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Quantifying the effects of heating temperature, and combined effects of heating medium pH and recovery medium pH on the heat resistance of *Salmonella typhimurium*

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Abstract

The influence of heating treatment temperature, pH of heating and recovery medium on the survival kinetics of *Salmonella typhimurium* ATCC 13311 are studied and quantified. From each non log linear survival curve, Weibull model parameters were estimated. An average shape parameter value of 1.67 was found, which is characteristic of downward concavity curves and is in agreement with values estimated from other *Salmonella typhimurium* strains. Bigelow type models quantifying the heating temperature, heating and recovery medium pH influences, are fitted on scale parameter δ data, (time of first decimal reduction), which reflects the bacterial heat resistance. The estimate of z_T (4.64°C) is in the range of values given in literature for this species. The influence of pH of the heating medium on the scale parameter (z_{pH}: 8.25) is lower than that of the recovery pH medium influence (z'_{pH}: 3.65)

Key words: Weibull, heating medium, recovery medium, pH, *Salmonella typhimurium*

Nomenclature

N_0: initial number of cells .

N: number of surviving cells after the heating time t.

δ: scale parameter : first decimal reduction of surviving spores or cells from N_0 to $N_0/10$.

p: shape parameter.

T: heating temperature.

pH: heating medium pH.

pH': recovery medium pH.

T^*: reference temperature fixed at 60°C.

pH^*: reference pH of the heating medium fixed to 7 .

pH'_{opt} : recovery medium:pH corresponding to the maximal apparent bacterial heat resistance.

z_T: distance of T from T^* which leads to a ten fold reduction in δ-value z_T quantifies the influence of the heating temperature on the bacterial heat resistance.

z_{pH} : distance of pH from pH^* which leads to a ten fold reduction in δ-value z_{pH} quantifies the influence of the pH of the heating medium on the bacterial heat resistance.

z'_{pH} : distance of pH' from pH'_{opt}, which leads a ten fold reduction in apparent δ -value. z'_{pH} characterises the influence of the pH on the recovery of the micro-organism after a heat treatment .

δ^* :estimated δ value corresponding to T*, pH^* and pH'_{opt} conditions.

Introduction

Salmonellae continue to be a major public health setting a great problem to the food industry. These bacterial species appear in a wide variety of foods and food ingredients. Various heat treatments implemented by the food processing industry are generally effective for destroying the vegetative bacteria. Taking into account not only temperature but other environmental factors is likely to allow significant reduction of heat treatment intensity with the same microbial safety and minimize damage of heat sensitive food. It is commonly agreed that the pH decrease of the heating medium is the main environmental factor after temperature, which reduces the bacterial heat resistance of spores (Bigelow and Esty, 1920) or vegetative form (White, 1963; Blackbrun, Curtis, Humpheson, Billon & McClure, 1997). The same effect was observed for *Salmonella enteritidis*, (Casadei, Ingram, Hitchings, Archer & Gaze, 2001) and for *Salmonella typhimurium* (Mazzotta, 2001).

Moreover the pH of the recovery medium highly influences the apparent heat resistance of bacterial spores for the same heat treatment condition. The *D* values decrease when pH values shift from an optimum (Cook and Brown, 1965). However, as far as we know, the influence of the recovery medium pH on the apparent heat resistance of Salmonella species has never been described. A decrease of pH of the heating medium or of the recovery medium both reduces the bacterial heat resistance (Couvert, Leguérinel & Mafart, 1999). Theses influences can be taken into account for reducing heat treatments. Such a cumulative effect is more or less specific of pH and is not observed for water activity where, oppositely, the protective effect of a low water activity of the heating medium tends to be balanced by the selectivity of a low water activity of the recovery medium towards injured cells (Coroller, Leguérinel & Mafart, 2001).

The aim of this study was to quantitatively characterise the impact of heating temperature, pH of the heating medium and pH of the recovery medium on the heat resistance of *Salmonella typhimurium* from relevant parameters of appropriated primary and secondary models

Materials and methods

Strain and culturing conditions

The studied strain was *Salmonella typhimurium* ATCC 13311 (NCTC 74). Cultures were stored in cryotube in mixing nutrient broth 50% glycerol 50% at -70°C.

