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2 **EFFECT OF PH ON THE HEAT RESISTANCE OF SPORES :**
3 **COMPARISON OF TWO MODELS**
4

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11
12 **Abstract**
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14 All published models describing the effect of pH on the heat resistance of spores can be
15 regarded either as a linear first degree equation or a linear second degree equation. This work
16 aimed to compare both models from 3 sets of published data for , *Clostridium sporogenes*
17 and *Bacillus stearothermophilus* respectively. The relative quality of fit of each model with
18 respect to the other depends on the species, the strain and the heating temperature. Parameter
19 estimation was more reliable for the second degree model than for of the simple first degree
20 equation. However, in the case of acidic foodstuffs, predictions obtained from the second
21 degree model are more sensitive toward errors of parameter values. The second degree model
22 is better from the point of view of safety at most frequent ranges of pH of foods. Moreover,
23 for *Clostridium botulinum* , the goodness of fit of this model is clearly higher than that of the
24 first degree equation. If this observation is confirmed by further work, the second degree
25 model in application of standard calculations of heat processes of foods would be preferred.

26 *Keywords:* spores; heat resistance ; pH ; model
27

28 **Introduction**
29

30 It has been recognised for several years that low pH values reduce spore resistance, but
31 available information related to the quantitative effect of this factor is scarce and can be
32 contradictory. Jordan and Jacobs (1948) observed a linear relationship between the D value
33 (decimal reduction time) of *Escherichia coli* and the pH of the heating menstruum. The same
34 linear relationship was found by several other researchers for *Bacillus cereus* (Mazas et al.,
35 1998) and *Clostridium butyricum* (Pirone et al., 1987). Regarding *Bacillus stearothermophilus*
36 and *Clostridium sporogenes*, Fernandez et al. (1996) proposed a simple first degree and a
37 quadratic polynomial model for describing effects of temperature and pH on the heat
38 resistance of spores. They did not carry out analysis of variance for selecting significant model
39 terms, but the fact that both models worked seems to indicate that the simple linear
40 relationship was sufficient for describing the effect of pH.

41 Davey et al. (1978) proposed a model describing the combined effect of temperature and pH
42 on the heat resistance of *Clostridium botulinum* spores which can be regarded as a quadratic
43 polynomial equation without an interaction term. Similarly, Mafart and Leguérinel (1998)
44 described the effect of temperature and pH on the decimal reduction time of *C. botulinum*, *C.*
45 *sporogenes* and *B. stearothermophilus* using an equation containing a squared term for pH:
46

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

where D^* is the decimal reduction time at the current heat temperature and at the pH of maximal heat resistance of spores, noted pH^* , while z_{pH} corresponds to the distance of pH from pH^* which leads to a ten fold reduction of the decimal reduction. T^* is the reference temperature (generally, 121.1°C) and z_T , the conventional z-value (increase of temperature which leads to a ten fold reduction of the decimal reduction).

The same equation fitted for *B.cereus* (Couvert et al., 1999).

Then, regardless of the effect of temperature, two incompatible models compete for describing the behaviour of the same spore species at various pH. One model is a first degree equation which can be written as follows (model 1):

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

The other model is a second degree equation. At isothermal conditions, the Mafart model can be reduced to the following expression (model 2):

This paper aims to compare both models according to the following criteria: goodness of fit, robustness and safety.

Materials and methods

Models were compared using three published sets of data, respectively obtained from *Clostridium botulinum* (Xenones and Hutchings, 1965) with range temperature from 110°C to 118.3°C and pH from 4 to 7, *Clostridium sporogenes* (Cameron et al., 1980) with ranges from 110°C and 121°C and pH from 5 to 7, and *Bacillus stearothermophilus* (Lopez et al., 1996) with temperature ranges from 115°C to 135°C and pH from 4 to 7.

Comparison of goodness of fit

Both models can be expressed in terms of the following equation (model n):

$$S = \frac{\partial \log D}{\partial z_{pH}}$$

where n is an additional parameter whose value indicates which of the two models presents the best accuracy. Parameters D^* , z_{pH} and n were then estimated according to a non linear regression by using the solver capability of the Excel software.

