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EFFECT OF PH ON THE HEAT RESISTANCE OF SPORES : COMPARISON OF TWO MODELS

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Abstract

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

Introduction

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

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

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

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):

$$\log D = \log D^* - \frac{T - T^*}{z_T} - \left(\frac{pl}{} \right.$$

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.

In addition, models1 and 2 were fitted from the three sets of data and the mean square errors were compared.

Comparison of robustness

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

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

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

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

and

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

Then, it follows that

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

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

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

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

Comparison of safety

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

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

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

According to model 1,

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

whereas, according to model 2,

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

Then, it follows that

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

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

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

The solution of this last equation is:

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

The safety of model 2 is then higher than that of model 1 when pH > pH_S

Results

Goodness of fit

The three sets of data related to *C. botulinum*, *C. sporogenes* and *B. stearothermophilus,* respectively, were fitted according to model n. Estimates of n and z_{pH} values are presented in table 1. Even inside the same species, a wide range of n values can be observed: for example, among the four strains of *C. sporogenes*, n values ranged from 0.90 to 2.35. However, this dispersion is obviously lied to the lack of robustness and the overparameterization of model n indicated by a strong structural correlation between n and z_{pH} (r = -0.978 for *C. botulinum* and –0.993 for *B. stearothermophilus*).
Tables 2a and 2b show the estimates of the parameters for *C. botulinum* and *B. stearothermophilus*, respectively, at each isothermal condition. Through both sets of data, a significant increase of n values can be observed at increasing heat treatment temperatures while z_{pH} values remain relatively stable (with a correlation coefficient of 0.749 between log n and heat temperature for *C. botulinum* and 0.701 for *B. stearothermophilus*). However, no significant effect of temperature on n values was detected in the case of *C. sporogenes*.
The relative goodness of fit of models 1 and 2 was compared by calculating their residual sums of squares (table 3). As expected, model 1 fitted better than model 2 when the estimated n value was close to 1. In some cases, when the n value was close to 1.5, the goodness of fit of both models was similar.

3.2. Robustness

Within each set of data, the standard deviation of estimated z_1 and z_2 from different media or different strains was calculated (table 3). It can be seen that in every case the standard deviation of z_1 values is higher than that of z_2 values, which tends to indicate a better stability of z_2 with respect to z_1 and a better robustness of model 2 with respect to model 1.
Another aspect of the robustness of a model is its sensitivity towards errors in its parameter estimation. The threshold pH (pH_R) above which model 2 is more robust than model 1 was calculated (table 4). While this value ranged from 5.5 to 6 for *C. botulinum* and *B. stearothermophilus*, it was between 6 and 7 for *C. sporogenes*.

Safety

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

4.Discussion

The pattern of the effect of pH on the heat resistance of spores is more variable and multiform than that of the effect of heat temperature which always can be described by either the Arrhenius or Bigelow model. As in some cases the quality of fit of model 1 can be better than that of model 2 and, in some other cases, the inverse situation can be observed, the more general model n could be preferred. However, this last model presents a number of drawbacks the main of which are its non linearity and its overparameterisation, which generates its instability and lack of robustness. The difference of goodness of fit of models 1 and 2 is reduced by the structural correlation between the exponent and the z_{pH} value: an increase of the exponent is partly balanced by a decrease of z_{pH}, so that it can occur that, in some particular situations, the goodness of fit of both models can be close. Neither model 1 or 2 takes interactions between temperature and pH into account, which can explain the dependence of estimated n values (according to model n) toward temperatures of heat treatments. It can be seen from table 2 that n increases with increasing temperatures. If this observation were confirmed by further work, it would suggest that at relatively low heating temperatures model 1 should be preferred, while at higher temperatures, model 2 would fit better.

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

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

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

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

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Table 1

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

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*

Table 2b

	7953		12980		15951		15952	
Heating temperature	n	z_{pH}	n	z_{pH}	n	z_{pH}	n	z_{pH}
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*		

Table 3

Species	Medium/Strain	Model 1		Model 2	
		z_{pH}	R.M.S.	z_{pH}	R.S.S.
C. botulinum	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
	Standard deviation	*0.214*		*0.056*	
C. sporogenes	Buffer	8.70	0.00273	4.29	0.00277
	Pea puree	5.11	0.00237	3.33	0.00302
	Standard deviation	*2.539*		*0.679*	
B. stearothermophilus	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
	Standard deviation	*0.824*		*0.457*	

Table 4

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

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).