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**On calculating sterility in thermal preservation methods :
application of the Weibull frequency distribution model.**

P. MAFART, O. COUVERT, S. GAILLARD and I. LEGUERINEL

ABSTRACT A simple and parsimonious model originated from the Weibull frequency distribution was proposed to describe non linear survival curves of spores. This model was suitable for downward concavity curves (*Bacillus cereus* and *Bacillus pumilus*) as well as for upward concavity curves (*Clostridium botulinum*). It was shown that traditional F-values calculated from this new model were no more additive, to such an extent that a heat treatment should be better characterized by the obtained decade reduction of spores. A modified Bigelow method was then proposed to assess this decade reduction or to optimize the heat treatment for a target reduction ratio.

KEYWORDS: spores, heat treatment, F-value, Weibull

INTRODUCTION

The conventional way of calculating the efficiency of heat treatments in food protection is based on the assumption that survival curves of microbial cells and bacterial spores are governed by a first order kinetic. Consequently, a linear relationship between the decimal logarithm of the number of surviving microorganisms and the treatment time at a given temperature is used to estimate the D-value (time of decimal reduction). However, in many cases, the survival curve of heated microorganisms is not linear and present a downward concavity (presence of a shoulder) or an upward concavity (presence of a tail). A number of models describing non linear survival curves were proposed. Some of them are mechanistic or

26 pseudo-mechanistic (Brynjolfsson, 1978; Casolari, 1988; Kilsby *et al.*, 2000; Rodriguez *et*
27 *al.*, 1988; Sapru *et al.*, 1992 and 1993; Shull *et al.*, 1963; Xiong *et al.*, 1999) while others are
28 purely empirical (Badhuri *et al.*, 1991; Baranyi, 1996; Buchanan *et al.*, 1997; Chiruta *et al.*,
29 1997; Cole *et al.*, 1993; Daughtry *et al.*, 1997; Geeraerd *et al.*, 1999; Linton *et al.*, 1995;
30 Whiting, 1993). These models generally present a satisfying goodness of fit, but they lack of
31 robustness and are adapted to some particular situations only. Moreover, parameters of
32 mechanistic models can be difficult to estimate, while parameters of empirical models have
33 generally no easily interpretable physical or biological significance. For both kinds of
34 equations, the number of parameters exceeds three or four, to such an extend that the
35 complexity of models prevents them from being applied to heat treatment calculations.

36 While the conventional first order model implicitly assumed that microbial populations are
37 homogeneous from the point of view of their heat resistance, some researchers (Fernandez *et*
38 *al.*, 1999; Peleg, 1999; Peleg and Cole, 1998 and 2000) assumed that , at a given temperature,
39 the time of heat exposure which caused the death of a microbial cell or a bacterial spore is
40 variable from one individual to the other, and that the dispersion of individual heat resistance
41 was governed by a Weibull distribution, the cumulative form of which yields:

$$42 \quad N = N_0 e^{-kt^p} \quad (1)$$

43 Where N represents the number of surviving cells after a duration of heat treatment t, while
44 N_0 is the initial size of the alive population. For a given temperature, parameter distribution
45 are k and p.

46 Peleg and Cole (1998) wrote out this model in the following decimal logarithmic form:

$$47 \quad \log \frac{N}{N_0} = -bt^p \quad (2)$$

48

49 The cited authors successfully checked the model for *Clostridium botulinum* and *Bacillus*
50 *stearothermophilus* spores and *Salmonella thyphimurium* and *Listeria monocytogenes* cells.
51 Similarly, Fernandez *et al.* (1999) successfully applied the same model to the heat destruction
52 of *Bacillus cereus*. Such a model presents the main advantage of remaining very simple and
53 being sufficiently robust to describe both downward concave survival curves ($p > 1$) and
54 upward concave curves ($p < 1$). Obviously, the model includes the traditional case where the
55 survival curve, originated from a first order, is linear ($p = 1$).

56 The present paper aims to improve the parameterization of the model , to propose a new
57 method of assessing the efficiency of heat treatments and to bring the traditional F-value
58 concept up to date.

