Quantification of spore resistance for assessment and optimization of heating processes: a never-ending story

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Abstract

The assessment and optimization of food heating processes require knowledge of the thermal resistance of target spores. Although the concept of spore resistance may seem simple, the establishment of a reliable quantification system for characterizing the heat resistance of spores has proven far more complex than imagined by early researchers. This paper points out the main difficulties encountered by reviewing the historical works on the subject.

During an early period, the concept of individual spore resistance had not yet been considered and the resistance of a strain of spore-forming bacterium was related to a global population regarded as alive or dead. A second period was opened by the introduction of the well-known *D* parameter (decimal reduction time) associated with the previously introduced *z*- concept. The present period has introduced three new sources of complexity: consideration of non log-linear survival curves, consideration of environmental factors other than temperature, and awareness of the variability of resistance parameters. The occurrence of non log-linear survival curves makes spore resistance dependent on heating time. Consequently, spore resistance characterisation requires at least two parameters. While early resistance models took only heating temperature into account, new models consider other environmental factors such as pH and water activity ("horizontal extension"). Similarly the new generation of models also considers certain environmental factors of the recovery medium for quantifying "apparent heat resistance" ("vertical extension").

Because the conventional *F*-value is no longer additive in cases of non log-linear survival curves, the decimal reduction ratio should be preferred for assessing the efficiency of a heating process.

Introduction

The assessment and optimization of food heating processes is clearly closely linked to the resistance of target pathogenic or spoilage spores, and the required intensity of any cooking, pasteurization or sterilization mainly depends on two factors:

- the *level of risk* which can be accepted by the operator and corresponds to a required reduction ratio, generally expressed as a decimal log-decrease,
- the *resistance of spores* which requires a relevant and, if possible, accurate quantification.

The establishment of a reliable quantification system for characterizing the heat resistance of spores has proven far more complex than imagined by early researchers. This paper aims to point out the main difficulties encountered by reviewing the historical concerned works on the subject, from the first attempts at spore resistance quantification, to an overview of the present situation. Similarly, the parallel evolution in the assessment of heating processes will be addressed.

1. Quantification of spore resistance

The history of spore resistance quantification can be arbitrarily fractionated into three periods. During an early period, the concept of individual spore resistance had not yet been considered and the resistance of a spore strain associated with a heating temperature or an exposure time, was related to a global population regarded as alive or dead.

The second period was opened by the introduction of the well-known *D* parameter (decimal reduction time) associated with the previously introduced *z*-concept. Today, calculations of food heating processes are still based on this quantification system and implicitly admit the two following assumptions:

- spore inactivation is assimilated to first order kinetic and survival curves are log-linear,
- the only environmental factor considered is heating temperature. In other words, it is assumed that spore resistance depends exclusively on the strain and temperature. Indeed, the effect of some other environmental factors such as pH or water activity were already qualitatively known, but not directly integrated in heat process calculations.

The third period which includes the present period introduced three new sources of complexity:

- consideration of non log-linear survival curves,
- taking into account of environmental factors other than temperature,
- awareness of the variability of resistance parameters.

1.1. First period: 1907-1942

Surprisingly, early authors who tried to quantitatively characterize the heat resistance of spores seem to have ignored the previous works of Madsen and Nyman (1907) and Chick (1908) who pointed out the first order nature of spore survival kinetics. More than 20 years after these works which should have imposed the specific rate of inactivation as the parameter characteristic of heat sensitivity, spore resistance was still regarded as the *death time of a global spore population at a given heating temperature* which corresponds to the famous TDT (Thermal Death Time) introduced by Bigelow in 1921. One of the main drawbacks of this simplistic concept was the fact that it was clearly dependent on the initial size of the living population. Aware of the need to standardize experimental determinations of spore heat resistance, Williams (1929) proposed the concept of *basic resistance* defined as the TDT of a 5.10^7 spore population aged 10 days and heated in a pH 7 phosphate buffer, at 95 or 100°C.

As early as the first works on survival kinetics, the famous Arrhenius equation (1889) was successfully applied for quantifying the effect of temperature on the specific rate of inactivation. Alternatively, ten years before the introduction of the *z*-concept by Bigelow (1921), Chick (1910) had already observed a linear relationship between the logarithm of the specific rate of inactivation and temperature. She then introduced the concept of *temperature coefficient* which corresponded to the multiplication factor of the specific rate of inactivation caused by an increase of 1°C of the heating temperature. The author could not detect any difference of goodness of fit between the latter relationship and the Arrhenius equation and, still nowadays, both models can be used indifferently.