The basic heating medium was tryptone salt broth (10g/l tryptone USP (Biokar Diagnostics, A1401HA) and 10g/l NaCl). The pH was adjusted with addition of H_2SO_4 and sterilised by filtration through 0.22µm porosity filter.

The recovery medium was nutrient agar (Biokar Diagnostics, BK021HA). The pH were adjusted with H_2SO_4 prior autoclaving at 121°C for 15 minutes. The pH values were checked after autoclaving.

Preparation of cells suspension

Nutrient broth (Biokar Diagnostics, BK003HA) in 200ml flask was inoculated with *Salmonella typhimurium* and incubated at 37°C for 24 hours under agitation (150 rpm). The culture (40ml) was centrifuged (2000g 15min at 20°C) and re-suspended in 3 ml of heating medium.

Thermal treatment of bacterial suspension and recovery conditions

Capillary tubes of 200 µl (Ringcaps® Duran®) were filled with 100µl of sample and submitted to a thermal treatment in a thermostated water bath. After heating, the tubes were cooled in water/ice bath. After rinsing, the ends were flamed with ethanol. The capillary tubes were broken at both ends and their contents poured into a tube containing 9 ml sterile tryptone salt broth (Biokar Diagnostics, BK014HA) by rinsing with 0.9 ml tryptone salt broth.

Viable cells were counted by duplicate plating in ajusted pH nutrient agar (Biokar Diagnostics, BK021HA) and incubated at 37°C for 48h.

Experimental design

To determine survival kinetic parameters, bacteria, for each sample corresponding to different heating times, were counted on nutrient agar plates.

Heating temperatures applied were 53, 55, 57 and 59°C (heating and recovery media pH equal 7). For studying the effect of pH, a complete factorial design was implemented according to the following levels of pH of the heating medium: (7, 6.5, 6, 5.3, 5, 4.4 and 3.8) and to the following levels of pH of the recovery medium (denoted pH'):7, 6.5, 6, 5.5 and 5 (controlled pH values are given table3) at 55°C.

Data from literature

Data taken from figures in literature were scanned and digitized using the software program DigXY 1.2 (Thunderhead Engineering, Manhattan, USA).

Primary and secondary models

Different authors considered the survival curve as a cumulative form of temporary distribution of lethality event frequency (Cunhan, Oliveira & Oliveira, 1998; Peleg & Cole, 1998; Fernandez, Salmeron, Fernandez & Martinez, 1999). In 2002, Mafart, Couvert, Gaillard, and Leguerinel proposed a new presentation of the Weibull frequency distribution model.

$$\log N = \log N_0 - \left(\frac{t}{\delta}\right)^p \quad \text{Eq1}$$

Parameter p characterises the shape of the curve: concave curves $p<1$, convex curves $p>1$ and linear curves $p=1$, in this case δ value corresponds to classical D value. This equation was taken up during the IFT summit in January 2003 (Heldman and Newsome, 2003), was used by different authors (Mafart et al., 2002; Gómez, García, Álvarez, Raso & Condón, 2005;

Geeraerd, Valdramidis & Van Impe, 2005; Virto, Sanz, Álvarez, Condón & Raso, 2005; Carlin et al., 2006) and was implemented in this work as primary model.

The influences of environmental factors on the Weibull model parameter estimated from survival kinetics related to bacterial spores or vegetative cells, were studied (Fernandez et al., 1999; Fernandez, Collado, Cunhan, Ocio & Martinez, 2002; van Boekel, 2002; Couvert, Gaillard, Savy, Mafart & Leguerinel 2005).These studies showed that the shape parameter was practically independent of the heating temperature and the pH of the heating medium. The conclusion of these studies leads us to determine a single average shape parameter value for a set of kinetics.

The δ value, first decimal reduction time, is highly influenced by heating temperature. The classical Bigelow model was used to describe the influence of heating temperature on the δ with the conventional z_T value.

The effect of the pH of the heating medium and the pH' of the recovery medium on the heat resistance was described according to the following equation (Leguérinel, Spegagne, Couvert, Gaillard & Mafart, 2005) :

$$\log\delta = \log\delta^* - \left|\frac{pH - pH^*}{z_{pH}}\right| - \left(\frac{pH' - pH'_{opt}}{z'_{pH}}\right)^2 \quad \text{Eq 2}$$

Curve fitting.

