

# Validation of an overall model describing the effect of three environmental factors on the apparent D-value of Bacillus cereus spores

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1	Validation of an overall model describing the effect of
2	three environmental factors on the apparent D-value of
3	Bacillus cereus spores
4	
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10 11	ABSTRACT
12	Several factorial models extending the famous Bigelow model to describe the influence of the
13	heating and recovery pH and $a_{\rm w}$ conditions on bacterial heat resistance have been developed.
14	These models can be associated in an overall multifactorial model describing the influences of
15	heating and recovery conditions on D values. For Bacillus cereus strain ADQP 407 the mo D
16	el parameters characterising the environmental factor influences (pH, Temperature, aw) were
17	evaluated. Determination of bacterial heat resistance in cream chocolate have been realised to
18	validate these parameter values and to evaluate the level of the influence of food texture or
19	different compounds not taken account of in the model.
20	
21	KEYWORDS:

22 Heat resistance, pH, aw, *Bacillus cereus*, secondary model Bigelow model.

#### 23 INTRODUCTION

Several environmental conditions influence bacterial heat resistance. In addition to the heating
temperature, it has been recognized that pH and water activities of the heating and recovery
medium are the main factors that affect the apparent heat resistance of bacteria (Esty and
Bigelow, 1920; Esty and Meyer, 1922; Murrel and Scott, 1966; Cook and Gilbert, 1968;
Harnulv et al., 1977; Lynch and Potter, 1988; Fernadez et al., 1996; Fernandez et al., 2002).

Different multifactorial models which describe the influence of the heating environmental
condition have been published (Davey et al., 1978; Reichart, 1994; Cerf et al., 1996;
Fernandez et al., 1996) Mafart and Leguérinel (1998) and Gaillard et al. (1999) have
developed an extension of the linear Bigelow model to the pH and aw influence Eq. 1.

33 
$$\log D = \log D^* - \left(\frac{T - T^*}{z_T}\right) - \left|\frac{pH - pH^*}{z_{pH}}\right| - \left(\frac{a_w - 1}{z_{a_w}}\right) Eq. 1$$

where  $T^*$  is the reference temperature (generally,  $T^*=121.1^{\circ}C$ ) and  $pH^*$  is the reference pHfixed at =7,  $D^*$  is the D-value at  $T^*$ ,  $pH^*$  and aw = 1,  $z_T$  is the conventional thermal z-value,  $z_{pH}$  is the difference of pH from  $pH^*$ , which leads to a ten fold reduction of D-value,  $z_{aw}$  is the difference of aw from  $aw^* = 1$  which leads to a ten fold reduction of D-value. As the Bigelow model, this imbricate model, taking temperature, pH and water activities into account, is used to evaluate the decimal reduction ratio and the sterilization value (F- value) Mafart (2000).

40 However, these models assume that the heat resistance is measured at optimal recovery 41 condition and do not take the influence of the non optimal condition of the recovery media 42 into account. It is well known that the count of survival bacteria after heating treatment is 43 greatly influenced by the characteristic of the recovery medium: temperature, pH, aw and 44 composition. When the recovery condition differs from the optimal condition both a decrease 45 in the number of heated stressed cells capable of producing colonies and a decrease in the 46 estimate decimal reduction time, are observed (Harris, 1963; Katsui et al., 1982; Mallidis and 47 Scholefield, 1986; Feeherry et al., 1987). Recently, according to the same approach as that adopted in the Mafart and Leguerinel model (1998), Couvert et al. (1999) and Coroller et al.
(2001), have developed similar Bigelow models to describe the influence of pH and water
activities respectively, of the recovery medium, on the apparent heat resistance of bacteria
Eq.2-3.

