictive dynamic model describing the effect of the ambient temperature and the convective heat transfer coefficient on bacterial growth. *International Journal of Food Microbiology*, Elsevier, 2009, 133 (1-2), pp.48-61. <10.1016/j.ijfoodmicro.2009.04.014>. <hal-00553650>

HAL Id: hal-00553650

<http://hal.univ-brest.fr/hal-00553650>

Submitted on 9 Jan 2012

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **A new predictive dynamic model describing the effect of**
2 **the ambient temperature and the convective heat transfer**
3 **coefficient on bacterial growth**

4
5 H. Ben Yaghlene^{a,b*}, I. Leguerinel^a, M. Hamdi^b, P. Mafart^a

6 ^a: Université Européenne de Bretagne, EA3882, Laboratoire Universitaire de Biodiversité et Ecologie
7 Microbienne, IFR148 ScInBioS, Université de Bretagne Occidentale, 6 rue de l'université, F29334 Quimper,
8 Cedex, France

9 ^b: Laboratoire d'Ecologie et de Technologie Microbienne, Institut National des Sciences Appliquées et de
10 Technologie (INSAT), 2 Boulevard de la terre, B.P. 676, 1080 Tunis, Tunisie

11
12 *Corresponding author: LUBEM, UBO, 6 rue de l'Université, F29334, Quimper Cedex, France. Tel.: +33 2 98
13 90 02 27; Fax: +33 2 98 90 85 44

14 E mail address: hana.benyaghlene@univ-brest.fr

15
16 **Abstract**

17 In this study, predictive microbiology and food engineering were combined in order to
18 develop a new analytical model predicting the bacterial growth under dynamic temperature
19 conditions. The proposed model associates a simplified primary bacterial growth model
20 without lag, the secondary Ratkowsky “square root” model and a simplified two-parameter
21 heat transfer model regarding an infinite slab. The model takes into consideration the product
22 thickness, its thermal properties, the ambient air temperature, the convective heat transfer
23 coefficient and the growth parameters of the micro organism of concern. For the validation of
24 the overall model, five different combinations of ambient air temperature (ranging from 8 °C
25 to 12 °C), product thickness (ranging from 1 cm to 6 cm) and convective heat transfer
26 coefficient (ranging from 8 W/(m².K) to 60 W/(m².K)) were tested during a cooling

27 procedure. Moreover, three different ambient air temperature scenarios assuming alternated
28 cooling and heating stages, drawn from real refrigerated food processes, were tested. General
29 agreement between predicted and observed bacterial growth was obtained and less than 5% of
30 the experimental data fell outside the 95 percent confidence bands estimated by the bootstrap
31 percentile method, at all the tested conditions. Accordingly, the overall model was
32 successfully validated for isothermal and dynamic refrigeration cycles allowing for
33 temperature dynamic changes at the centre and at the surface of the product. The major
34 impact of the convective heat transfer coefficient and the product thickness on bacterial
35 growth during the product cooling was demonstrated. For instance, the time needed for the
36 same level of bacterial growth to be reached at the product's half thickness was estimated to
37 be 5 and 16.5 h at low and high convection level, respectively. Moreover, simulation results
38 demonstrated that the predicted bacterial growth at the air ambient temperature cannot be
39 assumed to be equivalent to the bacterial growth occurring at the product's surface or centre
40 when convection heat transfer is taken into account. Our results indicate that combining food
41 engineering and predictive microbiology models is an interesting approach providing very
42 useful tools for food safety and process optimisation.

43

44 **Key words:**

45 Predictive microbiology; Heat transfer; Dynamic models; Bacterial growth; Cooling; Heating.

46 **Nomenclature**

47

A	Transfer area (m^2)
Bi	Biot number
E	Product thickness (m)
f'	Thermal proliferation value
Fo	Fourier number
h	Convective heat transfer coefficient ($W/(m^2.K)$)
j	Lag factor for reduced temperature profile
k	Slope of the linear portion of the semi-logarithmic plot of reduced temperature vs time.
l	Characteristic dimension (m)
N	Microbial cell density at time t (CFU/mL)
N_0	Initial microbial cell density (CFU/mL)
O	Observed microbial cell density (\log_{10} CFU/mL)
OD	Microbial optical density at time t
OD_0	Initial microbial optical density
P	Predicted microbial cell density (\log_{10} CFU/mL)
t	Time (s)
T	Temperature of the product ($^{\circ}C$)
T_{eq}	Equivalent temperature
T_{min}	Temperature below which no microbial growth occurs ($^{\circ}C$)
T_{opt}	Temperature at which the microbial growth is optimal ($^{\circ}C$)
T_0	Initial product temperature ($^{\circ}C$)
T_{∞}	Ambient temperature ($^{\circ}C$)
x	Distance from the centre (m)
x_{max}	Half-thickness for an infinite slab (m)
α	Thermal diffusivity of the product (m^2/s)
Γ	Overall gamma function
γ	Thermal gamma function
λ	Thermal conductivity of the product ($W/(m.K)$)
μ	Specific microbial growth rate (h^{-1})
μ_{opt}	Optimal specific microbial growth rate (h^{-1})
φ	First root of $\cot \varphi = \varphi / Bi$
φ_n	Roots of $\cot \varphi = \varphi / Bi$

48

49 **1. Introduction**

50 Predictive food microbiology involves knowledge of microbial growth responses to
51 environmental factors expressed in quantitative terms by mathematical equations (models)
52 (McMeekin et al., 1997). It is generally agreed that the most important environmental factor
53 that affects bacterial growth in food is temperature. This factor is constantly changing during
54 processing, storage and distribution of food products (Fujikawa et al., 2004). At the same
55 time, control organizations propose stringent requirements regarding monitoring of the
56 internal temperature of products being processed. For instance, according to the United States
57 Department of Agriculture, the allowable growth of *Clostridium perfringens* during the
58 cooling of certain meat and poultry products should be limited to 1 log (U.S.D.A, 1999). In
59 the case of cured products, the guidelines recommend that products' internal temperature
60 should be reduced from 54.4 °C to 26.6 °C in less than 5 h, and from 26.6 °C to 7.2 °C in the
61 next 10 h (15 h total cooling time).

62 Predictive microbiology is a useful tool for assessing and controlling food safety particularly
63 when models are able to cope with dynamic conditions such as changing temperatures.
64 Numerous published models, reported in Table 1, have dealt with the prediction of bacterial
65 growth under dynamic temperature conditions. Generally, these works were based on
66 predictive models corresponding to isothermal bacterial growths which were modified in
67 order to consider the effect of temperature changes. These studies primarily aimed at
68 assessing the feasibility of predicting the bacterial growth at changing temperature. Indeed,
69 predicting bacterial growth under dynamic conditions has been shown to be possible via the
70 dynamic transformation of existing static models. According to the authors of the cited studies
71 (Table 1), the models' predictions agreed well with experimental results after temperature
72 changes.

73 Nevertheless, the considered scenarios of changing temperatures within bacterial models were
74 various. In some cases, typical temperature profiles were considered either by the use of
75 hypothetical ones or by the adoption of real temperature scenarios recorded while the studied
76 products had been processed. In other cases, temperature profiles were simulated from heat
77 transfer models. Mathematical models that integrate effectively heat transfer phenomena and
78 dynamic bacterial growth relationships are scarce (Amezquita et al., 2005; de Jong et al.,
79 2005; Zwietering and Hasting, 1997). Armitage (1997) derived the temperature histories for
80 the deep leg and leg surface of a lamb carcass by using finite element models for dealing with
81 regular shapes. Temperature function integration technique (TFI) was applied to the
82 calculated temperature histories of five different cooling processes to simulate *Escherichia*
83 *coli* growth on the leg surface of each carcass during aging. TFI technique was previously
84 introduced by Gill and Harrison (1985). Data related to the growth of six strains of *E. coli*
85 isolated from commercially packed livers were fitted according to the Ratkowsky model
86 (Ratkowsky et al., 1982) linking the growth rate to temperature. Total growth was obtained by
87 summation of partial growth calculated within sequential 3.75-min periods from the growth
88 rate for the average temperature within each period. Estimated bacterial growth during offals
89 cooling (cooling profiles were directly recorded on several offals being chilled) agreed well
90 with observed data with *E. coli*. Bellara et al. (2000) experimentally validated bacterial
91 growth modelling involving a heat transfer model of temperature fluctuations within a solid
92 object. From data related to *E. coli* W3110 growth in agar, a model was set up describing
93 bacterial growth as a function of temperature. This was then used in conjunction with a finite
94 difference heat transfer model describing temperature change in a cylinder in order to
95 calculate the bacterial growth that occurs in agar gel inside a cylindrical glass vessel under
96 conduction cooling. Excellent agreement was found between model simulations and
97 experimental data. Alavi et al. (2001) determined the growth characteristics of *Listeria*

98 *monocytogenes* in sterilized whole milk. The parameter values of the Baranyi dynamic growth
99 model (Baranyi et al., 1995) were determined. Finite element software, ANSYS, was
100 implemented to determine temperature distributions in milk cartons subject to a time-varying
101 ambient temperature profile. The space-time-temperature data were input to the Baranyi
102 dynamic growth model, to predict the microbial population density distribution and the
103 average population density in the milk carton. Amezcuita et al. (2005) developed a computer
104 simulation scheme to analyze heat transfer phenomena and temperature-dependent *C.*
105 *perfringens* growth during cooling of cooked boneless cured ham. The temperature history of
106 ham was predicted from a finite element heat diffusion model. For *C. perfringens* growth, a
107 dynamic model was developed from the Baranyi's nonautonomous differential equation
108 (Baranyi and Roberts, 1994). The bacterium's growth model was integrated into the computer
109 program taking predicted temperature histories as input values. Validation of the model
110 predictions considered three different time-temperature cooling histories from 54.4 °C to 7.2
111 °C of the geometrical centre of a large cooked boneless ham. In a further work (Corradini et
112 al., 2006), the same data were fitted with the ad hoc empirical models in order to define the
113 three parameters temperature dependence of the modified version of the logistic primary
114 model. The continuous rate equation was solved incrementally by a numerical procedure
115 implemented in general purpose software. In both Amezcuita et al. (2005) and Corradini et al.
116 (2006) works, predicted *C. perfringens* growth curves obtained from dynamic modelling
117 showed good agreement with observed results for the tested cooling scenarios. The integrated
118 modelling approach for predicting microbial behaviour during processing was reviewed by
119 Lebert and Lebert (2006).

120 Combination of predictive microbiology and food engineering allows both the assessment of a
121 process in relation to risk and its optimisation (Mafart, 2005). In spite of all the advances
122 made in modelling microbial growth, proposing and improving new overall models which

123 combine predictive microbiology and heat transfer phenomena is an obvious necessity. The
124 more simple the proposed models and the more realistic the considered conditions are, the
125 easiest is their implementation to food processes.

126 In the present study, we aimed to develop a new analytical model combining bacterial growth
127 prediction and food engineering. Our goal was for the overall model to be simple and robust,
128 with minimum involved parameters, and to integrate the process conditions. The proposed
129 model is the association of three equations: a simplified primary model consisting of a simple
130 exponential growth equation without lag time, the Ratkowsky “square root” model linking the
131 specific growth rate to temperature (Ratkowsky et al., 1982), and a simplified two-parameter
132 heat transfer model regarding an infinite slab. While classical models assume that ambient
133 temperature is immediately reached at the growth medium, this work takes into account, not
134 only the air temperature, but also the thickness of the medium, its thermal properties and the
135 convective heat transfer coefficient. In addition, the proposed model may be easily
136 implemented to various micro organisms having known growth parameters.