In a first time, N_0,δ and p values are estimated from each survival curve to assess the influence of environmental factors on these parameters.

In a second time a single shape parameter p value was estimated from the corresponding whole set of experimental kinetics and from set of kinetics taken from the literature. Scale parameter δ values and $\log N_0$ values were determined for each curve.

Bigelow parameter z_T was estimated from scale parameter δ values obtained from the temperature mono factorial design. Equation 2 parameter values: z_{pH} and z'_{pH} were estimated from scale parameter δ values obtained from pH factorial design.

The parameter values and their associated confidence interval were estimated by using a non-linear module (nlinfit and nlparci Matlab 6.1, Statistical Toolbox, The Mathworks).

Results and discussion

Survival kinetics curves of *Salmonella typhimurium* showed a clear downward concavity. The same pattern of curves was elsewhere observed for different *Salmonella typhimurium* strains (Garibaldi, Ljichi & Bayne, 1969; Mackey and Derrick, 1986; Jäckle, Geiges & Schmidt-Lorenz, 1987). Such non linear curves were fitted according to the Weibull model.

The influences of the heating temperature, the pH of the heating and of the recovery medium on the shape parameter *p* are shown Figure 1. *p* values appear to be not clearly influenced by these environmental factors. This observation is in agreement with those of Fernandez *et al*. (2002), Collado, Fernandez, Rodrigo, Camats and Martinez Lopez. (2003) regarding *Bacillus cereus*, Couvert *et al*. (2005) ,and *Bacillus pumilus* and van Boekel's (2002). These authors did not observe any significant influence of the temperature on the shape parameter *p*. The presented results (Figure 1) show no clear influence of the heating medium or the recovery pH on estimates of *p* for the lower pH. However structural correlation between parameters *p* and δ could explain the variability of *p* values (Couvert *et al*., 2005).

Then, a single average *p* value was estimated, regardless of the heating temperature, the pH of the heating and of the recovery medium. The three parameters (N_0, δ and *p*) were globally estimated from the whole set of data by using the least square regression method (nlinfit Matlab 6.1). The single *p* value estimated from our set of data (1.677 ± 0.065) is close to the p value estimated from other sets of data from literature for the same Salmonellae species:

1.648 ± 0.313 (Jäckle *et al*.,1987), 1.538 ± 0.187 (Garibaldi *et al.*, 1969), 1.429 ± 0.295 (Mackey & Derrick, 1986). $log\ N_0$, the scale parameters δ and their confidence interval coefficients as functions of different pH and temperature conditions, are presented in Table 1 and 2. The scale parameter δ values are influenced by environmental factors: heating temperature, pH of the heating and the recovery medium. Within the investigated temperature range (53°C -59°C) the classical Bigelow relationship was kept to quantify the effect of temperature on δ value. The corresponding z_T value 4.64°C (Table 3) is lower than z_T values determined from Jäckle *et al*. (1987) data for the same bacterial strain but are in agreement with z_T value estimated from Mackey and Derrick data (1986) and with other values found in literature. Doyle and Mazzotta (2000) reviewed z_T values concerning different *Salmonella typhimurium* strains heated in different media. These z_T values, ranging from 3.24°C to 9.5°C with a mean of 5.56°C, illustrates the large variability of z_T values reported in literature. For the same *Salmonella typhimurium* strain ATCC13311 Casadei *et al.* (2001) reported a z_T-value of 4.6°C (Table 4).

Both the pH of the heating and the recovery medium affect the heat resistance of *Salmonella typhimurium* as shown Figure 2. A decrease of the pH of the heating medium reduces the salmonella heat resistance. This observation has been reported by Blackburn *et al*. (1997) and Casadei *et al.* (2001). Concerning the influence of the recovery medium, low pH reduces heat resistance parameter value δ of *Salmonella typhimurium*. A similar effect was observed for the thermal inactivation of bacterial spore (Cook and Brown, 1965; Lopez, Gonzalez, Mazas, Gonzalez, Martin & Bernardo, 1997) but never for vegetative bacteria cells.