59 **Material and methods**

60 *Microorganism and spore production.* The strain of *Bacillus cereus* was isolated from dairy
61 food line process, the strain of *Bacillus pumilus* from eggs powder. Spores were kept in
62 distilled water at 4°C. Cells were precultivated at 37°C during 24 hrs in Brain Heart Infusion
63 (Difco). The preculture was used to inoculate nutritive agar plates (Biokar Diagnostics
64 BK021) added with MnSO_4 40mg l⁻¹ and CaCl_2 100 mg l⁻¹ on the surface area. Plates were
65 incubated at 37°C for 5 days. Spores were then collected by scraping the surface of the agar
66 and suspended in sterile distilled water and washed three times by centrifugation (10000xg for
67 15 min) (Bioblock Scientific, model Sigma 3K30). The pellet was then resuspended in 5 ml
68 distilled water and 5 ml ethanol. The obtained suspension was then kept at 4°C during 12
69 hours in order to eliminate vegetative non sporulated bacteria, and washed again three times
70 by centrifugation.

71 Lastly the final suspension (about 10¹⁰ spores ml⁻¹) was at last distributed in sterile Eppendorfs
72 microtubes and kept at 4°C.

73 *Thermal treatment of spore suspension.*

74 First, 30µl of spore suspension was diluted in 3 ml heating medium. Capillary tubes of 25 µl
75 (vitrex) were filled with 10µl of sample and submitted to a thermal treatment in a
76 thermostated oil bath. After heating, the tubes were cooled in water/ice bath, washed in a
77 solution of soap and rinsed with sterile distilled water. Finally, ends were flamed with
78 ethanol. The capillary tubes were broken at both ends and their contents poured into a tube
79 containing 9 ml sterile tryptone salt broth (Biokar Diagnostics) by rinsing with 1 ml tryptone
80 salt broth contained in a needle-equipped syringe.

81 *Data analysis*

82 For each spore species, a single p value was estimated from the corresponding whole set of
83 data according to a non-linear regression by using the solver capability of the Excel software.
84 Each survival curve was then fitted according to Eqn 3 by a linear regression.

85

86 **Results and discussion**

87

88 *1. Improvements of the model*

89

90 Parameter b of the last equation has no immediate physical significance and has the
91 dimensions of a time power $-p$, so we preferred to reparameterize the model into the
92 following form:

$$93 \quad \log \frac{N}{N_0} = -\left(\frac{t}{\delta}\right)^p \quad \text{or} \quad \log N = \log N_0 + \left(\frac{-1}{\delta^p}\right) \times t^p \quad (3)$$

94 Or

$$95 \quad n = \left(\frac{t}{\delta}\right)^p \quad (4)$$

96

97 Where n represents the decimal reduction ratio. Parameter δ which has now the simple
98 dimensions of a time, can be called *time of first decimal reduction*: contrarily to the
99 conventional D-value which is originated from the first order kinetic and which represents the
100 time of decimal reduction, regardless of the time of heating, the significance of the δ -value is
101 restricted to first decimal reduction of surviving spores or cells from N_0 to $N_0/10$.

102 This model, if unmodified, presents two major drawbacks: first, assessment of parameters
103 requires a non linear regression. Secondly, p which is a shape parameter, is structurally
104 strongly correlated with δ values. That is to say, both parameters are not independent: an error
105 on δ will be balanced by an error on p in the same way. Such an autocorrelation causes a
106 certain instability of parameter estimates. See for example p values estimated by Peleg and
107 Cole (1998) for *C botulinum* (table 1).

108 As p values are expected to be dependent on temperature, we calculated correlation
109 coefficients between p and heating temperature (Table 2). It can be seen that, for the three sets
110 of data, correlation coefficients are poor. For *B. cereus*, the correlation is not significant,
111 while for *C botulinum* and *B. pumilus*, correlation coefficients just reach the significance
112 threshold (at the level $p = 0.05$). Then, it seems worthwhile to fix p at an average value,
113 characteristic of a strain, so that N_0 and δ values can be estimated from a linear regression.
114 Obviously, the fixation of p will have repercussions on δ values which, as expected, are
115 governed by the Bigelow relationship as a function of temperature. For the previously cited
116 set of data regarding *C botulinum*, when p and δ were estimated together from a non linear
117 regression, we obtained the following results:

118

119 $z = 7.09^\circ\text{C}$; $r = 0.969$

120

121 When p was fixed to its average value (p = 0.346) and δ estimated from a linear regression, it
122 yielded:

123

124 $z = 8.58^{\circ}\text{C}$; $r = 0.989$

125

126 Because the fixation of p causes a better stability of δ estimates, the clear improvement of the
127 concerned correlation coefficient was expected. Similarly, average p-values regarding *B.*
128 *cereus* and *B. pumilus* were determined and respective z-values assessed. Results are
129 presented in Table 3. It is then confirmed that δ -values have the same dependence
130 relationship towards temperature as conventional D-values:

$$131 \quad \delta = \delta^* 10^{\frac{T-T^*}{z}} \quad (5)$$

132

133 Where δ^* is the time of first decimal reduction at the reference temperature T^* .

134

135 *2. Application of the model to calculations of heat treatment efficiency*

136

137 The traditional sterilization value (F-value) is defined as the time of a heat treatment at the
138 reference temperature (generally, $T^* = 121.1^{\circ}\text{C}$), or as any equivalent heat treatment which
139 would cause the same destruction ratio. The target F-value which depends both on the
140 required level of safety and on the heat resistance of the target species of spore or bacterial
141 cell, is:

142

$$143 \quad F = nD^* \quad (6)$$

144

145 Where n is the ratio of decimal reduction (safety level) and D^* , the time of decimal reduction
146 at the reference temperature (heat resistance).

147 At a constant temperature, the actual F-value is the product of the heating time and the so
148 called Biological Destruction value L , which is a function of temperature:

149

$$150 \quad F = L(T) t \quad (7)$$

151 With

$$152 \quad L(T) = 10^{\frac{T-T^*}{z}} \quad (8)$$

153 In standard calculations, the z -value is assumed to be 10°C , which corresponds to that of *C.*
154 *botulinum*. Then, the traditional F-value is implicitly applied to an ideal strain of *C.*
155 *botulinum*, the destruction curve of which would be governed by a first order kinetic, and
156 which would be characterized by a z -value of 10°C . Because F-values are additive, in the case
157 of a variable temperature heat treatment, it can be written:

$$158 \quad F = \int_0^t L(T) dt \quad (9)$$

159 Bigelow numerically solved this equation by writing it in the following discrete form:

$$160 \quad F = \sum L(T_i) \Delta t_i \quad (10)$$

161 Where increments Δt_i were equally fixed at 1 minute.

162

163 If it is assumed that, instead of obeying to a first order kinetic, survival curves of spores are
164 governed by the Weibull frequency distribution model, F-values are no more additive. Let F
165 be the overall sterilization value resulting from two successive heat treatments whose
166 sterilization values would be F_1 and F_2 respectively. The first heat treatment would cause a
167 decimal destruction ratio:

168

169 $n_1 = \log \frac{N_0}{N_1}$ (11)

170 where N_1 is the number of surviving cells after the first heating. Similarly, the second
 171 treatment would cause a decimal destruction ratio:

172 $n_2 = \log \frac{N_1}{N_2}$

173 $n = \log \frac{N_0}{N_2} = \log \frac{N_0}{N_1} \frac{N_1}{N_2} = \log \frac{N_0}{N_1} + \log \frac{N_1}{N_2} = n_1 + n_2$ (12)

174 where N_2 is the number of surviving cells after the second heating. Lastly, the overall heat
 175 treatment would yield:

176

177 According to the new model,

178 $n = \left(\frac{t}{\delta} \right)^p = \left(\frac{F}{\delta^*} \right)^p$ (13)

179 So that

180 $\left(\frac{F}{\delta^*} \right)^p = \left(\frac{F_1}{\delta^*} \right)^p + \left(\frac{F_2}{\delta^*} \right)^p$ (14)

181 And

182 $F^p = F_1^p + F_2^p$ (15)

183 The F-value being no more additive, it is clear that the destruction ratio is no more
 184 proportional to this value, so that the F concept loses a great part of its relevance.

185 Consequently, the decimal reduction ratio becomes the only convenient indicator of the heat
 186 treatment efficiency.

187 At constant temperature,

188 $n = \left[\frac{L(T)t}{\delta^*} \right]^p$ (16)

189 Then,

$$190 \quad dn = p \left[\frac{L(T)}{\delta^*} \right]^p t^{p-1} dt \quad (17)$$

191 So

$$192 \quad n = p \int_0^t \left[\frac{L(T)}{\delta^*} \right]^p t^{p-1} dt \quad (18)$$

193 that, at variable temperature,

194 A procedure similar to that of Bigelow can then be applied to solve numerically this last
195 equation from the following discrete form:

$$196 \quad n = p \sum_{i=1}^m \left[\frac{L(T_i)}{\delta^*} \right]^p t_i^{p-1} \Delta t_i \quad (19)$$

197 An *adjusted F-value* (adjusted according to the p and z values of the target microorganism)
198 can then be calculated from the following equation:

$$199 \quad F = n^{\frac{1}{p}} \delta^* \quad (20)$$

200 Indeed, the conventional F-value, which could be called the *standard F-value* remains an
201 interesting criterion, as it allows to intrinsically compare several heat treatments, regardless of
202 the target species which is to be destroyed.