137 **2. Development of the model**

138 **2.1. Heat transfer modelling**

139 All cooling processes for solid materials exhibit similar behaviour. After an initial ‘lag’, the
140 reduced temperature at the thermal centre of the food item decreases exponentially. The linear
141 portion of the cooling curve (obtained by plotting, on semi-logarithmic axes, the reduced
142 temperature $(T_{\infty} - T)/(T_{\infty} - T_0)$ versus time (Becker and Fricke, 2004), see Fig. A1 in the
143 appendix) can typically be described by the simplified linear asymptotic form of the general
144 heat transfer model, valid for Fourier number $Fo \geq 0.2$ with $Fo = \alpha \cdot t/l^2$ where α is thermal
145 diffusivity of the product, l is the characteristic dimension of the product, T is the local
146 temperature of the product at the time t , T_0 is the initial temperature of the product, T_{∞} is the

147 cooling medium temperature (pulsed air). Consequently, the heating or cooling kinetic is
148 expressed as follows:

$$149 \quad T = T_{\infty} (1 - je^{-kt}) + T_0 je^{-kt} \quad (1)$$

150 Where j is the lag factor and k is the slope of the linear portion of the semi-logarithmic plot of
151 reduced temperature vs time. The value of the parameter k depends upon the product shape, l ,
152 α and the Biot number $Bi = hl/\lambda$, where h is the convective heat transfer coefficient between
153 the product surface area and the ambient air and λ is the thermal conductivity of the product.
154 A low Biot number ($Bi < 0.1$) indicates that the internal resistance to heat transfer is
155 negligible, and thus, the temperature within the object is uniform at any given instant in time
156 while the external thermal resistance can be neglected for $Bi > 40$ (Mafart, 1997).

157 The governing differential equation for infinite slab is given as follows with the initial
158 (uniform distribution of temperature) and boundary condition (surface convection):

$$159 \quad \frac{\partial T}{\partial t} = \alpha \frac{\partial^2 T}{\partial x^2} \quad (2)$$

160 where x is distance from the centre. For initial condition of uniform temperature and boundary
161 conditions of central symmetry and convective heat transfer at the surface, solution for Eq. (2)
162 is supplied by the infinite series given by Carslaw and Jaeger (1986) (see Eq. (20) in the
163 appendix). It is a general knowledge that use of the first term of this series would be enough
164 when the Fourier number is greater than 0.2 since the temperature change after that certain
165 time would be linear (Becker and Fricke, 2004; Bimbenet et al., 2002; Caro-Corrales et al.,
166 2002; Dincer, 1996; Erdogan, 2005). As long as the thermal diffusivity is constant, this first
167 term approach may be easily used to determine the temperature change with Eq. (1) and with
168 the known heat transfer coefficient value:

169 $k = \varphi^2 \cdot \alpha / l^2$ at any given position in the infinite slab where φ is the first root of the following
170 equation:

171
$$\cot \varphi = \frac{\varphi}{Bi} \quad (3)$$

172 At the half thickness of the infinite slab,

173
$$j = \frac{2 \sin \varphi}{\varphi + \sin \varphi \cos \varphi} \quad (4a)$$

174 At the surface of the infinite slab,

175
$$j = \frac{2 \sin \varphi \cos \varphi}{\varphi + \sin \varphi \cos \varphi} \quad (4b)$$

176 With respect to the cooling/heating of the product during the initial ‘lag’ period ($Fo < 0.2$), we
 177 assumed a straight linear link between T_0 and temperature at time corresponding to $Fo = 0.2$.

178 **2.2. Bacterial growth modelling**

179 As a primary model, a simple exponential growth without lag time was assumed:

180
$$N = N_0 e^{\mu t} \quad (5)$$

181 Where N is the cell number at time t , N_0 is the initial cell number and μ the specific bacterial
 182 growth rate at time t .

183 The gamma function corresponds to a comparison between the growth rate of microbial cells
 184 growing at given environmental conditions and the optimum growth rate that would be
 185 measured at optimal conditions (Zwietering et al., 1992):

186
$$\Gamma = \frac{\mu}{\mu_{opt}} \quad (6)$$

187 In the framework of standard calculations aiming to compare processes to each others, a
 188 simplified gamma function depending only on temperature can be derived from the “square
 189 root” model of Ratkowsky et al. (1982) modified by Zwietering et al. (1992)

190
$$\gamma(T) = \left(\frac{T - T_{min}}{T_{opt} - T_{min}} \right)^2 \quad (7)$$

191 Where T_{min} is the temperature below which no microbial growth occurs and T_{opt} is the
192 temperature at which the microbial growth is optimal.

193 If a process has to be intrinsically assessed regardless of the characteristics of the foodstuff, a
194 partial proliferation value may be involved. If the local temperature at which the bacteria are
195 growing is the single considered factor, Mafart (2005) defined a “thermal proliferation value”
196 as:

$$197 \quad f' = \int_0^t \gamma(T) dt \quad (8)$$

198 This value has the dimension of a time and corresponds to a time-temperature cycle that
199 would yield the same proliferation ratio than a growth of f' units of time at the optimal and
200 constant temperature (Mafart, 2005).

201 The original differential form of equation (6) is:

$$202 \quad \frac{1}{N} \frac{d(N)}{dt} = \mu(t) = \mu_{opt} \gamma[T(t)] \quad (9)$$

203 the solution of which yields:

$$204 \quad \frac{N}{N_0} = \exp \left[\mu_{opt} \int_0^t \left(\frac{T - T_{min}}{T_{opt} - T_{min}} \right)^2 dt \right] \quad (10)$$

205 This model can be easily combined with heat transfer models permitting the obtainment of an
206 analytic solution yielding the overall model. Note that, even for complex foodstuff shapes, an
207 analytic solution of the model is possible, provided that thermal parameters of equation (1) (j
208 and k) can be empirically determined from a temperature registration and a linear regression
209 of reduced temperature.

210 The concept of thermal proliferation value may be usefully completed by that of equivalent
211 temperature, which corresponds to the constant temperature that would yield the same
212 proliferation during the duration of a thermal cycle. A cooling/heating process can then be

213 characterised and be compared with others by its duration and its equivalent temperature
 214 (Mafart, 2005).

$$215 \quad \gamma(T_{eq})t = f' \quad (11)$$

216 where time is expressed in hours. The combination of this last equation with Eq. (7) yields:

$$217 \quad T_{eq} = T_{min} + (T_{opt} - T_{min}) \sqrt{\frac{f'}{t}} \quad (12)$$

218 2.3. Overall model

219 The overall model developed in the present study aims to consider the convective heat
 220 transfer coefficient and the temperature of the ambient medium when predicting bacterial
 221 growth under dynamic conditions of temperature.

222 Combining Eqs. (1) and (7) the obtained time-dependent $\gamma(T)$ function is employed in Eq. (8)
 223 in order to express, by integration, f' as a function of t :

$$224 \quad f'(T) = \frac{k(T_{\infty} - T_{min})^2 t + 2j(T_{\infty} - T_{min})(T_0 - T_{\infty})(1 - e^{-kt}) + \frac{j^2}{2}(T_0 - T_{\infty})^2(1 - e^{-2kt})}{k(T_{opt} - T_{min})^2} \quad (13)$$

225 The substitution of f' in Eq. (13) by its above-cited expression enables the calculation of the
 226 equivalent temperature as follows:

$$227 \quad T_{eq} = T_{min} + \sqrt{\frac{k(T_{\infty} - T_{min})^2 t + 2j(T_{\infty} - T_{min})(T_0 - T_{\infty})(1 - e^{-kt}) + \frac{j^2}{2}(T_0 - T_{\infty})^2(1 - e^{-2kt})}{kt}} \quad (14)$$

228 The implementation of the Eq. (10) to T_{eq} of the thermal process yields:

$$229 \quad \frac{N}{N_0} = \exp \left[\mu_{opt} \left(\frac{T_{eq} - T_{min}}{T_{opt} - T_{min}} \right)^2 t \right] \quad (15)$$

230 The overall mathematical model corresponds to the association of these two last equations.

231 The determination of the j value at the centre (Eq. (4a)) or at the surface (Eq. (4b)) of an

232 infinite slab allows the calculation of the corresponding T_{eq} values. The parameter k is

233 determined from Eq. (3) at any given position in the product and with Bi which includes the
234 convective heat transfer coefficient h that will be changed in the experimental validation of
235 the overall model.

236

237 **3. Materials and methods**

238 **3.1. Micro-organism and inoculum preparation**

239 *E. coli* SOR 201 (isolated from cheese), provided by SOREDAB Laboratory (France), was
240 stored at -80 °C in nutrient broth supplemented with 50% glycerol. The inoculum was
241 prepared by subculturing the bacterial strain in 100 mL of nutrient broth (tryptone 10 g/L,
242 meat extract 5 g/L and sodium chloride 5 g/L. pH 7.2±0.2) at 37 °C on a rotary shaker (100
243 rpm), subsequently for 8 h and 16 h.

244 **3.2. Experimental setup for the model validation at constant air temperature and** 245 **changing convective heat transfer coefficient**

246 **3.2.1. Growth measurement**

247 Growth curves were determined by the measurement of absorbance changes with a
248 spectrophotometer (Milton Roy, Spectronic 301) at 600 nm wavelength. Absorbance was
249 measured with sterile nutrient broth as a blank. Samples were filled in glass “growth flasks”
250 specially designed for the measurement of the absorbance of the content without sampling. A
251 calibration experiment was done to determine the correlation between viable counts and
252 absorbance data in nutrient broth permitting the estimation of the bacterial concentrations
253 from absorbance values. Samples were incubated at 30 °C in a shaking water bath and
254 bacteria were grown until stationary phase. The optical density was periodically measured and
255 one mL was simultaneously removed from the solution to be analyzed by the plate count
256 method. The experimental dataset was fitted with the exponential model proposed by Juarez
257 Tomas et al. (2002) to fit the calibration equation which can be written as follows:

258
$$\ln N = a(OD)^b \quad (16)$$

259 Where N is the bacterial concentration, OD is the corresponding optical density (600 nm)
260 measured at time t and a and b are empirical parameters.

261 Fig. 1 depicts the experimental data and the calibration curve relating OD to viable counts.
262 Estimates of parameters a and b were respectively assessed to 22.8 [22.6 .. 22.9] and 0.090
263 [0.087 .. 0.093] ($R^2 = 0.999$ and $MSE = 0.009$).

264 Francois et al. (2005) demonstrated that a temperature of 4 °C had no significant statistical
265 effect on the linear calibration curve compared to optimal conditions (T 30 °C, pH 7,4 and a_w
266 0.995) in BHI medium. Consequently, we didn't perform the calibration experiment at other
267 temperatures than 30 °C (the initial product temperature at all the experiments).

