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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.

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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:

- 138 - spore inactivation is assimilated to first order kinetic and survival curves are log-
139 linear,
140 - the only environmental factor considered is heating temperature. In other words, it is
141 assumed that spore resistance depends exclusively on the strain and temperature.
142 Indeed, the effect of some other environmental factors such as pH or water activity
143 were already qualitatively known, but not directly integrated in heat process
144 calculations.
- 145 The third period which includes the present period introduced three new sources of
146 complexity:
- 147 - consideration of non log-linear survival curves,
 - 148 - taking into account of environmental factors other than temperature,
 - 149 - awareness of the variability of resistance parameters.

150

151 ***1.1. First period: 1907-1942***

152

153 Surprisingly, early authors who tried to quantitatively characterize the heat resistance
154 of spores seem to have ignored the previous works of Madsen and Nyman (1907) and
155 Chick (1908) who pointed out the first order nature of spore survival kinetics. More
156 than 20 years after these works which should have imposed the specific rate of
157 inactivation as the parameter characteristic of heat sensitivity, spore resistance was
158 still regarded as the *death time of a global spore population at a given heating*
159 *temperature* which corresponds to the famous TDT (Thermal Death Time) introduced
160 by Bigelow in 1921. One of the main drawbacks of this simplistic concept was the fact
161 that it was clearly dependent on the initial size of the living population. Aware of the
162 need to standardize experimental determinations of spore heat resistance, Williams
163 (1929) proposed the concept of *basic resistance* defined as the TDT of a $5 \cdot 10^7$ spore
164 population aged 10 days and heated in a pH 7 phosphate buffer, at 95 or 100°C.

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

175

176 **1.2. Second period: 1942-1978**

177

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

182

183 *System I:*

184

185 - Primary model: (first order kinetic):

$$186 \quad N = N_0 e^{-kt} \quad (1)$$

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

$$189 \quad - \text{Secondary model: } k = k^* \exp \left[-\frac{E_a}{R} \left(\frac{1}{T} - \frac{1}{T^*} \right) \right] \quad (2)$$

190 This is the Arrhenius equation where k^* is the k -value at the T^* reference temperature.

191 E_a is the so-called activation energy and R , the perfect gas constant.

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

194

195 *System II*

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

197 (first order kinetic rewritten in decimal base)

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

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

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

203

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

209

210 **1.3. Third period: 1978 to date**

211

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

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

220

221 1.3.1. Primary quantification

222

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

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

233

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

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

240 Any comparison of resistances then becomes quite difficult.

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

243

$$244 \quad HR = -\frac{dt}{d(\log N)} \quad (5)$$

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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:

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

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

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

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

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

278

279 - *Horizontal extension*

280

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

287

$$288 \quad Lnk = C_0 + \frac{C_1}{T} + C_2 pH + C_3 pH^2 \quad (8)$$

289

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

295

$$296 \quad Lnk = C_0 + \frac{C_1}{T} + C_2 pH + C_3 pH^2 + C_4 a_w^2 \quad (9)$$

297

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

301

$$302 \quad \log D = \log D^* - \frac{T - T^*}{z_T} - \left(\frac{pH - pH^*}{z_{pH}} \right)^2 \quad (10)$$

303

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

311

$$312 \quad \log D = \log D^* - \frac{T - T^*}{z_T} - \left(\frac{pH - pH^*}{z_{pH}} \right)^2 - \frac{a_w - 1}{z_{a_w}} \quad (11)$$

313

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

320

321
$$\log D = \log D^* - \frac{T - T^*}{z_T} - \frac{|pH - pH^*|}{z_{pH}} \quad (12)$$

322

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

331

332 *Davey model:*

333

334 $C_0 = 105.23$

335 $C_1 = - 3.7041 \cdot 10^{-4} \text{ }^\circ\text{K}$

336 $C_2 = - 2.3967$

337 $C_3 = 0.1695$

338

339 *Mafart model:*

340

341 $D^* = 0.139 \text{ min}$

342 $z_T = 9.32^\circ\text{C}$

343 $z_{pH} = 3.61$

344

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

359

360 - *Vertical extension*

361

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

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

372

$$373 \quad \log D' = \log D - \left(\frac{X' - X'_{opt}}{z'_X} \right)^2 \quad (13)$$