203

204 Figure 1 represents registrations of a retort temperature (with an average value of 115.3°C)
205 and inside temperature of a canned tomato sauce. The Bigelow procedure (1920) allows to
206 calculate a conventional F-value of 7.31. Assuming a D*-value of 0.21 minutes, the decimal
207 destruction ratio which theoretically would be reached after the sterilization run would be n =
208 34.8. Data showed in Table 3 yield for *C. botulinum* the following estimates:

$$209 \quad p = 0.346$$

$$210 \quad \delta^* = 0.00527$$

211 $z = 8.58^{\circ}\text{C}$

212 An actual decimal destruction ratio of 6.18 and an adjusted F-value of 1.02 can be then
213 calculated. A 12 decade reduction of *C. botulinum* being conventionally proposed, for
214 reaching this reduction ratio, the actual sterilization would have to be prolonged to obtain a
215 further decimal reduction Δn :

216 $\Delta n = 12 - 6.18 = 5.82$

217

218 From Eqn 17, it can be deduced that the corresponding prolongation time would be:

219
$$t = \frac{\Delta n^{\frac{1}{p}} \delta^*}{L(T)} \quad (21)$$

220

221 At an assumed heat stationary product temperature of 115.3°C , the needed prolongation time
222 of sterilization would then be 3.85 minutes, while the adjusted F-value would become 4.09
223 (for a standard F-value which would become 8.32)

224 Indeed, applications of the Weibull frequency distribution are not likely to render traditional
225 concepts out of date: the conventional F-value concept remains highly useful. However heat
226 treatment calculations require some modifications when survival curves of spores or bacterial
227 cells are not linear. We presented an adaptation of the Bigelow method based on the Weibull
228 frequency distribution method for assessing the efficiency of sterilization. Similarly, further
229 useful investigations to adapt the analytical approach of Ball (1923) would be possible.

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279

280

281

282 Legend figure

283

284 Fig 1:

285 The fit of Equ 3 to the data for the death kinetics of *Bacillus pumilus* heat injured at 89°C

286

287 Fig 2

288 The fit of Equ 3 to the data of Anderson et al. (1996) for the death kinetics of *Clostridium*

289 *botulinum* heat injured at 111°C

290

291

292 Fig 3

293 Registrations of a retort temperature (with an average value of 115.3°C) and inside

294 temperature of a canned tomato sauce

295

296

297

298

299

Table1

Temperature (°C)	p
103	0.364
105	0.349
107	0.432
109	0.392
111	0.319
113	0.314
115	0.312
117	0.295
119	0.337
121	0.324

300

301

302

303

304

Table 2

305

Type of spore	Number of data	Correlation coefficient between temperature and p- value
<i>C. botulinum</i>	10	0.600 (0.60)
<i>B. cereus</i>	5	0.453 (0.81)
<i>B. pumilus</i>	6	0.751 (0.75)

306

307

308

309

310

Table 3

311

Type of spore	p-value	z-value	r
<i>C. botulinum</i>	0.346	8.58°C	0.989
<i>B. cereus</i>	1.37	8.57°C	0.997
<i>B. pumilus</i>	2.24	8.04°C	0.998