268 **3.2.2. Simulation design**

269 Five combinations of thickness of an infinite slab, convective heat transfer coefficient and
270 chilling temperature were input (Fig. 2) for the calculation of thermal parameters j and k . A
271 simulated growth curve related to each combination was then calculated from Eqs. (14) and
272 (15). An h value of 8 W/(m².K) corresponds to the absence of fan (particularly in unventilated
273 zones or in equipment inside which heat is transferred by natural convection) while an h value
274 of 60 W/(m².K) corresponds to a strong air fan (in the case of forced convection). Moreover,
275 according to Kondjoyan (2006), average heat transfer coefficient values (W/(m².K))
276 calculated under different air velocity (0.2 – 5.0 m s⁻¹) and free-stream turbulence intensity
277 conditions (5 – 40 %) ranged from 1.3 to 42.0 for a circular cylinder and from 2.3 to 60.0 for
278 meat products (Beef carcass, pork hindquarter and lamb carcass (loin)). Furthermore, Ben
279 Amara et al. (2004) studied the effect of various factors (air velocity, position, air-product
280 temperature difference) on the transfers during cooling with a low air velocity (< 0.2 m/s), of
281 an in-line stack of spheres and measured h values ranging from 8 to 19 W/(m².K) for air
282 velocity varying from 0.03 to 0.19 m s⁻¹.

283 3.2.3. Conditions of growth for the validation of the model

284 Corresponding to each combination of slab thickness and convective heat transfer coefficient,
285 cooling profiles and bacterial growth were simulated at the centre and at the surface of an
286 infinite slab shaped product. The product thermal diffusivity and thermal conductivity values
287 input in the calculations were assumed to be equal to those of water determined at 25 °C, i.e.
288 $1.43 \cdot 10^{-7} \text{ m}^2/\text{s}$ and $0.6 \text{ W}/(\text{m}\cdot\text{K})$ respectively.

289 Furthermore, input μ_{opt} , T_{min} and T_{opt} values of *E. coli* SOR 201 were respectively equal to
290 2.66 h^{-1} , 3.28 °C and 42.03 °C in nutrient broth. These values were estimated from forty six
291 growth kinetics conducted at 11 constant temperatures ranging from 10 °C to 46 °C .
292 Experimental datasets were fitted and growth curve parameters (maximum growth rate (μ_{max}),
293 lag time, initial and maximum population densities) were estimated from the primary growth
294 model of Baranyi and Roberts (1994). The obtained μ_{max} values were fitted with the
295 secondary cardinal model (Rosso et al., 1995) in order to estimate the optimal growth rate and
296 the cardinal temperatures of *E. coli* SOR 201 (see Fig. A2 in the appendix).

297 In order to validate the overall mathematical model developed in the present work,
298 experiments were conducted under a changing temperature program which corresponds to the
299 simulated cooling profiles of the surface or the centre of the product. All the prepared material
300 was pre-chilled/heated to the initial temperature (30 °C). Subculture was diluted to provide an
301 inoculation level ranging from 10^6 to 10^7 CFU/mL at the starting of the test. Samples were
302 filled in glass “growth flasks” allowing the measurement of the absorbance of the content,
303 during the experiment, without sampling. Their shaking was guaranteed by a magnetic
304 shaking table. Samples were instantly incubated and periodically removed so as to measure
305 optical densities (at 600 nm) in the course of the experiment. Experiments were carried out in
306 triplicate. The chilling was monitored with a refrigerated and heating circulator (FP40-HE,
307 Julabo Labortechnik GmbH, Germany) allowing a temperature stability of $\pm 0.01 \text{ °C}$.

308 **3.3. Experimental setup for the model validation at changing air temperature and**
309 **constant convective heat transfer coefficient**

310 For a further validation of the model, more complicated temperature scenarios were tested
311 assuming alternated cooling and heating stages. Moreover, an inoculation level ranging from
312 10^3 to 10^4 CFU/mL was used in order to consider more realistic bacterial concentrations
313 encountered in food industry processes.

314 Three different ambient air temperature scenarios, drawn from real refrigerated food
315 processes, were tested (cf. Table 2).

316 Corresponding to each air temperature scenario, temperature profiles and bacterial growth
317 were simulated at the centre and at the surface of an infinite slab shaped product. At all the
318 tested temperature scenarios, the initial product temperature, the product thickness and the
319 convective heat transfer coefficient were respectively equal to 15 °C, 0.12 m and 8 W/(m².K).
320 Input μ_{opt} , T_{min} and T_{opt} values of *E. coli* SOR 201 are mentioned above. Typical beef meat
321 thermal diffusivity and thermal conductivity values, respectively equal to $1.25 \cdot 10^{-7}$ m²/s and
322 0.42 W/(m.K), were input in the calculations.

323 Experiments were conducted under thermal programs which corresponded to the simulated
324 temperature profiles of the surface or the centre of the product. Subculture was diluted,
325 samples were filled in glass “growth flasks”, instantly incubated and periodically removed so
326 as to perform the viable count measurement in the course of the experiment. Experiments
327 were carried out in duplicate. The thermal program was monitored with the refrigerated and
328 heating circulator (FP40-HE, Julabo Labortechnik GmbH, Germany). At each sampling time,
329 1-mL aliquots were aseptically removed from each “growth flask”, serially diluted in tryptone
330 salt broth and plated on nutrient agar (15 g/L) with a double layer. Petri dishes were incubated
331 at 37 °C for 24 h and colonies were counted.

332 **3.4. Validation and assessment of the quality of the overall model**

333 Model accuracy was assessed by the estimation of confidence bands of predictions by using
334 the bootstrap percentile method (Efron and Tibshirani, 1993). Forty six growth kinetics were
335 performed in nutrient broth at 11 constant temperatures ranging from 10 °C to 46 °C (data not
336 shown). Bootstrap of each kinetic was made in order to take the experimental errors of
337 kinetics into account. The appropriate residuals of each kinetic were drawn with replacement.
338 Twenty five thousands bootstrap set of primary parameters were obtained. Each set of
339 bootstrapped μ_{max} values were used to fit the secondary model. Then bootstrapped values of
340 parameters T_{min} , T_{opt} and μ_{opt} were used to predict the bacterial growth with the overall model.
341 Secondary observed residuals were drawn with replacement and added to the μ predicted
342 value. Primary residuals were added to the predicted kinetics to consider the experimental
343 error of cell number estimation. Twenty five thousands bootstrapped growth kinetics were
344 obtained for each validation experiment and sorted in ascending order at each calculation
345 time. The quartiles 2.5% and 97.5% of the sorted bootstrapped kinetics were taken to give the
346 inferior and superior limits. These points were linked to give an approximation of the 95
347 percent confidence bands of the predicted growth kinetics. To validate the assumptions made
348 and the model, less than 5% of the points must fall outside the confidence bands.
349 With regard to the assessment of the quality of the overall model predictions various
350 statistical criteria were calculated at all the tested conditions of validation experiments.
351 Correlation coefficients (R) between observations and predictions were calculated using the
352 ‘corrcoef’ function of MATLAB 6.5. The correlation coefficient is related to the covariance
353 cov by:

$$354 \quad R(i, j) = \frac{cov(i, j)}{\sqrt{cov(i, i)cov(j, j)}} \quad (17)$$

355 where i is the observations index and j is the variables index.

356 Bias factor B_f (Eq. (18)) and Accuracy factor A_f (Eq. (19)) were calculated as proposed by
357 Jeyamkondan et al. (2001). These factors were initially introduced by Ross (1996) but here,
358 the predicted values are normalized.

$$359 \quad B_f = 10^{\sum \log(P/O)/n} \quad (18)$$

$$360 \quad A_f = 10^{\sum |\log(P/O)|/n} \quad (19)$$

361 where O and P are observed and predicted microbial populations in \log_{10} CFU/mL and n is
362 the number of observations. The bias factor indicates the relative average deviation of
363 predicted and observed bacterial growth. However it has to be kept in mind that this deviation
364 does not directly concern the size of the population, but its logarithmic transformation which
365 reduces the effect of outliers.

366 A bias factor of 1 indicates perfect agreement between observed and predicted values.
367 However, $B_f > 1$ or < 1 indicates that the model predicts N upper than or lower than observed
368 values. ~~For example, $B_f = 1.1$ means that predictions are on average upper than observed~~
369 ~~values by 10%.~~ The accuracy factor gives indication of the spread of the results about the
370 predicted value. An accuracy factor of 1 represents perfect agreement between observed and
371 predicted values. This parameter quantifies the difference between observed and predicted
372 values. ~~For example, $A_f = 1.25$ indicates that the average deviation of the predicted values~~
373 ~~from the observed values is 25%. It has to be kept in mind that this deviation does not directly~~
374 ~~concern the size of the population, but its logarithmic transformation.~~

375 4. Results

376 4.1. Model validation at constant air temperature and changing convective heat transfer 377 coefficient

378 4.1.1. Growth simulation

379 Thermal programs tested at the validation stage were utilized at five different combinations of
380 product thickness (E), convective heat transfer coefficient (h) and refrigeration temperature

381 (T_∞) (Fig. 2).

382 The obtained cooling profiles and growth predictions are presented in Fig. 3. As a rule,
383 bacterial growth kinetics were faster at the product centre than at its surface. In cases of
384 lowest thickness, simulated computing profiles (1c and 2c) were practically identical leading
385 to the same bacterial growth predictions (1g and 2g) at the centre and at the surface of the
386 product. In fact, in these two experiments the product thickness was sufficiently low to
387 prevent a clear observation of an internal temperature gradient and the internal heat resistance
388 may had been neglected. In contrast, the difference between simulated theoretical temperature
389 kinetics at the centre and the surface of the product were more marked in the cases of the
390 highest thickness leading to an important thermal gradient within the product (3c and 4c). For
391 that matter, the most important differences between internal and surface growth kinetics were
392 obtained in these two cases (3g and 4g), particularly in the case of low heat transfer
393 coefficient.

394 On the other hand, additional simulated growth curves at constant temperatures equal to T_∞
395 were presented in Fig. 3. The aim of these further experiments was to compare the bacterial
396 growth that really occurs when temperature dynamic changes at the centre and at the surface
397 of the cooled product are considered, with the growth that would occur if external and internal
398 resistances were neglected.

399 **4.1.2. Validation results**

400 Each validation experiment regarding each (T_∞, h, E) combination (Fig. 2) was conducted
401 under two different refrigeration programs which corresponded to the simulated cooling of the
402 surface and the centre of the infinite slab-shaped product yielding a total of eight validation
403 tests. These tests lasted 24 hours with an initial temperature T_0 of 30 °C. In order to validate
404 the overall mathematical model, Fig. 4 depicts predicted and observed *E. coli* SOR 201
405 growth during the simulated cooling at the centre or the surface of the product for the five

406 cases presented in Fig. 2. A fair agreement between predicted and observed growth can be
407 noted, and the observed growth data of *E. coli* SOR 201 were found to be adequately
408 predicted by the overall mathematical model. For each predicted growth kinetic, 95 percent
409 confidence bands calculated with bootstrap method are also presented in Fig. 4. All the
410 experimental data fell inside the confidence bands showing that the overall model was
411 successfully validated at the tested conditions.