Equation 2 was used to describe the effect of the heating medium pH and the recovery medium pH on δ values. This model does not take interactions between these two environmental factors into account. To estimate the weight of the possible interaction, a variance analysis was performed. For the studied pH range, the weight of heating and recovery medium pH represent 62.9% and 34.7% respectively, while the unexplained variance comprising interactions represented only 2.4% of the total variability. This observation concerning *Salmonella typhimurium* is in agreement with Couvert et al. (1999) results related to *Bacillus cereus* spores. Such observations led us to neglect interactions and to retain the simple Equation 2 without crossed term. Model parameters (Eq 2) determined for *Salmonella typhimurium* (Table 5) were estimated from the whole set of δ values, according to factorial design.

The determination coefficient between experimental and calculated values (R^2 :0.958) and Figures 3a and 3b illustrate the goodness of fit of the model. The high z_{pH} parameter value 8.25, indicates a poor influence of the heating medium pH on the heat resistance for this Salmonella strain. For other salmonella strains few data allow to evaluate this z_{pH} parameter. From Blackburn et al. (1997), Casadei et al. (2001) and Mañas, Pagán, Raso and Condón (2003) data, an optimal pH value appears to be variable and often lower than 7. This observation leads to replace a fixed reference pH^*, equal to 7, by a variable optimal pH parameter (pH_{opt}) to be estimated Eq 3.

$$\log\delta = \log\delta_{opt} - \left(\frac{T - T^*}{z_T}\right) - \left|\frac{pH - pH_{opt}}{z_{pH}}\right| \quad \text{Eq 3}$$

Parameters z_{pH}, pH_{opt} and z_T estimated from published values are shown Table 4. Regarding the studied bacterial strain and different bacterial species, z_{pH} values estimated from data of literature and our own data are lower than z_{pH} obtained in this work, even for the same *Salmonella typhimurium* strain ATCC 11331. This difference could be explained by errors on *D* value estimates due to the fitting of concave curves by linear regressions or growth condition and physiology state of bacteria which can have produced stress protein.

Concerning the effect of the pH of the recovery medium, the z'_{pH} value (3.6) indicates a higher influence of this factor than that of the heating medium pH. It is generally accepted that the pH of the recovery medium exerts a large influence on the apparent heat resistance of spores: *D*-values decrease as pH is reduced (Cook and Brown, 1965; Yokoya and York, 1965; Cook and Gilbert, 1968; Mallidis and Scholefield, 1986; Santos and Zarzo, 1996; Lopez et al., 1997). Observed z'_{pH} values, which characterized the influence of the recovery medium pH, for *Salmonella typhimurium* cannot be compared with values regarding other Salmonella strains or species. As far as we know, no data which could be compared to our results, are available from literature.

The primary model derived from the Weibull distribution and describing non linear survival kinetics associated with Bigelow type secondary models, can be easily used to optimise heat treatment process calculations taking the heating and recovery pH influences into account. For example, compared to a heat treatment in food at pH 7, a heat treatment for *Salmonella typhimurium* in food at pH 5, could reduce the heating time to a 3.5 ratio, or, with the same heating time, could reduce the heating temperature of 2.25°C with the same lethal efficiency.

In practice, to ensure safety of acid foods, heating pH is the pH of food before heat treatment. Because foods represent both the heating and the recovery medium, the input recovery pH is likely to keep the value of the heating pH. However, it frequently occurs that a decrease of food pH is observed during the heat treatment. In this case, for safety reasons, it is recommended to retain the value of the pH which is measured immediately after the heat treatment.

This work confirms the impact of low recovery medium pH on the apparent heat resistance. This influence allows reducing heat treatments with the same safety objective to keep better nutritional and sensory quality of foods. This approach could be extended to other vegetative strains and species which would require further data: *p* and *z* parameter values, related to heating temperatures and heating and recovery medium pH. From these data, standard values of z_{pH} and z'_{pH} could be defined according to a similar approach to the one that was implemented for the standard z_T value equal to 7°C for most resistant vegetative cells and input in pasteurisation process calculations.

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Table legends

Table1

log N_0 and δ estimates and their confidence interval coefficients as functions of the heating temperature.