374

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

382 Couvert et al. (2000) applied this equation to fit the effect of incubation
383 temperature on the apparent heat resistance of *B. cereus* with the following
384 estimates: $D_{95^\circ C} = 2.85$ min; $T'_{opt} = 23.6^\circ C$; $z'_T = 33.7^\circ C$ ($R^2 = 0.95$). The
385 authors validated the model on other types of spore from data in the literature.
386 Equation (13) was equally successfully applied to describe the effect of the pH
387 of the recovery medium on the heat resistance of *B. cereus* (Couvert et al.
388 1999) with the following estimates: $D_{max} = 2.33$ min; $pH'_{opt} = 6.78$; $z'_{pH} = 1.81$
389 ($R^2 = 0.983$). Coroller et al. (2001) applied the same equation to describe the
390 effect of the water activity of the recovery medium on the apparent D -value of
391 the same strain of *B. cereus*. They found an optimal water activity close to 0.98-
392 0.99, whereas the z'_{aw} value was dependent on the involved depressor which

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

395

396 - *Multi factorial combination of unit-models*

397

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

$$405 \quad \log D = \log D^* - \sum \left(\frac{X_i - X_i^*}{z_{X_i}} \right)^n \quad (14)$$

406 where the n exponent can be equal to 1 or 2. Note that X_i^* does not correspond to a
407 parameter to be estimated, but to a reference value such as $T^* = 121.1^\circ\text{C}$, $pH^* = 7$ or $a_w^* = 1$.

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

410

$$411 \quad \log D' = \log D - \sum \left(\frac{X'_i - X'_{iopt}}{z'_{X_i}} \right)^2 \quad (15)$$

412

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

415

416

417
$$\log D' = \log D_{(X^*, X'_{opt})} - \sum \left(\frac{X_i - X^*_i}{z_{X_i}} \right)^n - \sum \left(\frac{X'_i - X'_{iopt}}{z'_{X_i}} \right)^2 \quad (16)$$

418

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

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

430

431

432 *1.3.3. Variability of spore resistance*

433

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

441

442 **2. Assessment and optimization of heating processes**

443

444 The most simple and direct criterion for assessing the efficiency of a heating process is
445 indeed the obtained inactivation ratio, which is commonly expressed as the decimal
446 log decrease of alive spores, $n = \log N_0/N$. The major advantage of this criterion is the
447 fact that it is additive whatever the pattern of the survival curve. Its main limit is that it
448 is dependent on the target strain and the heating medium, so that it does not
449 intrinsically allow comparison of two heating processes. Because of the considerable
450 variability of spore resistance, such a comparison requires arbitrary assumptions and
451 standard calculations. As early as 1927, Ball introduced the popular concept of *F*-
452 value which corresponds to the time (in minutes) of heating at a reference temperature
453 (250°F or 121.1°C for sterilization), *or to any time/temperature combination which*
454 *would yield the same destruction ratio.* The reference z-value, equal to 10°C, which is
455 that of the reference strain (*Clostridium botulinum* 62A), is associated with the
456 reference temperature. Note that the determination of the *F*-value does not require the
457 knowledge of any *D*-value. The *F* concept can be applied both for the assessment of a
458 given process (*observed F-value*) and for the optimization of a heating process (*target*
459 *F-value*). Both applications encounter specific difficulties.

460

461

462 **2.1. Observed F-value**

463

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

465

466
$$F = \int L(T)dt \qquad (17)$$

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

468

469 where T is the core temperature of the exposed foodstuff, T^* is the reference
470 temperature and $L(T)$ corresponds to the so called lethality factor. Because T is itself a
471 function of time, the solution of the integral requires the knowledge of the heat
472 transfer kinetic $T = f(t)$, then a core temperature registration. The numerical approach
473 of Bigelow consisted of a graphic determination of the integration area of the curve
474 $L(T) = f(t)$, whereas the analytical approach of Ball involved simplified heat transfer
475 equation. The empirical approach of Bigelow can be regarded as a *measurement tool*
476 and as the reference method, whereas the theoretical approach of Ball can lead to
477 some errors due to some simplifying assumptions, although it is an efficient
478 *simulation tool*.

479 **2.2. Target F-value**

480

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

483

$$484 \quad F = nD^* \quad (18)$$

485

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

497

498 **2.3. *Limits of the F concept and alternatives***

499

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

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

509 What can be done to circumvent these drawbacks?

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

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

521 Traditional calculations regarding heating processes were mainly devoted to *F*-values
522 determinations and optimisation but rarely to risk assessment which is rather difficult
523 on account of the dissuasive variability which can be observed everywhere: heat
524 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.

528

529

530

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