412 On the other hand, the quality of the overall model prediction was assessed by the calculation
413 of correlation coefficient, B_f and A_f (see Table 3). The correlation coefficients were higher
414 than 0.975 showing that the bacterial growth was satisfactorily predicted by the overall
415 model. At all the instances, bias factors ranged from 0.98 to 1.00 indicating that ~~on average,~~
416 ~~the observed growth was at the most 2% higher than the predicted growth and that~~ the overall
417 predictions agreed well with observed data with a slight under-prediction tendency by the
418 model. ~~In all the tested conditions, the percentage of predicted values which were different~~
419 ~~(either above or below) than the observed values was at the most equal to 5% as can be noted~~
420 ~~from A_f values ranging from 1.01 to 1.05.~~

421 Agreement between model predictions and experimental validation results was also assessed
422 by plotting predicted bacterial growth versus observed bacterial growth (Fig. 5). A perfect
423 agreement between predicted and measured growth was observed for 30.80% of experimental
424 data. A percentage of 25.36% of the observed–predicted values’ pairs were laying under the
425 line of equivalence while 43.84% were laying above the line of equivalence.

426 **4.2. Model validation at changing air temperature and constant convective heat transfer** 427 **coefficient**

428 **4.2.1. Growth simulation**

429 Fig. 6 illustrates the simulated thermal profiles and bacterial growth kinetics according to the
430 conditions presented in Table 2 and §3.3. Thermal and bacterial growth simulation results

431 showed important differences of temperature profiles and growth between the product centre,
432 the product surface and the ambient air. During heating, the highest bacterial growth was
433 observed when simulations were carried out at air temperature, followed by the product's
434 surface and then by the product's centre. Conversely, an opposite trend was observed during
435 cooling. From a safety point of view, neglecting heat transfer phenomena that occur inside the
436 product and between the ambient air and the product is more dangerous at cooling stages than
437 at heating stages. Nevertheless this hazard depends on the temperature and duration history of
438 the cooling/heating stages. Consequently it's very important to take into account the actual
439 temperature of the product surface or centre rather than the ambient air temperature for a
440 reliable bacterial growth prediction.

441 **4.2.2. Validation results**

442 Validation results regarding the three tested ambient scenarios are presented in figures 7 and
443 8. The observed growth data of *E. coli* SOR 201 were found to be adequately predicted by the
444 overall mathematical model. On the other hand, less than 5% of the experimental data fell
445 outside the 95 percent confidence bands showing that the overall model was successfully
446 validated at the tested conditions. Table 4 presents the statistical criteria related to the data of
447 validation at changing air temperature and shows correlation coefficients higher than 0.944 at
448 all the tested conditions. Bias factors obtained at all the assays were higher than 1 showing a
449 slight over-prediction tendency by the model. A_f values (lower than 1.13 at all the instances)
450 indicate that the overall predictions agreed well with observed data. Fig. 8 shows predicted
451 bacterial growth versus observed bacterial growth and confirms that the overall model was
452 successfully validated, with a slight over-prediction tendency, for the tested dynamic
453 refrigeration cycles.

454 **5. Discussion**

455 Traditionally, when simulating a bacterial growth during a product cooling or heating,
456 predictive microbiology took only the ambient air temperature into account, ignoring delays
457 and temperature gradients due to external and internal thermal resistances linked to the effects
458 of the medium thickness and of the convective heat transfer coefficient on the rate of heat
459 transfer. All the studies cited in Table 1 have evaluated the prediction of bacterial growth
460 under dynamic temperature conditions taking into account only the temperature of the
461 cooling/heating medium. Assuming that the bacterial growth directly occurs at the
462 temperature of air is not valid. In fact, the local temperature at any given position in the
463 product depends upon both T_{∞} and h . In the present study, predictive microbiology was
464 combined with heat transfer phenomena in order to develop an overall mathematical model
465 describing the effect of the ambient temperature and the convective heat transfer coefficient
466 on bacterial growth. The proposed method in this work is different than those in previous
467 studies which combined predictive microbiology and heat transfer (Alavi et al., 2001;
468 Amezcuita et al., 2005; Bellara et al., 2000; Corradini et al., 2006). An experimental method
469 was implemented in order to validate the model results. Thermal profiles were firstly
470 simulated at the centre and at the surface of the infinite slab shaped product. Then,
471 experiments were conducted under a changing temperature program corresponding to the
472 simulated temperature kinetics. The validation of the overall model was performed by the
473 comparison of the measured bacterial growths with model predictions at constant ambient air
474 temperature and changing convective heat transfer coefficient and product thickness (five
475 different combinations of product thickness, convective heat transfer coefficient and cooling
476 air temperature were tested), then at changing ambient air temperature and fixed convective
477 heat transfer coefficient and product thickness (three different ambient air scenarios assuming
478 alternated cooling and heating stages, drawn from real refrigerated food processes, were
479 tested).

480 As expected, the results of the computer simulation related to single cooling stage
481 experiments showed that the bacterial growth kinetics were faster at the product centre than at
482 its surface. Moreover, the importance of the impact of the convective heat transfer coefficient
483 and of the product thickness on the bacterial growth during the cooling of an infinite slab
484 shaped product by pulsed air was pointed out. As an example, at the product half thickness,
485 the level of bacterial growth accomplished after 5 hours of cooling with $h = 8 \text{ W}/(\text{m}^2 \cdot \text{K})$ (case
486 3) was the same after 16.5 hours with $h = 60 \text{ W}/(\text{m}^2 \cdot \text{K})$ (case 4). For these cases the product
487 thickness was the same and the cooling air temperature was in case 3 4°C lower than in case
488 4. Similarly, at product half thickness, the cooling time needed to reach a same bacterial
489 growth with low or high product thickness (1 cm in case 2 and 6 cm in case 4) was estimated
490 to 17.5 hours and 8 hours, respectively, for the same convection level ($60 \text{ W}/(\text{m}^2 \cdot \text{K})$). The
491 slowest simulated bacterial growth was observed with intermediate product thickness and
492 convective heat transfer coefficient (case 5). Moreover, it obviously appears that the validity
493 of the assumption of an instantaneously thermal equilibrium between the product and the
494 cooling medium with regard to bacterial growth is depending on the level of convection. In
495 fact, predicted growth kinetics at the surface of the product were close to those predicted at T_∞
496 for high heat transfer coefficients (h) equal to $60 \text{ W}/(\text{m}^2 \cdot \text{K})$, which corresponds to a strong air
497 fan (Fig. 3: 2g and 4g). At this instance, neglecting the external heat resistance may be
498 accepted particularly for low product thickness (1 cm) where there's no internal heat
499 resistance too. Oppositely, for low heat transfer coefficients (h) equal to $8 \text{ W}/(\text{m}^2 \cdot \text{K})$, which
500 corresponds to the absence of fan, (Fig. 3: 1g and 3g) important dissimilarities between
501 predicted growth kinetics at the surface of the product and at T_∞ may be observed. Indeed,
502 assuming no external heat transfer resistance is not valid especially for high product thickness
503 of 6 cm (Fig. 3: 3g) where the bacterial growth that occurs when the product is cooled from T_0
504 to T_∞ was clearly higher than that at an immediately reached temperature T_∞ . In this case, a

505 difference of $1.35 \log_{10}(\text{CFU/mL})$ between the bacterial populations at the product surface
506 and at T_{∞} was reached after 24 hours of growth. Besides, using the bootstrap percentile
507 method for the estimation of confidence bands of predictions, the model was successfully
508 validated at all the tested conditions.

509 Concerning the results related to alternated cooling and heating stage experiments, the model
510 was successfully validated at more complicated ambient air scenarios and lower inoculation
511 levels by plate count measurement method. These results showed that, in predictive
512 microbiology, assuming an instantaneously thermal equilibrium between the product and the
513 ambient medium is not valid and may have large consequences on risk assessment of
514 refrigerated food processes (see Fig. 9).

515 To assess the quality of the overall model, predicted bacterial growth was compared to
516 observed growth according to several statistical criteria related to the data of validation. Our
517 results pointed out a good agreement between predicted and observed growth.

518 On the other hand, in the overall model, as a primary bacterial model, a simple exponential
519 growth without lag time was assumed. In other words, it is supposed that the bacterial growth
520 occurred without delay when the product temperature was continuously changing. Our
521 assumption is in agreement with several previous studies. Although the fact that organisms
522 need to adapt to the new temperature, so that they go through a lag phase caused by the stress
523 of the temperature shift, was largely noticed in literature (Amezquita et al., 2005; Baranyi et
524 al., 1995; Koutsoumanis, 2001; Zwietering et al., 1994), the lag phase duration was rarely
525 considered in predictive models. In accordance with our work, no further delay occurring after
526 temperature shifts, once a cell population is growing exponentially, was frequently assumed
527 (Baranyi et al., 1995; Bovill et al., 2000; Koutsoumanis et al., 2006). Although, Swinnen et al.
528 (2005) demonstrated that temperature shifts crossing a lag/no lag transition zone (positioned
529 more or less between 22.78 and 23.86 °C for *E. coli* K12 MG1655) will cause an intermediate

530 lag phase, we consider that neglecting contingent intermediate lag phase by our model may be
531 a valid approach. In fact, simulated thermal profiles at product surface or centre due to heat
532 transfer phenomena (see Fig. 3 and Fig. 6) progressively changed with time and cannot be
533 regarded as instantaneous temperature shifts with given amplitude which may generate
534 intermediate lag phases.

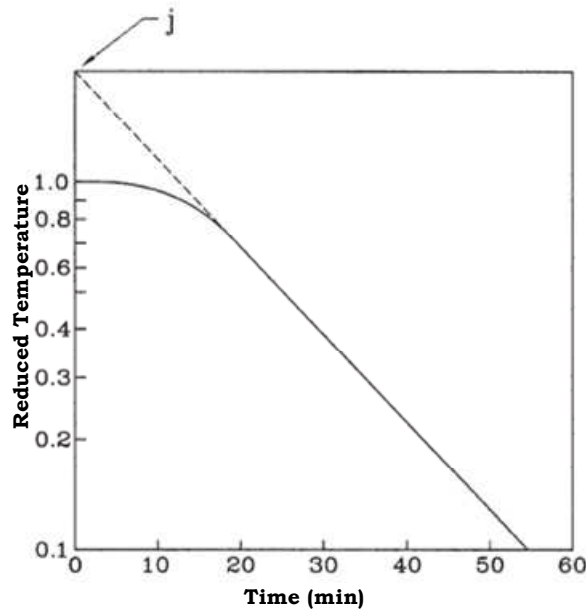
535 Paradoxically, the complexity of foods' shapes and the lack of knowledge about their physical
536 properties dictate the utilization of simple model. We took as an example the case of infinite
537 slab, but the model can be generalised to complex shapes. A classical and relevant approach is
538 of course, the implementation of numerical modelling. Another approach is related to the
539 well-known Ball method (Ball, 1923) with its two empirical parameters, for heat treatment
540 processes calculations. Because foodstuffs shapes are complex and because their thermal
541 properties are often unknown, it can be relevant to implement a simple model including only
542 two parameters (j and k of Eq. (1)) which can be easily empirically estimated from a simple
543 temperature registration and a simple linear regression of the reduced temperature (which is
544 linearised from a logarithmic transformation). Note that errors which can be generated by
545 "simplistic" heat transfer models are minor compared to errors which are linked to the
546 "biological background". Moreover, the overall model can be used for any bacterial strain
547 having known growth parameters T_{min} , T_{opt} and μ_{opt} . Accordingly, the proposed model can be
548 used for designing safe cooling or heating processes and may be considered as a very useful
549 tool for risk assessment regarding food safety and process optimisation.