312

313

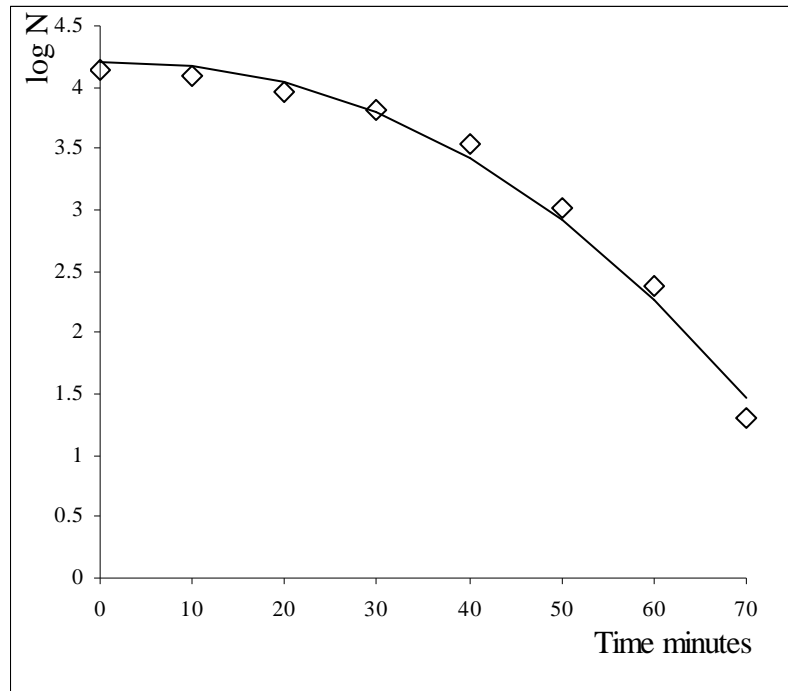
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Figure1



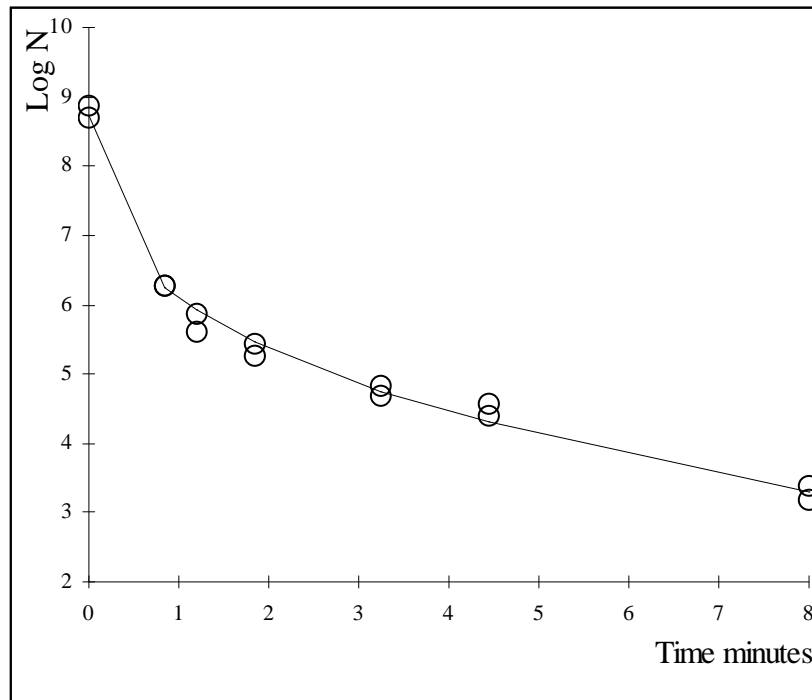
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Figure 2



322

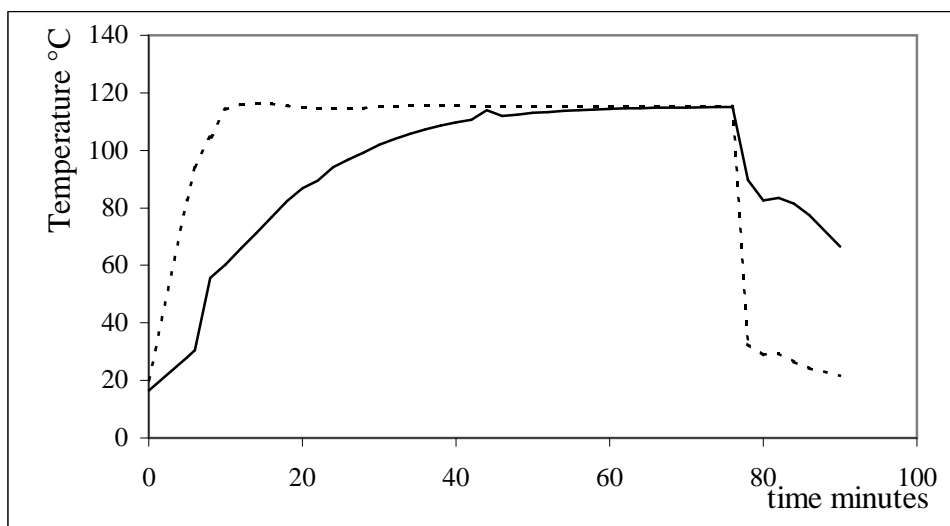
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Figure 3



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328