Table 2

log N_0 and δ estimates and their confidence interval coefficients as functions of the heating and the recovery medium pH.

Table 3

Parameters z_T estimated (from log δ values determined) from our own (data) and published data for different *Salmonella* strains

Table 4

$z_{T,}$ z_{pH} and pH_{opt} estimates from published classical D values for different *Salmonella* species

Table 5

z_{pH}, $z'_{pH,}$ pH'_{opt} estimates for *Salmonella Typhimurium* ATCC 13311 (log δ values)

Figure legends

Figure 1

Weibull shape parameter values and their confidence interval 95% evaluated for each kinetic of heating temperature, and heating and recovery medium pH

Figure 2

Survival kinetics experimental data and fitted curves, with p–value equal1.67, at different heating medium pH for different recovery pH (pH 7 ●, pH 6.5 □, pH6▲, pH5.5 ▽, pH 5 ◆) and at different temperatures (59°C7 ●, 57°C □, 55°C ▲, 53°C ▽) heating and recovery pH fixed at 7

Figure 3a 3b

Observed and calculated log *δ* values for different conditions of heating and recovery medium pH

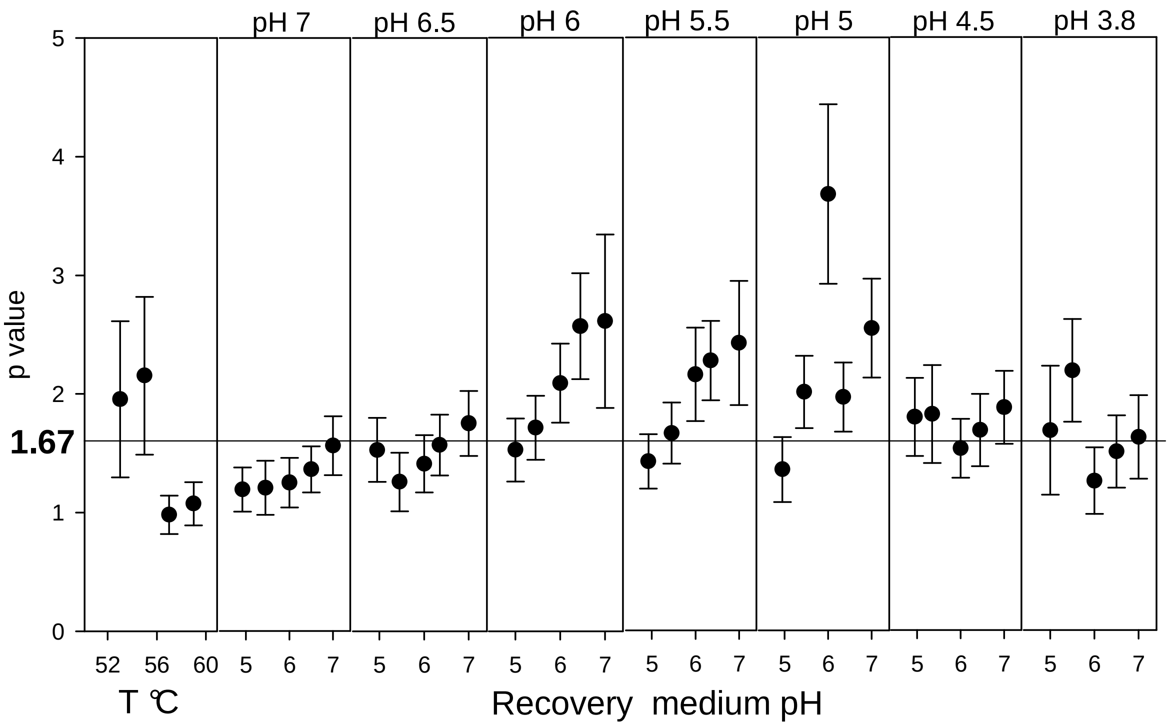
pH 7
pH 6.5
pH 6
pH 5.5
pH 5
pH 4.5
pH 3.8
p value
1.67
0
1
2
3
4
5
52
56
60
5
6
7
T ℃
Recovery medium pH