550 **6. Appendix**

551 Equation of the the infinite series given by Carslaw and Jaeger (1986):

552
$$\frac{T_{\infty} - T}{T_{\infty} - T_0} = \sum_{n=1}^{\infty} \frac{2 \sin \varphi_n}{\varphi_n + \sin \varphi_n \cos \varphi_n} \cos\left(\varphi_n \frac{x}{x_{\max}}\right) \exp(-\varphi_n^2 Fo) \quad (20)$$

553 with x_{\max} is half-thickness for an infinite slab and φ_n are the roots of the equation $\cot \varphi = \frac{\varphi}{Bi}$.

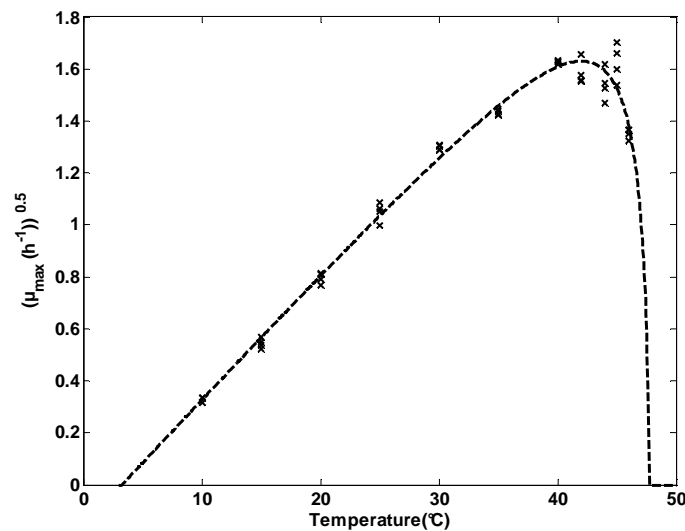


554

555

Fig. A1. Typical cooling curve (Becker and Fricke, 2004)

556



557

558 Fig. A2. Fit of the cardinal model on μ_{\max} values measured in nutrient broth (\times). The dashed

559

line denotes the fitted model on the experimental data.

560 **References**

561

562 Alavi, S.H., Puri, V.M., Mohtar, R.H. 2001. A model for predicting the growth of
563 *Listeria monocytogenes* in packaged whole milk. Journal of Food Process Engineering 24,
564 231–251.

565 Amezcuita, A., Weller, C.L., Wang, L., Thippareddi, H., Burson, D.E. 2005.
566 Development of an integrated model for heat transfer and dynamic growth of *Clostridium*
567 *perfringens* during the cooling of cooked boneless ham. International Journal of Food
568 Microbiology 101, 123-144.

569 Armitage, N.H. 1997. Use of predictive microbiology in meat hygiene regulatory
570 activity. International Journal of Food Microbiology 36, 103-109.

571 Ball, C. 1923. Thermal process times for canned foods. Bulletin 7-1, 37 (National
572 Research Council, Washington, DC).

573 Baranyi, J., Roberts, T.A. 1994. A dynamic approach to predicting bacterial growth in
574 food. International Journal of Food Microbiology 23, 277-294.

575 Baranyi, J., Robinson, T.P., Kaloti, A., Mackey, B.M. 1995. Predicting growth of
576 *Brochothrix thermosphacta* at changing temperature. International Journal of Food
577 Microbiology 27, 61-75.

578 Becker, B.R., Fricke, B.A. 2004. Heat transfer coefficients for forced-air cooling and
579 freezing of selected foods. International Journal of Refrigeration 27, 540-551.

580 Bellara, S.R., McFarlane, C.M., Thomas, C.R., Fryer, P.J. 2000. The growth of
581 *Escherichia coli* in a food simulant during conduction cooling: combining engineering and
582 microbiological modelling. Chemical Engineering Science 55, 6085-6095.

583 Ben Amara, S., Laguerre, O., Flick, D. 2004. Experimental study of convective heat
584 transfer during cooling with low air velocity in a stack of objects. *International Journal of*
585 *Thermal Sciences* 43, 1213-1221.

586 Bimbenet, J.J., Duquenoy, A., Trystram, G. 2002. *Génie des procédés alimentaires. Des*
587 *bases aux applications*. Dunod ed., Paris, France.

588 Bovill, R., Bew, J., Cook, N., D'Agostino, M., Wilkinson, N., Baranyi, J. 2000.
589 Predictions of growth for *Listeria monocytogenes* and *Salmonella* during fluctuating
590 temperature. *International Journal of Food Microbiology* 59, 157–165.

591 Bovill, R.A., Bew, J., Baranyi, J. 2001. Measurements and predictions of growth for
592 *Listeria monocytogenes* and *Salmonella* during fluctuating temperature II. Rapidly changing
593 temperatures. *International Journal of Food Microbiology* 67, 131–137.

594 Caro-Corrales, J., Cronin, K., Abodayeh, K., Gutiérrez-López, G., Ordorica-Falomir, C.
595 2002. Analysis of random variability in biscuit cooling. *Journal of Food Engineering* 54, 147-
596 156.

597 Carslaw, H.S., Jaeger, J.C. 1986. *Conduction of Heat in Solids*. 2nd ed. Oxford
598 University Press.

599 Corradini, M.G., Amézquita, A., Normand, M.D., Peleg, M. 2006. Modeling and
600 predicting non-isothermal microbial growth using general purpose software. *International*
601 *Journal of Food Microbiology* 106, 223–228.

602 Dalgaard, P. 1995. Modelling of microbial activity and prediction of shelf life for
603 packed fresh fish. *International Journal of Food Microbiology* 26, 305-317.

604 de Jong, A.E.I., Beumer, R.R., Zwietering, M.H. 2005. Modeling growth of *Clostridium*
605 *perfringens* in pea soup during cooling. *Risk Analysis: An International Journal* 25, 61-73.

606 Dincer, I. 1996. An exact solution on the estimation of heat-transfer rates during deep-
607 freezing of slab products. *Journal of Food Engineering* 30, 417-423.

608 Efron, B., Tibshirani, R.J. 1993. Confidence intervals based on bootstrap percentiles. In:
609 An introduction to the Bootstrap. Chapman and Hall, New York, pp. 168-177.

610 Erdogdu, F. 2005. Mathematical approaches for use of analytical solutions in
611 experimental determination of heat and mass transfer parameters. Journal of Food
612 Engineering 68, 233-238.

613 Francois, K., Devlieghere, F., Standaert, A.R., Geeraerd, A.H., Cools, I., Van Impe,
614 J.F., Debevere, J. 2005. Environmental factors influencing the relationship between optical
615 density and cell count for *Listeria monocytogenes*. Journal of Applied Microbiology 99, 1503-
616 1515.

617 Fujikawa, H., Kai, A., Morozumi, S. 2004. A new logistic model for *Escherichia coli*
618 growth at constant and dynamic temperatures. Food Microbiology 21, 501–509.

619 Gibson, A.M., Bratchell, N., Roberts, T.A. 1987. The effect of sodium chloride and
620 temperature on the rate and extent of growth of *Clostridium botulinum* type A in pasteurized
621 pork slurry. Journal of Applied Bacteriology 62, 479-490.

622 Gill, C.O., Harrison, J.C.L. 1985. Evaluation of the hygienic efficiency of offal cooling
623 procedures. Food Microbiology 2, 63-69.

624 Gumudavelli, V., Subbiah, J., Thippareddi, H., Velugoti, P.R., Froning, G. 2007.
625 Dynamic predictive model for growth of *Salmonella Enteritidis* in egg yolk. Journal of Food
626 Science 72, M254-M262.

627 Huang, L. 2003. Dynamic computer simulation of *Clostridium perfringens* growth in
628 cooked ground beef. International Journal of Food Microbiology 87, 217–227.

629 Jeyamkondan, S., Jayas, D.S., Holley, R.A. 2001. Microbial growth modelling with
630 artificial neural networks. International Journal of Food Microbiology 64, 343-354.

631 Juarez Tomas, M.S., Bru, E., Wiese, B., de Ruiz Holgado, A.A.P., Nader-Macias, M.E.
632 2002. Influence of pH, temperature and culture media on the growth and bacteriocin

633 production by vaginal *Lactobacillus salivarius* CRL 1328. Journal of Applied Microbiology
634 93, 714-724.

635 Juneja, V.K., Marks, H., Thippareddi, H. 2008. Predictive model for growth of
636 *Clostridium perfringens* during cooling of cooked uncured beef. Food Microbiology 25, 42-
637 55.

638 Kondjoyan, A. 2006. A review on surface heat and mass transfer coefficients during air
639 chilling and storage of food products. International Journal of Refrigeration 29, 863-875.

640 Koseki, S., Isobe, S. 2005. Prediction of pathogen growth on iceberg lettuce under real
641 temperature history during distribution from farm to table. International Journal of Food
642 Microbiology 104, 239–248.

643 Koutsoumanis, K. 2001. Predictive modeling of the shelf life of fish under
644 nonisothermal conditions. Applied and Environmental Microbiology 67, 1821–1829.

645 Koutsoumanis, K., Stamatiou, A., Skandamis, P., Nychas, G.J.E. 2006. Development of
646 a microbial model for the combined effect of temperature and pH on spoilage of ground meat,
647 and validation of the model under dynamic temperature conditions. Applied and
648 Environmental Microbiology 72, 124-134.

649 Lebert, I., Lebert, A. 2006. Quantitative prediction of microbial behaviour during food
650 processing using an integrated modelling approach: a review. International Journal of
651 Refrigeration 29, 968-984.

652 Mafart, P. 1997. Génie industriel Alimentaire. Tome 1: Les procédés physiques de
653 conservation. Lavoisier Techniques & Documentations ed., Paris, France.

654 Mafart, P. 2005. Food engineering and predictive microbiology: on the necessity to
655 combine biological and physical kinetics. International Journal of Food Microbiology 100,
656 239-251.

657 McMeekin, T.A., Brown, J., Krist, K., Miles, D., Neumeier, K., Nichols, D.S., Olley, J.,
658 Presser, K., Ratkowsky, D.A., Ross, T., Salter, M., Soontranon, S. 1997. Quantitative
659 Microbiology: A Basis for Food Safety. *Emerging Infectious Diseases* 3, 541-550.

660 McMeekin, T.A., Olley, J.N., Ross, T., Ratkowsky, D.A. 1993. *Predictive*
661 *Microbiology: theory and application* Research Studies Press, Taunton, Somerset, England.

662 Ratkowsky, D.A., Olley, J., McMeekin, T.A., Ball, A. 1982. Relationship between
663 temperature and growth rate of bacterial cultures. *Journal of Bacteriology* 149, 1-5.

664 Ross, T. 1996. Indices for performance evaluation of predictive models in food
665 microbiology. *Journal of Applied Bacteriology* 81, 501-508.

666 Rosso, L., Lobry, J.R., Bajard, S., Flandrois, J.P. 1995. Convenient model to describe
667 the combined effects of temperature and pH on microbial growth. *Applied and Environmental*
668 *Microbiology* Vol. 61, p. 610–616.

669 Swinnen, I.A.M., Bernaerts, K., Gysemans, K., Van Impe, J.F. 2005. Quantifying
670 microbial lag phenomena due to a sudden rise in temperature: a systematic macroscopic
671 study. *International Journal of Food Microbiology* 100, 85.