52 
$$\log D' = \log D'_{opt} - \left(\frac{pH' - pH'_{opt}}{z'_{pH}}\right)^2 \text{Eq. } 2$$

53 
$$\log D' = \log D'_{opt} - \left(\frac{a'_{w} - a'_{wopt}}{z'_{a_{a}}}\right)^{2}$$
 Eq. 3

54 Where *pH*' or *aw*' are the *pH* or the water activity of the recovery medium, *D*' is the apparent 55 reduction time at *pH*' or *aw*', *pH'*<sub>opt</sub> and *aw'*<sub>opt</sub> correspond to the maximal *D*'value and  $z'_{pH}$ 56 and  $z'_{aw}$  are the distance from the *pH'*<sub>opt</sub> or *aw'*<sub>opt</sub> respectively, which leads to a ten fold 57 reduction of the apparent reduction time *D*'.

58 Mafart and Leguerinel, Gaillard et al., Couvert et al. and Coroller et al. models can be 59 associated in an overall nested model which describes the influences of heating and recovery 60 conditions on the estimated D value of bacteria. This model (eq 4) can be used like the 61 Bigelow model to estimate the heat resistance and decimal reduction rate of bacterial 62 population.

63 
$$\log D = \log D^* - \left(\frac{T - T^*}{z_T}\right) - \left|\frac{pH - pH^*}{z_{pH}}\right| - \left(\frac{a_w - 1}{z_{a_w}}\right) - \left(\frac{pH' - pH'_{opt}}{z'_{pH}}\right)^2 - \left(\frac{a'_w - a'_{wopt}}{z'_{a_a}}\right)^2 Eq 4$$

64 The aim of this paper is to obtain the model's parameters for *Bacillus cereus* spores and65 validate these parameter values in the food product of chocolate.

66

#### 67 Material and methods

#### 68 *Micro-organism and spore production*

The strain of *Bacillus cereus* ADQP407 isolated from shrimp was obtained from the
ADRIA (France). Spores were kept in distilled water at 4°C.

Cells were precultivated at  $37^{\circ}$ C for 24 hrs in Brain Heart Infusion (Difco ). The preculture was used to inoculate nutritive agar plates (Biokar Diagnostics BK021) added with MnSO<sub>4</sub> 40mg l<sup>-1</sup> and CaCl<sub>2</sub> 100 mgl<sup>-1</sup> on the surface area. Plates were incubated at  $37^{\circ}$ 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 final suspension (about  $10^{10}$  spores ml<sup>-1</sup>) was finally distributed in sterile Eppendorfs microtubes and kept at 4°C.

78

#### 79 Thermal treatment of spore suspension and recovery conditions.

In basic condition the heating medium was a tryptone salt broth ( $10gl^{-1}$  tryptone 80 81 Biokar and 10gl<sup>-1</sup> sodium chloride) at pH 7 with no sucrose added, the heating temperature 82 was 100°C. The heating medium was sterilized by filtration. The influence of heating 83 temperature was studied ranging from 95°C to 102°C, the heating pH ranging from 4.5 to 7 84 adjusted with HCL and the heating water activities ranging from 1 to 0.92 were adjusted using 85 sucrose. The previous molarities of the different solutes were determined using curves from 86 model UNIFAC-LARSEN ( Achard et al. 1992). The aw values were controlled with an aw-87 meter (FA-st1 GBX France Scientific Instrument).

Firstly,  $30\mu$ l of spore suspension was diluted in 3 ml adjusted heating medium. Capillary tubes of 200 µl (vitrex) were filled with 100µl of sample, sealed, and submitted to a thermal treatment in a thermostated glycerol bath for different heating times. The heat treatment was stopped by cooling capillary tubes in water / ice bath. Then they 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.

94 The viable spores were counted by duplicate plating in nutritive agar (10g tryptone, 5g meat
95 extract, 5g sodium chloride, 15 g agar for 1000ml water)(Biokar Diagnostic) and incubated at

96 37°C for 6 days. The recovery medium pH ranging from 5 to 7 was adjusted with sterile 97 solution of HCl after autoclaving. The recovery medium water activity ranging from 1 to 0.95 98 was adjusted with added sucrose. To adjust a<sub>w</sub> values, the previous molarities of the different 99 solutes were determined using curves from model UNIFAC-LARSEN (Achard <u>et al.</u> 1992). 100 Nutritive agar and sucrose solutions were sterilized separately by autoclaving to avoid the 101 Maillard reaction. After sterilisation the two solutions were mixed, pH was adjusted to 7 and 102 a<sub>w</sub> value was controlled.