1.2. Second period: 1942-1978

The popular *D* concept (required heating time for a survival ratio of 10%) was introduced as late as 1943 by Katzin and Sandholzer who rewrote the first order survival kinetic in a decimal base. From this date, the quantification of spore resistance could be based on two alternative model systems:

System I:

- Primary model: (first order kinetic):

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

where N_0 is the initial number of spores and N the number of surviving spores after heating time t; k is the specific rate of inactivation

- Secondary model: $$k = k^* \exp\left[-\frac{E_a}{R}\left(\frac{1}{T}-\frac{1}{T^*}\right)\right] \qquad (2)$$

This is the Arrhenius equation where k^* is the k-value at the T^* reference temperature. E_a is the so-called activation energy and R, the perfect gas constant.

Within the frame of this system, each strain resistance can be quantified by the two parameters (k^*, E_a).

System II

- Primary model: $N = N_0 10^{-\frac{t}{D}}$ (3)

(first order kinetic rewritten in decimal base)

- Secondary model: $D = D^* 10^{-\frac{T-T^*}{z}}$ (4)

(Bigelow relationship) where z corresponds to the increase in temperature yielding a ten-fold *D* reduction.

Using this system, each strain resistance can be quantified by the two parameters (D^*, z).

Both systems are still very useful: for traditional reasons, the first one is preferentially applied in the field of industrial microbiology, whereas the second is more widely used in the field of food heat processes. Unfortunately, both are limited to the cases of log-linear survival curves and ignore all factors other than temperature and time of heating.

1.3. Third period: 1978 to date

The beginning of this era demonstrates a growing complexity in the problem of spore quantification resistance due to the consideration of non log-linear survival curves (primary modelling) and new environmental factors (secondary modelling). An extensively cited review of the cases of observed non log-linear survival curves was published by Cerf (1977), in which the author classified the curves according to their patterns and tried to biologically or physically interpret the different shapes. On the

other hand, Davey et al. (1978) published the first thermal resistance secondary model including not only temperature, but also pH of the heating medium.

1.3.1. Primary quantification

The primary quantification of spore heat resistance has to cope with several typical curve shapes:

- curves presenting a *shoulder*: an initial phase showing gradual acceleration of the inactivation followed by a linear portion,
- curves presenting a *tail*: an initial linear portion followed by a braking phase,
- *sigmoid* curves showing both a shoulder and a tail,
- curvilinear curves with a downward concavity,
- curvilinear curves with an upward concavity,
- *Biphasic* curves with two straight lines of different slopes
- Biphasic curves including a shoulder.

For a given strain and in equal environment conditions, one parameter (k or D) is sufficient to quantify and compare spore heat resistances in the case of a log-linear survival kinetics. The situation is far more complex when the kinetics are no longer linear for two reasons:

- quantification of the resistance requires at least two parameters,
- heat resistance becomes dependent on heating time.

Any comparison of resistances then becomes quite difficult.

Whatever the shape of the survival curve, a general expression of heat resistance can be:

$$HR = -\frac{dt}{d(\log N)} \qquad (5)$$

In the particular case of log-linear curves, it is obviously obtained $HR = D$.

Among the numerous published models for fitting non linear curves, the cumulative function of the Weibull frequency distribution model is used increasingly frequently on account of its simplicity and its flexibility (Peleg and Cole, 1998; Mafart et al., 2002). This model can be written as follows:

$$\log\frac{N}{N_0} = -\left(\frac{t}{\delta}\right)^p \qquad (6)$$

In this example, the heat resistance of spores is quantified by the two following parameters: δ (scale parameter) and p (shape parameter):

$$HR = p\delta^p t^{p-1} \qquad (7)$$

Let us consider two strains characterized by the couples (δ_1, p_1) and (δ_2, p_2) respectively. Which one is the most heat resistant? A simple answer to this question is not possible because heat resistance is dependent on heating time, so one strain may be more resistant than the other at the beginning of the heating and more sensitive by the end of the exposure. For want of a better solution, a number of authors simply characterize heat resistance by the so-called *tDn*, which is defined as the required time of heating for obtaining n decimal reductions (most frequently, $n = 4$).