1 In addition, models 1 and 2 were fitted from the three sets of data and the mean square errors
 2 were compared.

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 5 *Comparison of robustness*

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 7 Two complementary criteria were taken into account. First, at each isothermal condition, z_{pH}
 8 values were estimated according to models 1 and 2 (corresponding estimated values were
 9 noted z_1 and z_2 respectively). The stability of z_{pH} values for each model was assessed by
 10 calculating standard deviations of z_1 and z_2 values. Secondly, the sensitivity of D values
 11 toward variations of z_{pH} was assessed using the following criterion

12

$$S = \frac{\partial \log D}{\partial z_{pH}}$$

13
 14 The relative sensitivity of model 2 with respect to model 1 can be assessed from the ratio
 15 S_2/S_1 , where

16

$$S_1 = \frac{\partial \log D}{\partial z_1} = \frac{pH^* - pH}{z_1^2}$$

17
 18 and

19

$$S_2 = \frac{\partial \log D}{\partial z_2} = \frac{2(pH^* - pH)^2}{z_2^3}$$

20
 21 Then, it follows that

22

$$\frac{S_2}{S_1} = 2(pH^* - pH) \frac{z_1^2}{z_2^3}$$

23
 24 The relative sensitivity of both models is then dependent on the pH of the heating medium and
 25 equals unity for a particular pH value which is:

26

$$pH_R = pH^* - \frac{z_2^3}{2z_1^2}$$

27
 28 When $pH > pH_R$, model 2 is more sensitive toward variations of z_{pH} than model 1 while, on
 29 the contrary, model 2 is more robust than model 1 when $pH < pH_R$

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 35 *Comparison of safety*

1 The concept of partial biological destruction value (BDV) related to pH was defined as the
 2 ratio of the D value at the standard pH ($pH^* = 7$) and the D value at the current pH (Mafart,
 3 1999):

$$\lambda(pH) = \frac{D^*}{D}$$

5 An overestimated partial BDV indicates a fail safe model because it corresponds to an
 6 overestimation of the effect of the acidity of the medium on the decrease of heat resistance of
 7 spores. The safety of both models can be compared using the ratio λ_2/λ_1 , where λ_1 and λ_2
 8 represent partial BDV calculated from models 1 and 2 respectively.

9 According to model 1,

$$\lambda_1 = 10^{\frac{pH^* - pH}{z_1}}$$

11 whereas, according to model 2,

$$\lambda_2 = 10^{\left(\frac{pH^* - pH}{z_2}\right)^2}$$

15 Then, it follows that

$$\frac{\lambda_2}{\lambda_1} = 10^{\left[\left(\frac{pH^* - pH}{z_2}\right)^2 - \frac{pH^* - pH}{z_1}\right]}$$

17 The relative safety of both models is then dependent on the pH of the heating medium and
 18 equals unity when

$$\left(\frac{pH^* - pH}{z_2}\right)^2 - \frac{pH^* - pH}{z_1} = 0$$

21 The solution of this last equation is:

$$pH_s = pH^* - \frac{z_2^2}{z_1}$$

24 The safety of model 2 is then higher than that of model 1 when $pH > pH_s$

33 Results

1 Goodness of fit

2
3 The three sets of data related to *C. botulinum*, *C. sporogenes* and *B. stearothermophilus*,
4 respectively, were fitted according to model n. Estimates of n and z_{pH} values are presented in
5 table 1. Even inside the same species, a wide range of n values can be observed: for example,
6 among the four strains of *C. sporogenes*, n values ranged from 0.90 to 2.35. However, this
7 dispersion is obviously linked to the lack of robustness and the overparameterization of model n
8 indicated by a strong structural correlation between n and z_{pH} ($r = -0.978$ for *C. botulinum* and
9 -0.993 for *B. stearothermophilus*).

10 Tables 2a and 2b show the estimates of the parameters for *C. botulinum* and *B.*
11 *stearothermophilus*, respectively, at each isothermal condition. Through both sets of data, a
12 significant increase of n values can be observed at increasing heat treatment temperatures
13 while z_{pH} values remain relatively stable (with a correlation coefficient of 0.749 between log n
14 and heat temperature for *C. botulinum* and 0.701 for *B. stearothermophilus*). However, no
15 significant effect of temperature on n values was detected in the case of *C. sporogenes*.