The validation of the heating sensibility parameters had been realized by heating *Bacillus cereus* spores scattered in "chocolate cream" (pH: 6.76 and aw: 0.968), included in capillary
tubes

106

#### 107 Experimental design and data analysis

For each environmental factor studied a monofactorial experimental design was carried out. D values were determinate by linear regression on the straight portion of curves obtained when the log number of survivors was plotted against heating time. The sensibility parameters of the models "z" were fitted on experimental values using Excel software.

112

#### 113 **Results and discussion**

For the strain of *Bacillus cereus* ADQP 407 studied, all survival curves present a log linear relation between the number of colony forming units and heating time. The classical D values were determined by linear regression. One example is presented Fig1.The whole set of data values is presented table 1.

118 The fitting of Bigelow model, related to the heating temperature, on the experimental D 119 values (Fig 2), gives a  $z_T$  value equal to 7.1°C, which corresponds to the values currently 120 given for *Bacillus cereus* spores (Bergere and Cerf, 1992). 121 The decrease of heating and recovery medium pH values reduces the apparent bacterial heat 122 resistance. (Fig 3-4). The fitting of parameters (eq 1-2) and associated correlation coefficients 123 were computed Table 2. The decrease in recovery pH medium ( $z'_{pH}$ : 2.18) appears to have 124 more influence on the apparent heat resistance than a decrease in heating medium pH ( $z_{pH}$ 125 3.45).

126 The dominating influence of recovery pH medium has been observed for other bacterial127 species (Couvert et al., 1999; Couvert thesis 2002)

Regarding the water activity influences, a decrease in aw value leads to a thermo-protective effect (Fig 5). In the recovery medium heated spores show an apparent maximum heat resistance at an optimum close to 0.985. Under this optimal value, an increase in sucrose concentration reduces the bacterial heat resistance (Fig 6). The aw decrease of recovery medium ( $z_{aw}$ , 0.092) presents a more pronounced effect than the protective effect of heating medium ( $z_{aw}$ , 0.156).

134 These values correspond to the parameters determined for other Bacillus cereus strain with 135 sucrose as the same water depressor (Coroller et al., 2001). For the different models the  $D^*$  or 136 D'opt correspond to the D value evaluated to the reference or the optimal conditions 137 respectively, and could not be considered the same. To get one and only D value, the overall 138 equation (Eq 4) was fitted on the whole set of data. The  $D^*$  value correspond closely to those 139 determined at heating condition: T\*: 121.1°C, pH\*: 7, aw\*: 1 and the evaluated optimal recovery conditions  $pH'_{opt}$  and  $aw'_{opt}$ . The heat resistance parameters;  $z_T$ ,  $z_{pH}$ ,  $z'_{pH}$ ,  $z_{aw}$  and  $z'_{aw}$ 140 141 obtained in Table 3, show the parameter values determined from each monofactorial design. 142 Fig 7 illustrates the relationship between experimental and calculated D values

143 In food product the main factors that influence the apparent heat resistance are the 144 temperature, pH and water activities. Moreover different compounds or food textures can 145 influence the bacterial heat resistance. However, previous studies, not published, have shown 146 that these secondary factors had a low influence on the sensibility parameters "*z*". The part of 147 these factors is evaluated by the ratio between the experimental heat resistance determined in 148 food and the corresponding calculated values.

149 This comparison is made on the heat treatment only on the one hand and, on the overall 150 apparent heat resistance, heating and recovery on the other hand. Fig 8 shows the comparison 151 of the experimental and calculated *Bacillus cereus* death kinetics determined in chocolate 152 cream (pH 6.76 and aw:0.968). The figure and ratio 1.73, higher than 1, shown that the model 153 taking temperature, pH and water activities into account, underestimates the bacterial heat 154 resistance. However, on the overall apparent heat resistance heating and recovery, the 155 experimental result confirms the calculated forecast concerning the overall apparent heat 156 resistance (Table 4); after heat treatment at 100°C for 35 minutes no growth was observed in 157 cream chocolate incubated at 37°C for 7 days.

