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

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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 extend 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

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

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

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

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

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

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

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

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

Material and methods

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

Lastly the final suspension (about 10^{10}spores ml^{-1}) was at last distributed in sterile Eppendorfs microtubes and kept at 4°C.

Thermal treatment of spore suspension.

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

Data analysis

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

Results and discussion

1. Improvements of the model

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

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

Or

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

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

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

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

$z = 7.09°C$; $r = 0.969$

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

z = 8.58°C ; r = 0.989

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

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

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

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

The traditional sterilization value (F-value) is defined as the time of a heat treatment at the reference temperature (generally, T* = 121.1°C°), or as any equivalent heat treatment which would cause the same destruction ratio. The target F-value which depends both on the required level of safety and on the heat resistance of the target species of spore or bacterial cell, is:

$$F = nD^{*} \quad (6)$$

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

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

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

With

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

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

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

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

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

Where increments Δt_i were equally fixed at 1 minute.

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

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

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

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

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

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

According to the new model,

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

So that

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

And

$$F^p = F_1^p + F_2^p \quad (15)$$

The F-value being no more additive, it is clear that the destruction ratio is no more proportional to this value, so that the F concept loses a great part of its relevance. Consequently, the decimal reduction ratio becomes the only convenient indicator of the heat treatment efficiency.

At constant temperature,

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

Then,

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

So

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

that, at variable temperature,

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

$$n = p\sum_1^m \left[\frac{L(T_i)}{\delta^*}\right]^p t_i^{p-1}\Delta t_i \quad (19)$$

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

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

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

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

p = 0.346

δ* = 0.00527

z = 8.58°C

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

$\Delta n = 12 - 6.18 = 5.82$

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

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

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

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

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Legend figure

Fig 1:

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

Fig 2

The fit of Equ 3 to the data of Anderson et al. (1996) for the death kinetics of *Clostridium botulinum* heat injured at 111°C

Fig 3

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

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

Table 2

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

Table 3

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

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316 Figure1

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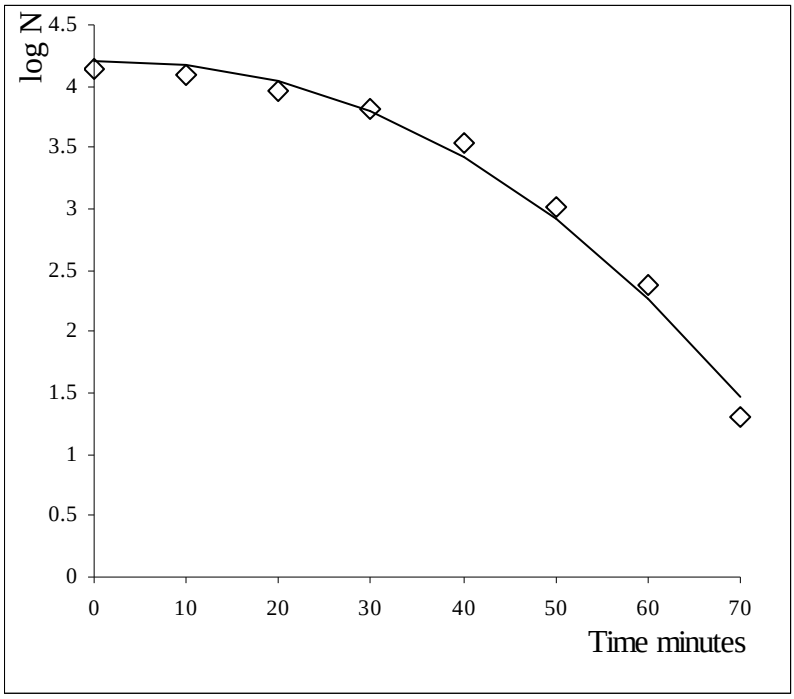

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Figure 2

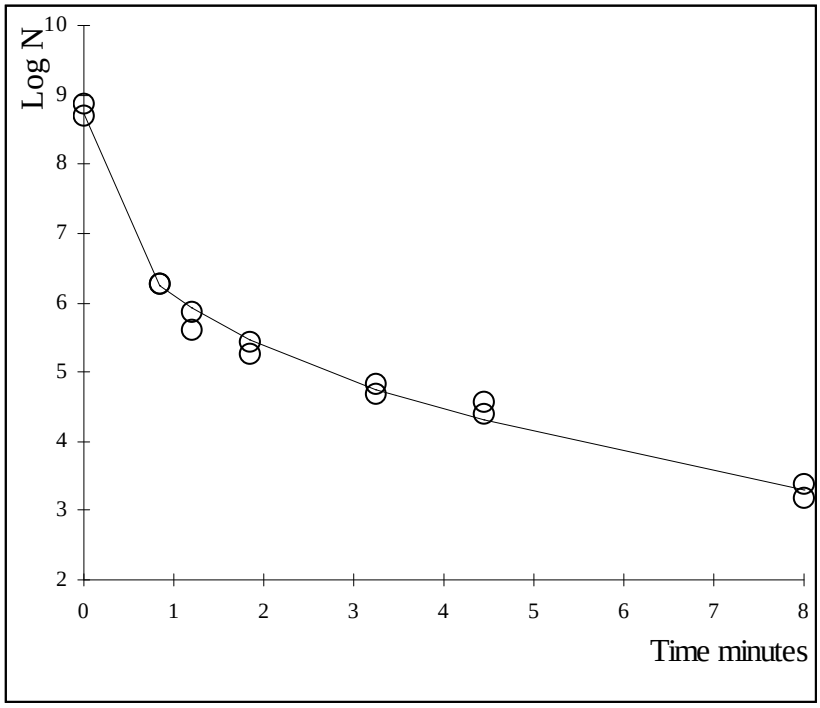
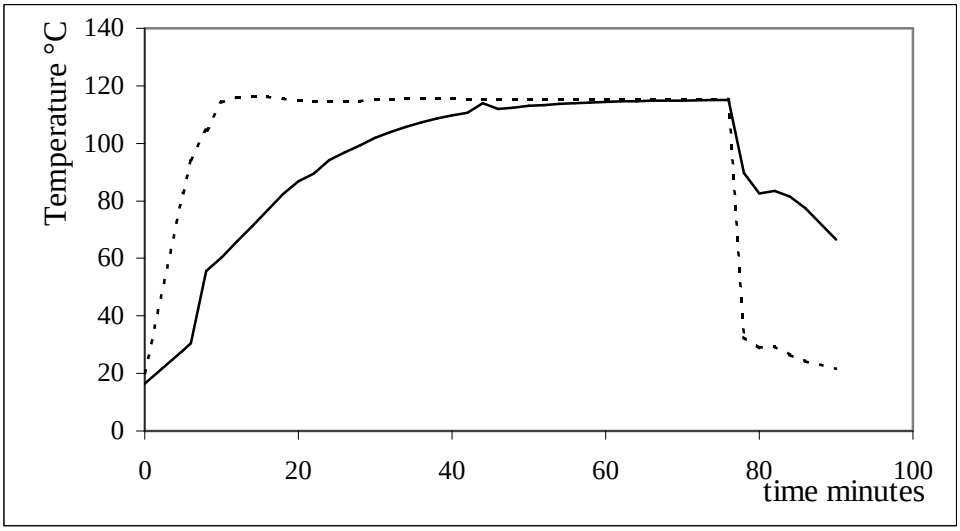


Figure 3


Temperature °C
140
120
100
80
60
40
20
0
0
20
40
60
80
100
time minutes