16 The relative goodness of fit of models 1 and 2 was compared by calculating their residual
17 sums of squares (table 3). As expected, model 1 fitted better than model 2 when the estimated
18 n value was close to 1. In some cases, when the n value was close to 1.5, the goodness of fit of
19 both models was similar.

20 21 3.2. Robustness

22
23 Within each set of data, the standard deviation of estimated z_1 and z_2 from different media or
24 different strains was calculated (table 3). It can be seen that in every case the standard
25 deviation of z_1 values is higher than that of z_2 values, which tends to indicate a better stability
26 of z_2 with respect to z_1 and a better robustness of model 2 with respect to model 1.

27 Another aspect of the robustness of a model is its sensitivity towards errors in its parameter
28 estimation. The threshold pH (pH_R) above which model 2 is more robust than model 1 was
29 calculated (table 4). While this value ranged from 5.5 to 6 for *C. botulinum* and *B.*
30 *stearothermophilus*, it was between 6 and 7 for *C. sporogenes*.

31 32 Safety

33
34 The threshold pH (pH_S) above which model 2 is safer than model 1 was calculated (table 4).
35 In most cases, this value kept close to 4, which indicates that between this pH and 7, model 2
36 is safer than model 1.

37 38 39 40 41 42 4. Discussion

43
44 The pattern of the effect of pH on the heat resistance of spores is more variable and multiform
45 than that of the effect of heat temperature which always can be described by either the
46 Arrhenius or Bigelow model. As in some cases the quality of fit of model 1 can be better than
47 that of model 2 and, in some other cases, the inverse situation can be observed, the more
48 general model n could be preferred. However, this last model presents a number of drawbacks
49 the main of which are its non linearity and its overparameterisation, which generates its

1 instability and lack of robustness. The difference of goodness of fit of models 1 and 2 is
2 reduced by the structural correlation between the exponent and the z_{pH} value: an increase of
3 the exponent is partly balanced by a decrease of z_{pH} , so that it can occur that, in some
4 particular situations, the goodness of fit of both models can be close. Neither model 1 or 2
5 takes interactions between temperature and pH into account, which can explain the
6 dependence of estimated n values (according to model n) toward temperatures of heat
7 treatments. It can be seen from table 2 that n increases with increasing temperatures. If this
8 observation were confirmed by further work, it would suggest that at relatively low heating
9 temperatures model 1 should be preferred, while at higher temperatures, model 2 would fit
10 better.

11 As the comparison of dispersions of z_1 and z_2 values (table 3) shows a better stability of z_2
12 with respect to z_1 , it can be deduced that a reliable estimation of z_{pH} is easier with model 2
13 than with of model 1. Moreover, when pH exceeds 5.5 to 6, predictions obtained from model
14 2 are less sensitive to errors in z_{pH} values than those obtained from model 1. However, for
15 most acidic foodstuffs, model 1 is less sensitive toward z_{pH} variations than the other model
16 (table 4).

17 According to these last observations, the first degree model seems to fit better at low heating
18 temperatures and low pH, while the second degree model would be better suited at higher
19 treatment temperatures and for moderately acidic foodstuffs. However, from the point of view
20 of food industries, the main criteria to be considered is the safety of predictions obtained from
21 different models. It can be seen from pH_S values shown in table 4 that, inside the most
22 frequent range of pH of foods (4 to 7) the second degree model presents a better safety than
23 the first degree one.

24 Special attention must be paid to the behaviour of *C. botulinum* (62A), which is the reference
25 strain for heat processes standard calculations. According to results shown in tables 1-3, it
26 clearly appears that the second degree model applied to this strain presents a better goodness
27 of fit than model 1. Moreover, this last model is fail safe with respect to model 2. It can then
28 be concluded that for calculations of heat treatment optimisation, the second degree model
29 must be preferred to the first degree one. In other cases, when a particular target strain or
30 species has to be considered, model 1 can be preferred to the other only when it presents a
31 clearly better quality of fit.