672 U.S.D.A. 1999. Performance standards for the production of certain meat and poultry
673 products. Final rule. *Federal Register* 64, 732-749.

674 Zwietering, M.H., De Koos, J.T., Hasenack, B.E., De Wit, J.C., Van't Riet, K. 1991.
675 Modeling of bacterial growth as a function of temperature. *Applied and Environmental*
676 *Microbiology* 57, 1094-1101.

677 Zwietering, M.H., De Wit, J.C., Cuppers, H.G.A.M., Van't Riet, K. 1994. Modeling of
678 bacterial growth with shifts in temperature. *Applied and Environmental Microbiology* 60,
679 204-213.

680 Zwietering, M.H., Hasting, A.P.M. 1997. Modelling the hygienic processing of foods -
681 Influence of individual process stages. *Food and Bioproducts Processing* 75, 168-173.

682 Zwietering, M.H., Wijtzes, T., de Wit, J.C., Van't Riet, K. 1992. A decision support
683 system for prediction of the microbial spoilage in foods. *Journal of Food Protection* 55, 973-
684 979.
685
686

687 **Figure captions**

688

689 Fig. 1.

690

691 Calibration curve between viable counts and absorbance data depicted with solid line.
692 Experimental data measured at 30°C in nutrient broth are marked with (o).

693

694 Fig. 2.

695

696 Experimental design implemented for the validation of the overall model at different
697 combinations (mentioned with numbers from 1 to 5) of product thickness (E), convective heat
698 transfer coefficient (h) and refrigeration temperature (T_∞).

699

700 Fig. 3.

701

702 Cooling scenarios (from 1c to 5c) and bacterial growth curves (from 1g to 5g) obtained from
703 simulation of the overall model at the five cases of the experimental design. Symbols in
704 predicted cooling profiles: temperature of product centre (line —) and temperature of product
705 surface (line — · —). Symbols in predicted growth curves: bacterial growth at product centre
706 (line —), bacterial growth at product surface (line — · —) and bacterial growth that occurs at
707 T_∞ (line

708

709 Fig. 4.

710

711 Comparison of predicted and observed *Escherichia coli* SOR 201 growth during cooling. **a**:
712 case1 at the centre or the surface of the product. **b**: case2 at the centre or the surface of the
713 product. **c**: case3 at the product centre. **d**: case3 at the product surface. **e**: case4 at the product
714 centre. **f**: case4 at the product surface. **g**: case5 at the product centre. **h**: case5 at the product
715 surface. Symbols in experimental data: o, □, Δ. Predicted growth curves estimated by the
716 overall model are presented with (line —). 95 percent confidence bands obtained with
717 bootstrap method are presented with (line — —).

718

719

720 Fig. 5.

721

722 Predicted bacterial growth from the overall model versus observed bacterial growth obtained
723 with constant air temperature. Symbols: □ (case 1), o (case 2). Symbols related to product
724 surface experiments: * (case 3), Δ (case 4), ◇ (case 5). Symbols related to product centre
725 experiments: • (case 3), + (case 4), x (case 5). The line of equivalence between predicted and
726 observed growth is marked with (line —).

727

728 Fig. 6.

729

730 Thermal profiles (from 1c to 3c) and bacterial growth curves (from 1g to 3g) obtained by
731 simulation of the overall model at the three ambient air scenarios. Symbols in predicted
732 cooling profiles: temperature of product centre (line —) and temperature of product surface
733 (line — · —). Symbols in predicted growth curves: bacterial growth at product centre (line
734 —), bacterial growth at product surface (line — · —) and bacterial growth that occurs at T_{∞}
735 (line

736

737 Fig. 7.

738

739 Comparison of predicted and observed *Escherichia coli* SOR 201 growth during thermal
740 processing at changing air temperature. **a**, **c** and **e** depict the model validation results at the
741 product centre according to cycle N°1, cycle N°2 and cycle N°3, respectively. **b**, **d** and **f**
742 depict the model validation results at the product surface according to cycle N°1, cycle N°2
743 and cycle N°3, respectively. Symbols in experimental data: o, □. Predicted growth curves
744 estimated by the overall model are presented with (line —). 95 percent confidence bands
745 obtained with bootstrap method are presented with (line — —).

746

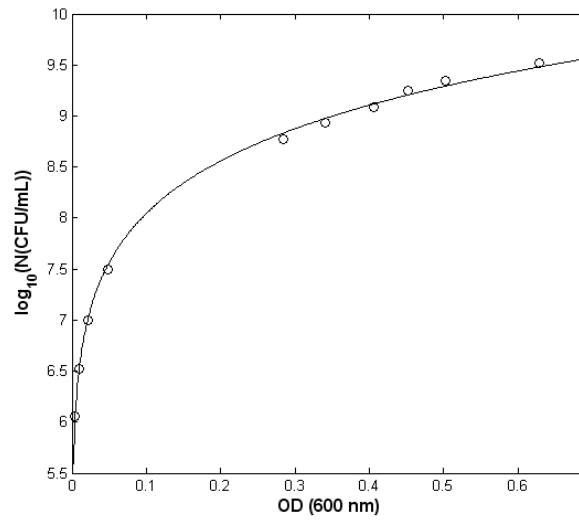
747 Fig. 8.

748 Predicted bacterial growth from the overall model versus observed bacterial growth obtained
749 with changing air temperature. Symbols related to product surface experiments: □ (Cycle
750 N°1), * (Cycle N°2), Δ (Cycle N°3). Symbols related to product centre experiments: o (Cycle
751 N°1), • (Cycle N°2), + (Cycle N°3). The line of equivalence between predicted and observed
752 growth is marked with (line —).

753 Fig. 9.
754 Comparison of the bacterial growth prediction at ambient temperature according to cycle N°1
755 with the model validation results at product's centre (*a*) and at product's surface (*b*). Symbols
756 in experimental data: o, □. Symbols in predicted growth curves: bacterial growth at product's
757 surface or centre (line —), bacterial growth that occurs at T_{∞} (line). 95 percent confidence
758 band of predictions at T_{∞} obtained with bootstrap method is presented with (line — —).

759

760

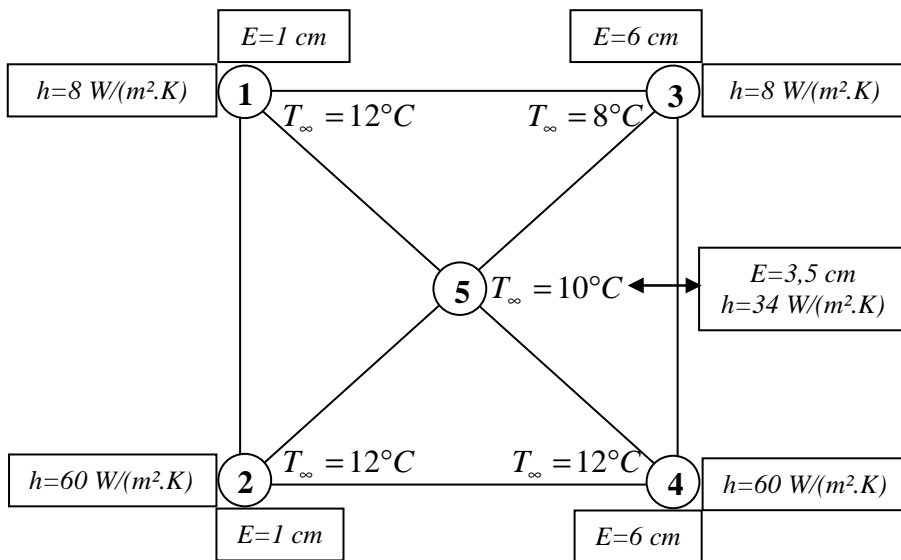


761

762

763 Fig. 1.

764



765

766

767 Fig. 2.

768

769

770

771

772

773

774

775

776

777

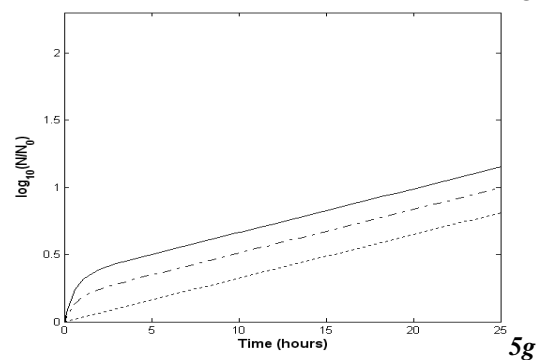
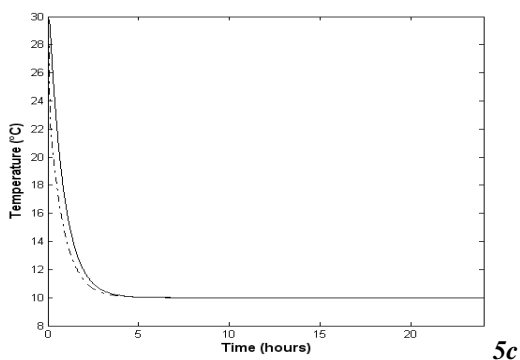
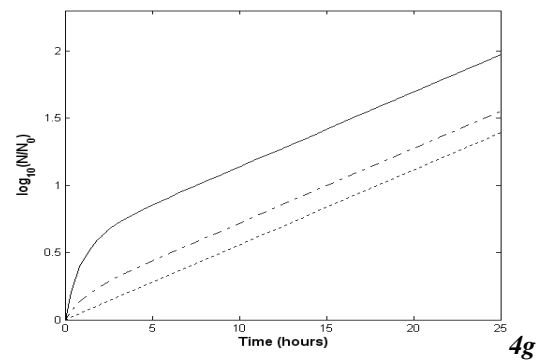
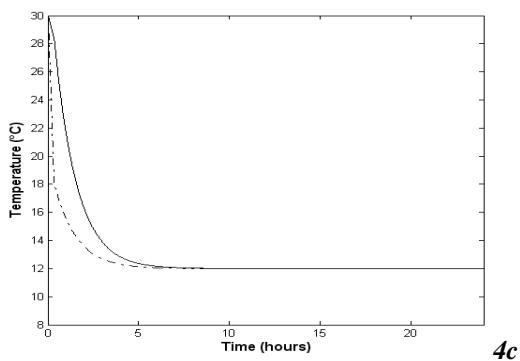
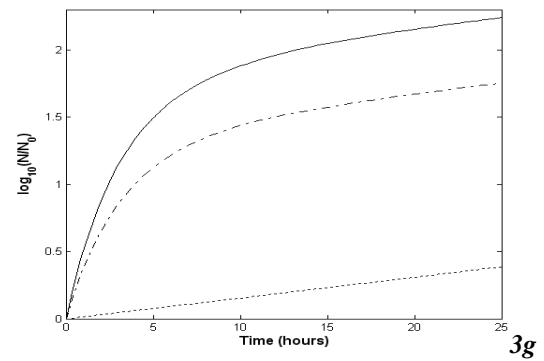
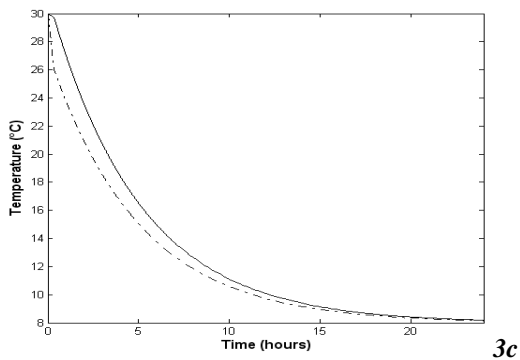
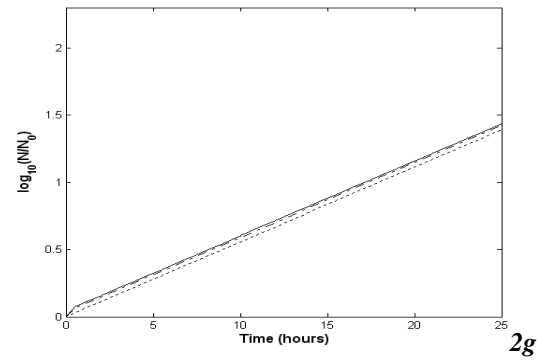
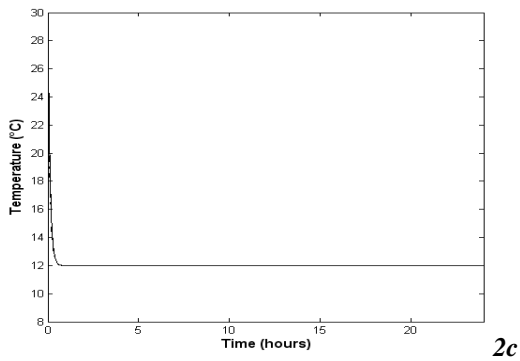
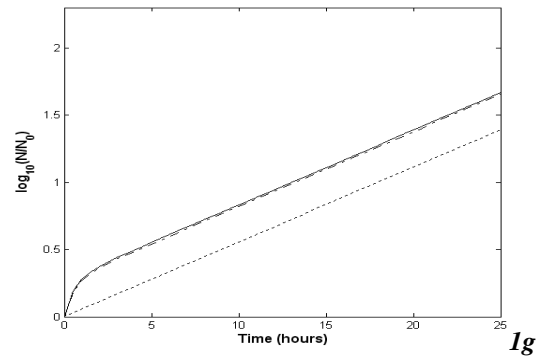
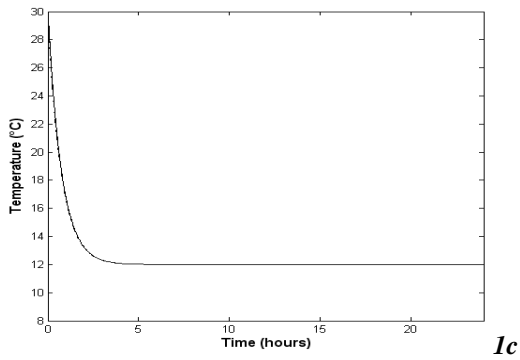
778