1.3.2. Secondary quantification

The new secondary models include not only heating temperature for estimating the spore heat resistance, but also some other main environmental factors such as pH, water activity or sodium chloride concentration (“horizontal extension”) (Davey et al., 1978; Cerf et al., 1996; Gaillard et al., 1998 a; Mafart et Leguérinel, 1998). On the

other hand, as the observed heat resistance depends not only on the heating conditions, but also on the recovery conditions of surviving cells, new generation models include factors which are related to the recovery medium. For example, pH of the heating medium and pH of the recovery medium are regarded as two distinct factors, even if cells are recovered in the heating medium, as is the case for heat processed foodstuffs ("vertical extension") (Coroller et al., 2001; Couvert et al., 1999; Couvert et al., 2000).

- *Horizontal extension*

The first non-thermal factor which was included in inactivation models was the pH of the heating medium. As early as 1948, Jordan and Jacobs observed a linear relationship between the logarithm of the decimal reduction time and pH, but the first model combining heating temperature and pH was proposed as late as 1978 by Davey et al. to describe the effect of these two factors on the specific inactivation rate of *Clostridium botulinum*:

$$Lnk = C_0 + \frac{C_1}{T} + C_2\,pH + C_3\,pH^2 \qquad (8)$$

where T represents the absolute heating temperature and C are empirical parameters. If the pH terms of this equation are dropped, the logarithmic form of the Arrhenius equation can be recognised. For this reason, Davey regarded his model as an extension of the Arrhenius equation. The Davey model was later further extended by the adjunction of a water activity term:

$$Lnk = C_0 + \frac{C_1}{T} + C_2\,pH + C_3\,pH^2 + C_4\,a_W^2 \qquad (9)$$

From the same bibliographic data as those used by Davey in 1993, regarding the heat resistance of *C. botulinum, C. sporogenes* and *Bacillus cereus*, Mafart and Leguérinel (1998) proposed a Bigelow-like model including a pH term:

$$\log D = \log D^* - \frac{T - T^*}{z_T} - \left(\frac{pH - pH^*}{z_{pH}} \right)^2 \qquad (10)$$

where T^* represents the reference temperature (most often 121.1°C) and pH^* the reference pH 7. The sensitivity parameters are z_T which simply corresponds to the conventional *z*-value, and z_{pH} which is the distance of pH from pH^* which leads to a ten-fold reduction in the decimal reduction time. Lastly, D^* represents the *D*-value in the reference conditions ($T = T^*$; $pH = pH^*$). This model was also further extended with the addition of a water activity term (Gaillard et al., 1998 a):

$$\log D = \log D^* - \frac{T - T^*}{z_T} - \left(\frac{pH - pH^*}{z_{pH}} \right)^2 - \frac{a_W - 1}{z_{a_W}} \qquad (11)$$

Regarding the pH terms of the models, Davey himself observed a strong self-correlation between his C_2 and C_3 parameters, which denotes a certain over-parameterization of his equation. On the contrary, the Mafart equation which includes one less parameter could be regarded as under-parameterized: in some cases (mild heat treatments, vegetative cells), a first degree instead of second degree equation may be more suitable:

$$\log D = \log D^* - \frac{T - T^*}{z_T} - \frac{|pH - pH^*|}{z_{pH}} \quad (12)$$

Moreover, the linearity of the Davey equation allows a very simple estimation of confidence intervals of each parameter, whereas the estimation of confidence intervals of Mafart parameters requires more sophisticated calculations. On the other hand, Davey parameters are difficult to use for quantifying heat resistance of a given spore strain because they lack robustness and do not have any biological significance. As an example, from the same set of data regarding *C.botulinum*, the following parameter estimates could be respectively obtained:

Davey model:

C_0 = 105.23

C_1 = - $3.7041.10^{-4}$ °K

C_2 = - 2.3967

C_3 = 0.1695

Mafart model:

D^* = 0.139 min

z_T = 9.32°C

z_{pH} = 3.61

The main drawback of both models is their absence of an interaction term while it is well known that interactions frequently occur between environmental factors. Gaillard et al. (1998 b) attempted to modify the equation (10) by adding a temperature/pH interaction term. Applying this modification to the inactivation of *Bacillus cereus*, they obtained a relatively poor improvement of goodness of fit (R^2 = 0.985 instead of 0.977). The authors then considered that this slight improvement was not sufficient to justify the implementation of an additional parameter and the loose of biological meaning of all parameters, except D^*. According to our results, values of the sensitivity parameters (z_T, z_{pH}, z_{aw}) seem to be independent of the food matrix. However, further works would be needed to confirm this property. Because of the possible occurrence of interactions between factors, it is recommended to estimate a sensitivity parameter linked to a factor, while the other considered factors are adjusted at their reference level.