32 Only temperature ranges of sterilisation were considered in this work. Further investigations
33 would be needed for milder heat treatments such as pasteurisation to check if tested models
34 are still suitable and which of the first degree or the second degree models presents the best
35 goodness of fit.

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Table 1

Species	Medium/Strain	Number of data	n	Z _{pH}
<i>C. botulinum</i>	Spaghetti	32	1.87	3.65
	Macaroni creole	32	1.95	3.56
	Spanish rice	32	2.10	3.48
<i>C. sporogenes</i>	Buffer	30	1.34	6.04
	Pea puree	30	1.25	4.29
<i>B. stearothermophilus</i>	7953	20	0.90	5.01
	12980	20	1.42	4.17
	15951	20	1.83	3.48
	15952	20	2.35	2.96

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Table 2a

	Spaghetti		Macaroni creole		Spanish rice	
Heating temperature	n	z_{pH}	n	z_{pH}	n	z_{pH}
110°C	1.59	3.58	1.72	3.49	1.96	3.44
112.8°C	1.83	3.56	2.00	3.45	2.08	3.40
115.6°C	1.91	3.77	2.15	3.57	2.10	3.53
118.3°C	2.20	3.65	2.01	3.70	2.23	3.55

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Table 2b

	7953		12980		15951		15952	
Heating temperature	n	<i>z_{pH}</i>	n	<i>z_{pH}</i>	n	<i>z_{pH}</i>	n	<i>z_{pH}</i>
115°C	0.51	4.33	0.79	3.50	1.24	3.08	1.72	2.59
120°C	0.89	4.33	1.12	4.25	0.89	3.78	2.12	2.99
125°C	0.83	4.48	1.10	4.22	1.44	3.54	2.34	2.92
130°C	1.87	4.53	3.76	3.41	3.09	3.34	2.28	3.11
135°C	2.00	4.50	2.09	4.73	3.86	3.59		

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Table 3

Species	Medium/Strain	Model 1		Model 2	
		z_{pH}	R.M.S.	z_{pH}	R.S.S.
<i>C. botulinum</i>	Spaghetti	4.52	0.00471	3.61	0.00130
	Macaroni creole	4.19	0.00529	3.54	0.00114
	Spanish rice	4.12	0.00587	3.50	0.00111
	<i>Standard deviation</i>	<i>0.214</i>		<i>0.056</i>	
<i>C. sporogenes</i>	Buffer	8.70	0.00273	4.29	0.00277
	Pea puree	5.11	0.00237	3.33	0.00302
	<i>Standard deviation</i>	<i>2.539</i>		<i>0.679</i>	
<i>B. stearothermophilus</i>	7953	4.79	0.00789	3.97	0.01353
	12980	4.66	0.01074	3.81	0.01044
	15951	3.91	0.01268	3.45	0.00811
	15952	3.00	0.02279	2.94	0.00632
	<i>Standard deviation</i>	<i>0.824</i>		<i>0.457</i>	

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Table 4

Species	Medium/Strain	pH _R	pH _S
<i>C. botulinum</i>	Spaghetti	5.85	4.12
	Macaroni creole	5.74	4.01
	Spanish rice	5.74	4.03
<i>C. sporogenes</i>	Buffer	6.99	4.88
	Pea puree	6.29	4.83
<i>B. stearothermophilus</i>	7953	5.64	3.71
	12980	5.73	3.88
	15951	5.66	3.96
	15952	5.59	4.12

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Legends of tables

Table 1: Estimated n and z_{pH} values according to model n.

Table 2a: Estimated n and z_{pH} values related to *C. botulinum* at isothermal heating conditions, (according to model n).(8 data per temperature and per food)

Table 2b: Estimated n and z_{pH} values related to *B. stearothermophilus* at isothermal heating conditions, (according to model n).(4 data per temperature and per strain)

Table 3: Comparison of z_{pH} values and residual mean squares according to models 1 and 2 respectively.

Table 4: Critical threshold values of pH related to the relative robustness (pH_R) and the relative safety (pH_S) of models (see Materials and Methods).