779

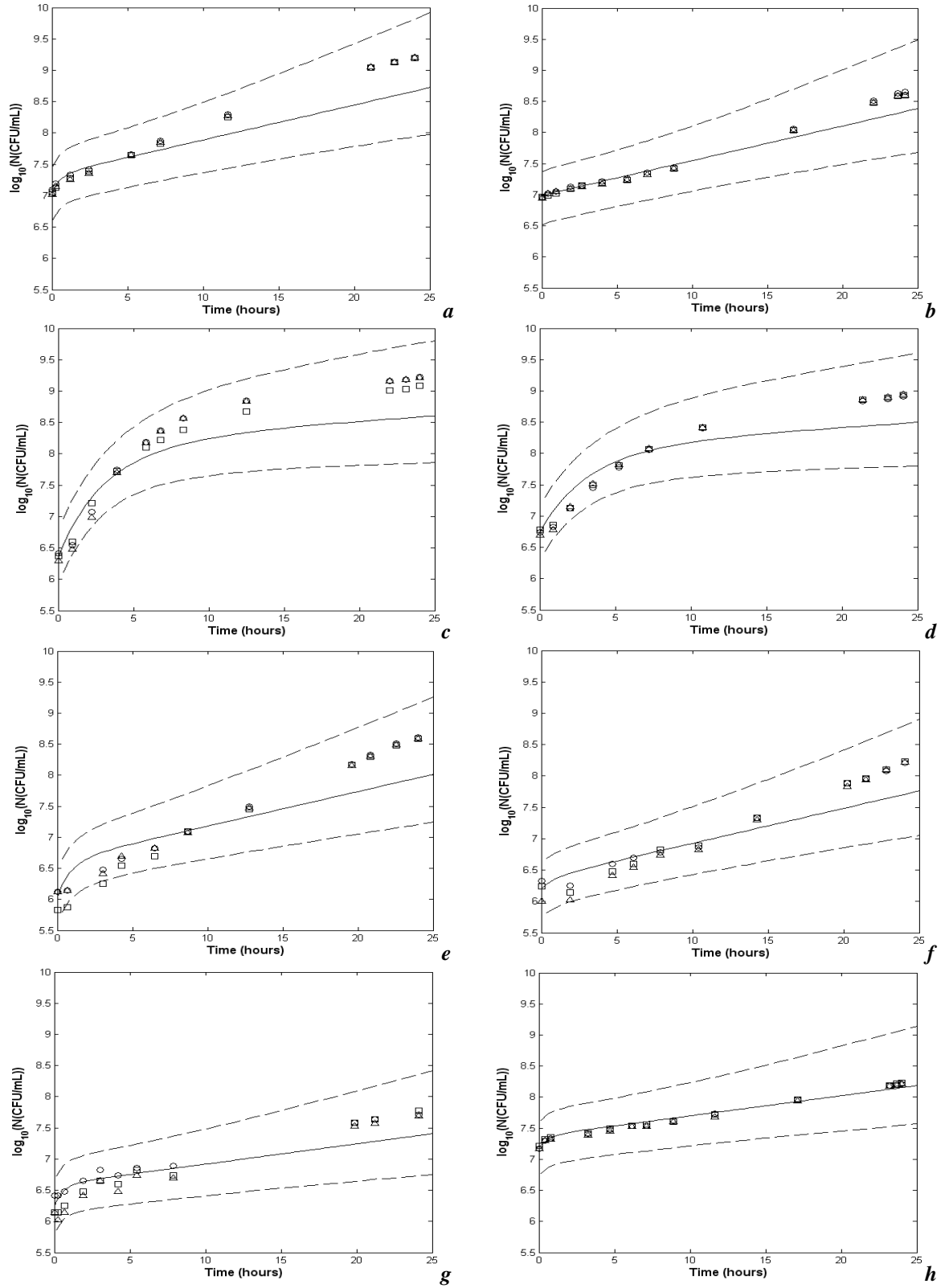
780

781

782



783 Fig. 3.

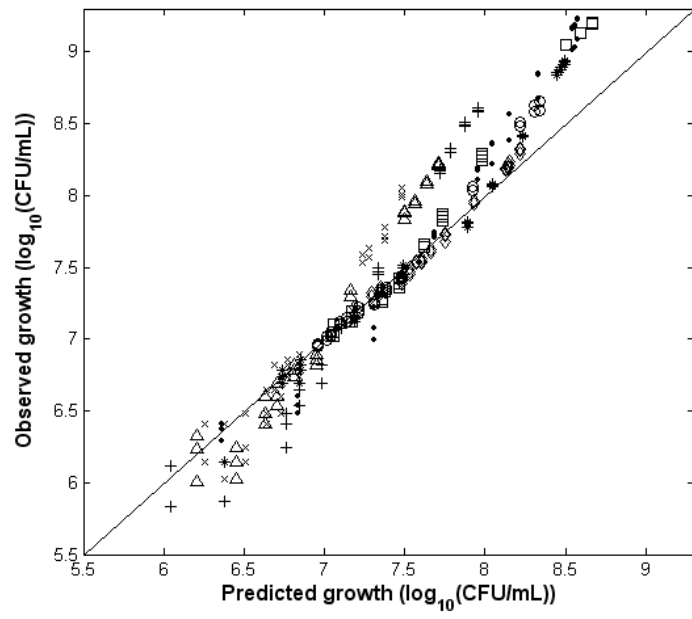


785 Fig. 4.

786

787

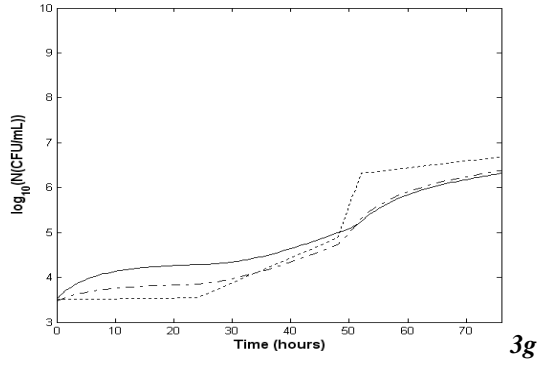
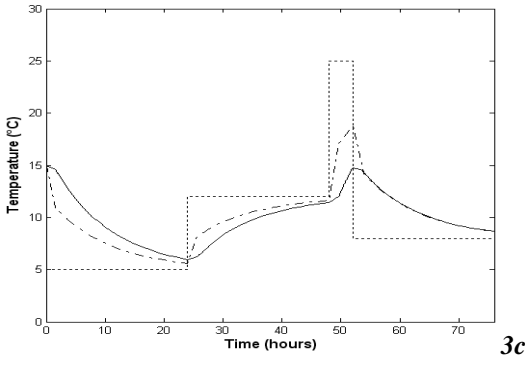
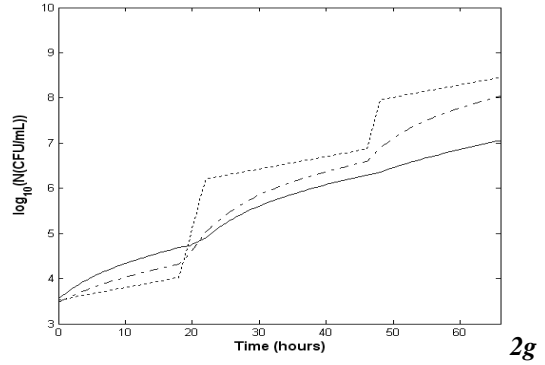
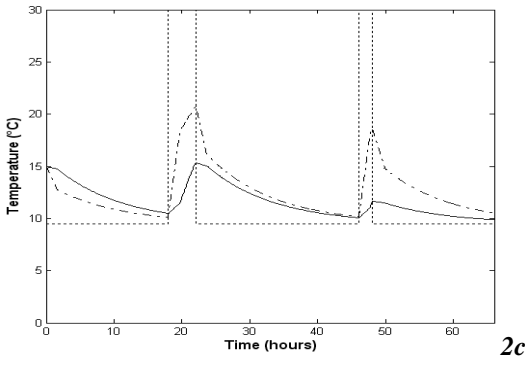
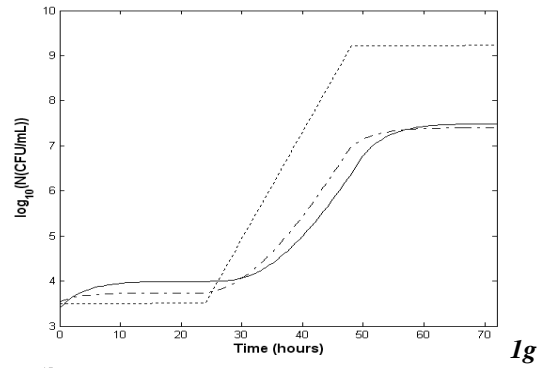
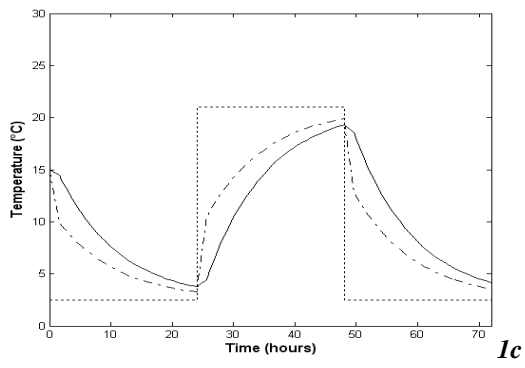
788