- *Vertical extension*

It has been long known that the measured "apparent" decimal reduction time is dependent on the recovery conditions. When the recovery medium diverges from optimal conditions of incubation temperature, pH or water activity, the measured apparent *D*-value (denoted *D'*) is always lower than the *D*-value which would have been measured in optimal recovery conditions. For this reason, any environmental factor *X* which is related to the heating medium has to be clearly distinguished from the factor *X'* of the same name which is related to recovery medium. As far as we know, the only models integrating recovery

environmental factors were derived from our laboratory and present the same form which is as follows:

$$\log D' = \log D - \left(\frac{X' - X'_{opt}}{z'_X} \right)^2 \quad (13)$$

where X'_{opt} corresponds to the optimal value of the considered factor and z'_X the distance from the optimal level of this factor, which leads to a tenfold reduction of the *D*-value. This simple equation presents the drawback of artificially assuming a symmetric pattern of apparent heat resistance with respect to its maximum level. However, it yields quite a fair goodness of fit and its main advantage is the requirement of as few as three parameters, each having a biological meaning.

Couvert et al. (2000) applied this equation to fit the effect of incubation temperature on the apparent heat resistance of *B. cereus* with the following estimates: $D_{95°C}$ = 2.85 min; T'_{opt} = 23.6°C; z'_T = 33.7°C (R^2 = 0.95). The authors validated the model on other types of spore from data in the literature. Equation (13) was equally successfully applied to describe the effect of the pH of the recovery medium on the heat resistance of *B. cereus* (Couvert et al. 1999) with the following estimates: D_{max} = 2.33 min; pH'_{opt} = 6.78; z'_{pH} = 1.81 (R^2 = 0.983). Coroller et al. (2001) applied the same equation to describe the effect of the water activity of the recovery medium on the apparent D-value of the same strain of *B cereus.* They found an optimal water activity close to 0.98-0.99, whereas the z'_{aw} value was dependent on the involved depressor which

was used to adjust the water activity: in the range of 0.1 for glucose or glycerol and close to 0.07 for sucrose.

- *Multi factorial combination of unit-models*

The structure of equations (9) and (11) is an illustration of the classical modular approach which is frequently adopted in the field of food predictive microbiology and consists of assuming a multiplicative effect of combined involved factors on spore heat resistance. Indeed, the yielded product of factorial unit-models becomes a sum when the resistance parameter is submitted to a logarithmic transformation. If any given environmental factor related to the heating medium is denoted X_i, the overall model can then be written as follows:

$$\log D = \log D^* - \sum \left(\frac{X_i - X^*_i}{z_{X_i}} \right)^n \qquad (14)$$

where the n exponent can be equal to 1 or 2. Note that X^*_i does not correspond to a parameter to be estimated, but to a reference value such as $T^* = 121.1°C$, $pH^* = 7$ or $a^*_w = 1$.

Similarly, if any given environmental factor related to the recovery medium is denoted X'_i, the overall model can then be written as follows:

$$\log D' = \log D - \sum \left(\frac{X'_i - X'_{iopt}}{z'_{X_i}} \right)^2 \qquad (15)$$

The combined effects of environmental factors linked to the heating and to the recovery medium can then be written as follows:

$$\log D' = \log D_{(X^*, X'_{opt})} - \sum \left(\frac{X_i - X^*_i}{z_{X_i}} \right)^n - \sum \left(\frac{X'_i - X'_{iopt}}{z'_{X_i}} \right)^2 \qquad (16)$$

From this last equation, it can be seen that the complete heat resistance characterization of a given strain requires three sets of parameters:

- a main resistance parameter such as $D_{(X^*, X'opt)}$ which is an overall parameter and may be depend on the food matrix.
- the sensitivity parameters *z* and *z'* which are assumed to be independent of the food matrix,
- the optimal level of each considered factor yielding the maximum apparent heat resistance. If needed, the reference values of factors linked to the heating medium can be replaced by estimated optimal values. For example, if the optimal pH of the heating medium is distant from 7, it can be estimated and input in the model instead of retaining pH* = 7.

1.3.3. Variability of spore resistance

Although the last cited models allow a clear improvement in spore heat resistance assessment, they still suffer considerable background noise due to the number of controlled or uncontrolled factors such as the strain, the composition and the texture of the medium, the thermal history of spores (pre-incubation or sporulation temperature), possible pre-adaptation to different types of stress, interaction between factors etc. Any conclusion or decision from calculations of heat processes therefore requires the greatest caution.