158 The confrontation between the validation ratio and the food texture and composition could 159 bring to the fore new factors or compounds that affect apparent heat resistance.

160

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- 220 <u>Legend of figure</u>
- Fig 1: log N versus heating time
- Fig 2: Log D versus heating temperature
- Fig 3: Log D versus heating medium pH
- Fig 4: Log D versus recovery medium pH
- Fig 5: Log D versus heating medium aw
- Fig 6: Log D versus recovery medium aw
- Fig 7 : correlation between experimentally log D values and theoretically log D values calculated
- from the overall model
- Fig 8: comparison of the experimental (---) and calculated (---) Bacillus cereus death kinetics,
- heating in chocolate cream and recovery in nutritive agar pH7, aw1.
- 231
- 232
- 233
- 234
- 235 <u>Table of legends</u>
- Table 1 Effects of heating temperature, heating and recovery medium pH and aw on D-values (min) of
- 237 Bacillus cereus
- Table 2: models parameters
- Table 3: fitting parameters on the whole set of data
- 240 Table 4: comparison of the experimental and calculated *Bacillus cereus* growth in capillary
- tube after heating and recovery in chocolate cream for different heating times

















$\mathbf{c}$	0	2
э	ð	3

		-		~		
Heating	Heating	Recovery	Heating	Recovery		
temperature	medium	medium	medium	medium	D value	Confidence
°C	pН	pH'	aw	aw'	minutes	interval
95	7	7	1	1	29.52	$\pm 2.31$
98	7	7	1	1	11.54	$\pm 0.78$
100	7	7	1	1	5.61	±0.46
102	7	7	1	1	3.11	±0.19
100	6.85	7	1	1	5.57	±0.57
100	6.62	7	1	1	5.22	±0.60
100	6.41	7	1	1	3.99	±0.32
100	5.92	7	1	1	3.14	±0.22
100	5.21	7	1	1	1.61	±0.19
100	4.56	7	1	1	1.89	±0.16
100	7	7	1	1	7.53	±0.73
100	7	6.52	1	1	6.06	±0.54
100	7	6.07	1	1	3.92	±0.44
100	7	5.8	1	1	2.98	±0.33
100	7	5.5	1	1	3.59	±0.37
100	7	5.35	1	1	1.04	±0.14
100	7	7	1	1	5.78	±0.48
100	7	7	0.98	1	8.71	±1.39
100	7	7	0.96	1	13.32	±1.67
100	7	7	0.94	1	15.07	±1.66
100	7	7	0.92	1	19.10	±2.31
100	7	7	1	1	6.55	±0.53
100	7	7	1	0.99	6.93	±0.61
100	7	7	1	0.98	6.58	±0.49
100	7	7	1	0.97	6.44	±0.60
100	7	7	1	0.96	5.79	±0.38
100	7	7	1	0.95	4.90	±0.47

387 388 Table1

	Values	r
z <sub>T</sub> °C	7.09	0.999
Z <sub>pH</sub>	3.44	0.967
z' <sub>pH</sub>	2.18	
pH' <sub>opt</sub>	6.96	0.920
Z <sub>aw</sub>	0.156	0.979
z' <sub>aw</sub>	0.092	
aw' <sub>opt</sub>	0.985	0.989



Table 2

	Fitting on the	
	whole set of data	
D* minutes	0.009	
logD*	-2.02	
$z_T \ ^\circ C$	7.32	
$Z_{pH}$	3.48	
z' <sub>pH</sub>	1.55	
Z <sub>aw</sub>	0.153	
z' <sub>aw</sub>	0.088	
pH'opt	6.78	
aw'opt	0.983	
r	0.990	

Table3

	Heating time	Tubes with observed growth	Calculated N value in tube
	minutes		
	20 min	2 / 2	54
	25 min	2/2	11
	30 min	1 / 2	2
	35 min	1 / 2	0
	40 min	0 / 2	0
	45 min	0 / 2	0
403 —			

Table4