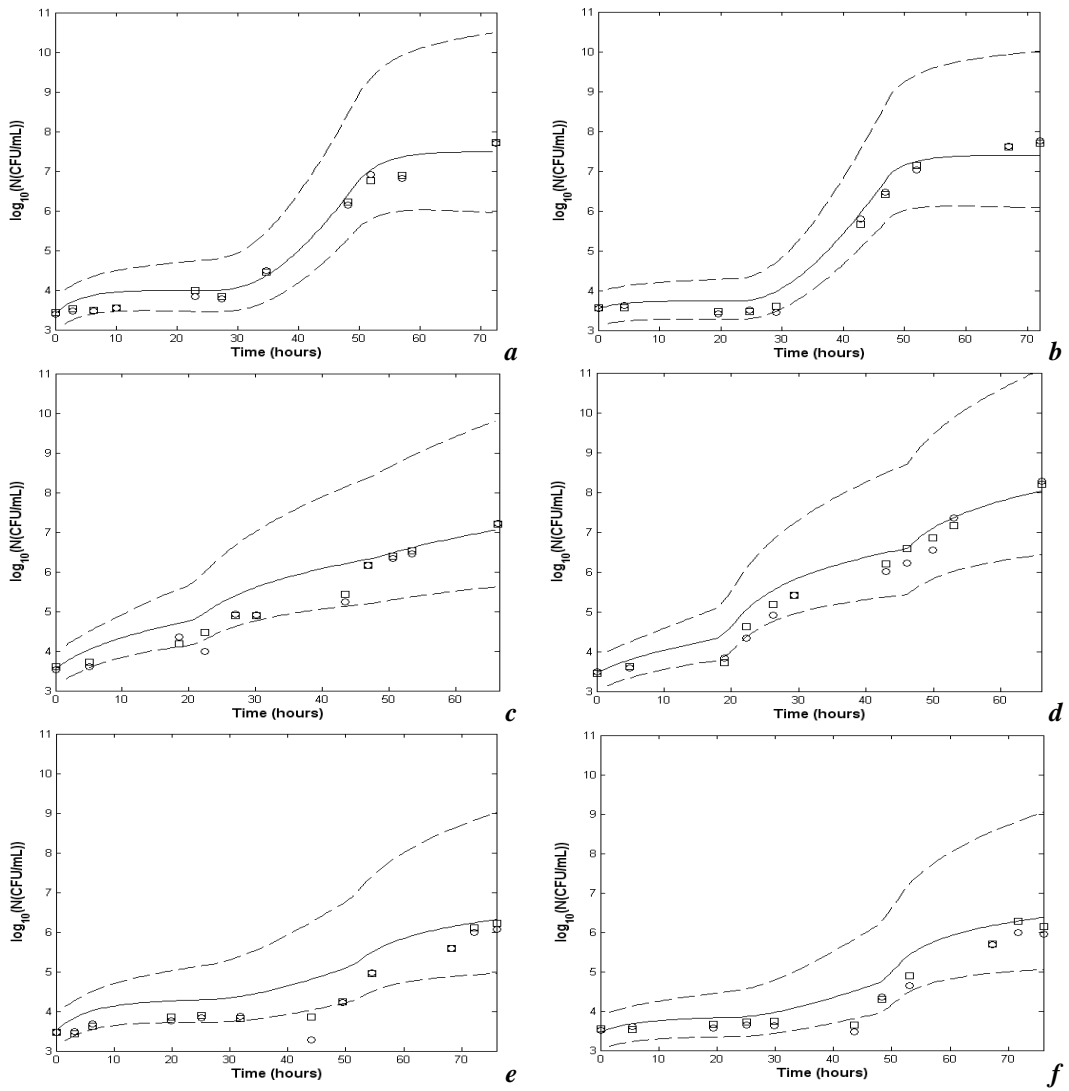
789

790 Fig. 5.

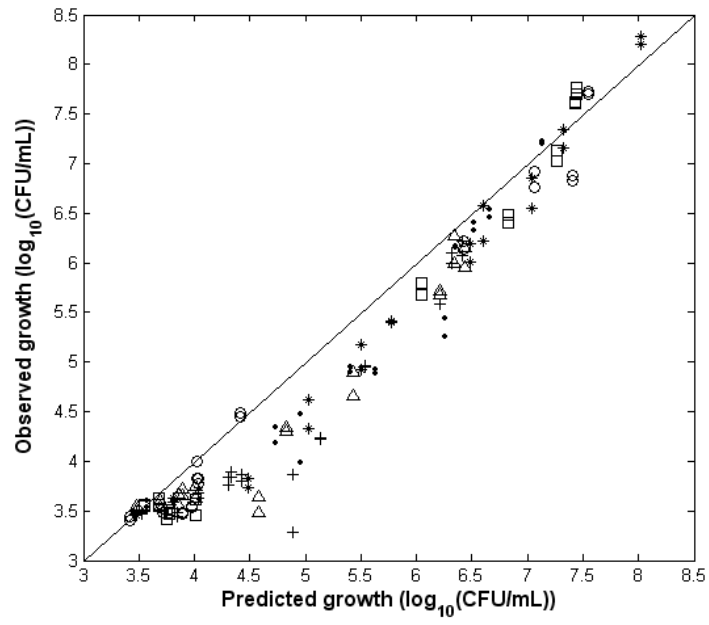
791



793 Fig. 6.



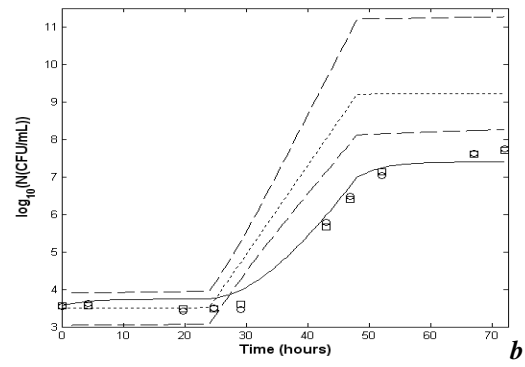
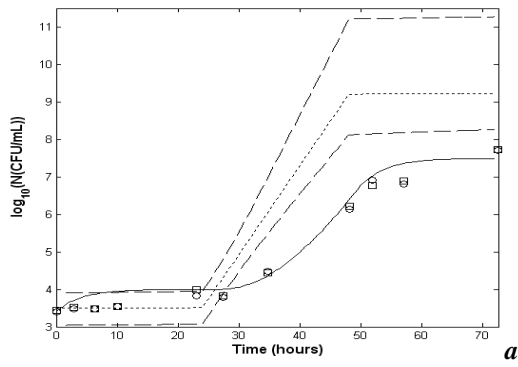
795 Fig. 7.



796

797 Fig. 8.

798



799

800

801 Fig. 9.

802

803 Table 1
 804 List of previous works dealing with models predicting the bacterial growth under dynamic
 805 temperature conditions
 806

Primary Model	Secondary model	Solution method	Validation conditions	Reference
and Roberts aranyi and (4).	Quadratic polynomial model.	Numerical solution by the fourth order Runge–Kutta method.	Rates of change of T ranging from $1.7^{\circ}\text{C}\cdot\text{h}^{-1}$ to a virtually instantaneous change: cooling from 25°C to $-2, 2, 5$ or 10°C and heating from 2°C to 25°C .	Bovil
istic model.	Arrhenius equation.	Numerical solution by the fourth order Runge–Kutta method.	Various types of a dynamic temperature history with various intervals were studied for of <i>Escherichia coli</i> 1952 growth prediction.	Fujik 2004
and Roberts aranyi and (4).	A modified Ratkowsky equation (Zwietering et al., 1991).	Numerical solution by the fourth order Runge–Kutta method.	Growth of <i>Salmonella Enteritidis</i> in egg yolk under varying temperature profiles (exponential and linear cooling, exponential heating and sinusoidal temperatures).	Gumu al., 20
form of the odel (Gibson	A modified Ratkowsky equation (Zwietering et al., 1991).	Numerical solution by the fourth order Runge–Kutta method.	Growth of <i>Clostridium perfringens</i> in cooked ground beef under fluctuating temperature conditions between 30°C and 45°C (square waved) and under continuous temperature changes from 51°C to 10°C (linear and exponential cooling).	Huan

807 Table 2

808 Tested temperature scenarios for the validation of the model at changing air temperature and

809 constant convective heat transfer coefficient equals to $8 \text{ W}/(\text{m}^2 \cdot \text{K})$

810

Stage of the cycle	Temperature (°C)	Duration (h)
Cycle N° 1		
Storage in a cold room	2.5	24
Refrigeration stopped due to an accidental failure of the installation	21	24
Repaired breakdown and remaining product in the cold room	2.5	24
Cycle N° 2		
Industrial storage and transport	9.5	18
Household doorstep delivery	30	4
Storage in refrigerator	9.5	24
Product on the table	30	2
Remaining product in the refrigerator	9.5	18
Cycle N° 3		
Storage and transport	5	24
Storage in the refrigerated cabinet	12	24
Household doorstep delivery	25	4
Storage in refrigerator	8	24

811 Table 3

812 Statistical criteria related to the data of validation at constant air temperature and changing
813 convective heat transfer coefficient

814

Test conditions	R	B_f	A_f
Case 1	0.996	0.98	1.03
Case 2	0.996	0.99	1.01
Case 3 at product centre	0.987	0.98	1.04
Case 3 at product surface	0.987	0.99	1.02
Case 4 at product centre	0.976	0.99	1.05
Case 4 at product surface	0.986	0.99	1.03
Case 5 at product centre	0.975	0.99	1.03
Case 5 at product surface	0.994	1	1.01

815

816

817 Table 4

818 Statistical criteria related to the data of validation at changing air temperature and constant

819 convective heat transfer coefficient equals to $8 \text{ W}/(\text{m}^2 \cdot \text{K})$

820

Test conditions	R	B_f	A_f
Cycle 1 at product centre	0.991	1.05	1.05
Cycle 1 at product surface	0.992	1.04	1.05
Cycle 2 at product centre	0.963	1.08	1.08
Cycle 2 at product surface	0.986	1.06	1.06
Cycle 3 at product centre	0.944	1.13	1.13
Cycle 3 at product surface	0.964	1.09	1.09

821

822

823