2. Assessment and optimization of heating processes

The most simple and direct criterion for assessing the efficiency of a heating process is indeed the obtained inactivation ratio, which is commonly expressed as the decimal log decrease of alive spores, $n = \log N_0/N$. The major advantage of this criterion is the fact that it is additive whatever the pattern of the survival curve. Its main limit is that it is dependent on the target strain and the heating medium, so that it does not intrinsically allow comparison of two heating processes. Because of the considerable variability of spore resistance, such a comparison requires arbitrary assumptions and standard calculations. As early as 1927, Ball introduced the popular concept of *F*-value which corresponds to the time (in minutes) of heating at a reference temperature (250°F or 121.1°C for sterilization), *or to any time/temperature combination which would yield the same destruction ratio*. The reference z-value, equal to 10°C, which is that of the reference strain (*Clostridium botulinum* 62A), is associated with the reference temperature. Note that the determination of the *F*-value does not require the knowledge of any *D*-value. The *F* concept can be applied both for the assessment of a given process (*observed F-value*) and for the optimization of a heating process (*target F-value*). Both applications encounter specific difficulties.

2.1. Observed F-value

The obtained *F*-value can be calculated from the following equation:

$$F = \int L(T)dt \qquad (17)$$

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

where T is the core temperature of the exposed foodstuff, T^* is the reference temperature and $L(T)$ corresponds to the so called lethality factor. Because T is itself a function of time, the solution of the integral requires the knowledge of the heat transfer kinetic $T = f(t)$, then a core temperature registration. The numerical approach of Bigelow consisted of a graphic determination of the integration area of the curve $L(T) = f(t)$, whereas the analytical approach of Ball involved simplified heat transfer equation. The empirical approach of Bigelow can be regarded as a *measurement tool* and as the reference method, whereas the theoretical approach of Ball can lead to some errors due to some simplifying assumptions, although it is an efficient *simulation tool.*

2.2. Target F-value

The required F-value for yielding n decimal reductions (or a n log-decrease) is as follows:

$$F = nD^* \qquad (18)$$

where D^* corresponds to the decimal reduction time at the reference temperature. The required F-value is therefore the product of two factors: a safety factor which is determined from a management decision, and a resistance factor which is linked to the target strain. This very simple equation is in reality extremely difficult to apply. The first difficulty is the choice of the target pathogenic or spoilage strain according to its prevalence and to its level of nuisance in a given factory. Secondly, provided that the initial concentration of contaminants is approximately known, it will be possible to make an arbitrary decision from the accepted level of risk. Even if the target organism is clearly identified and if the problem of the choice of the n value is solved, the difficulty for determining the D^*-value remains, the variability of which was discussed earlier.

2.3. Limits of the F concept and alternatives

While the *F* concept is a simple and convenient indicator allowing comparisons of cooking or sterilization procedures regardless of the target strains, it is not a suitable tool for accurately optimizing heat processes for the two following reasons:

- if the survival curve linked to the process is not log-linear, the *F*-value loses its property of additivity and conventional calculation can no longer be applied (Mafart et al.,2002),
- an optimization of a process from the *F*-value takes only heating temperature into account and ignores the other environmental factors such as the pH and the water activity of the medium.

What can be done to circumvent these drawbacks?

In the cases of non-log-linear survival curves, optimization calculations can be made from a suitable primary model and from log decrease values (*n*) instead of from *F*-values. Conventional calculation procedures can then be modified and adapted to the primary model that should preferably be sufficiently simple for allowing analytical solutions.

In the case of log-linear curves, the *F*-concept could be kept, provided that it is extended according to the main environmental factors other than temperature (see Mafart, 2000). According to this approach, *D** denotes the *D*-value, not only at the reference temperature, but also at reference levels of other environmental factors (for example, $pH^* = 7$, $a_w^* = 1$). Similarly, the conventional concept of the lethality factor *L(T)* is extended into a multifactorial function such as $L(T, pH, a_w)$.

Traditional calculations regarding heating processes were mainly devoted to *F*-values determinations and optimisation but rarely to risk assessment which is rather difficult on account of the dissuasive variability which can be observed everywhere: heat transfer inside foodstuffs, *F*-values, food matrix, levels of initial contamination, spore

525 resistance etc. However, the contribution of statisticians and the presence of powerful
526 computers at every desk make it possible to conduct simulations taking the distribution
527 of each input variable into account.
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