Figure 1

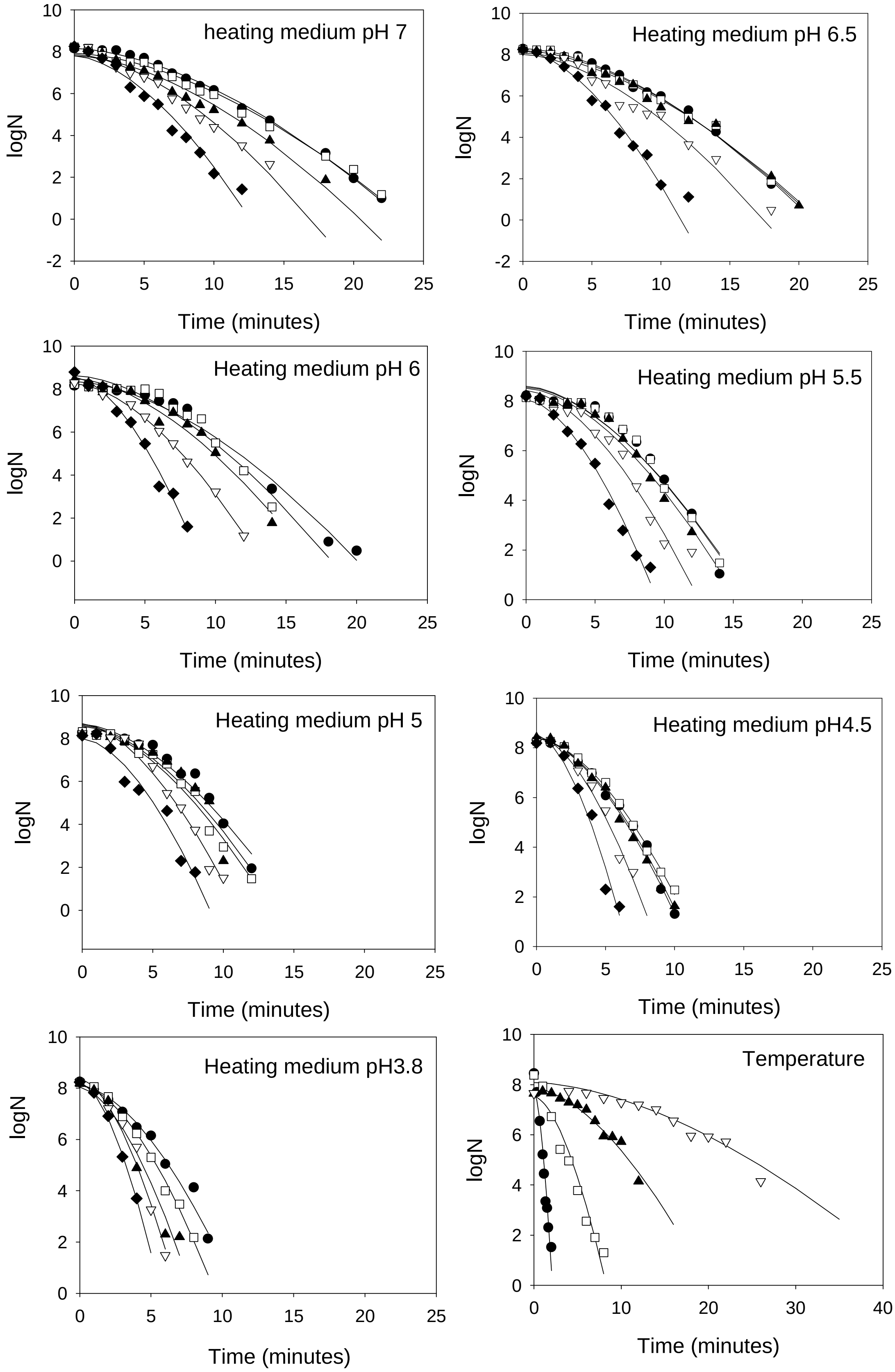
heating medium pH 7
Heating medium pH 6.5
Heating medium pH 6
Heating medium pH 5.5
Heating medium pH 5
Heating medium pH4.5
Heating medium pH3.8
Temperature
logN
Time (minutes)

Figure2

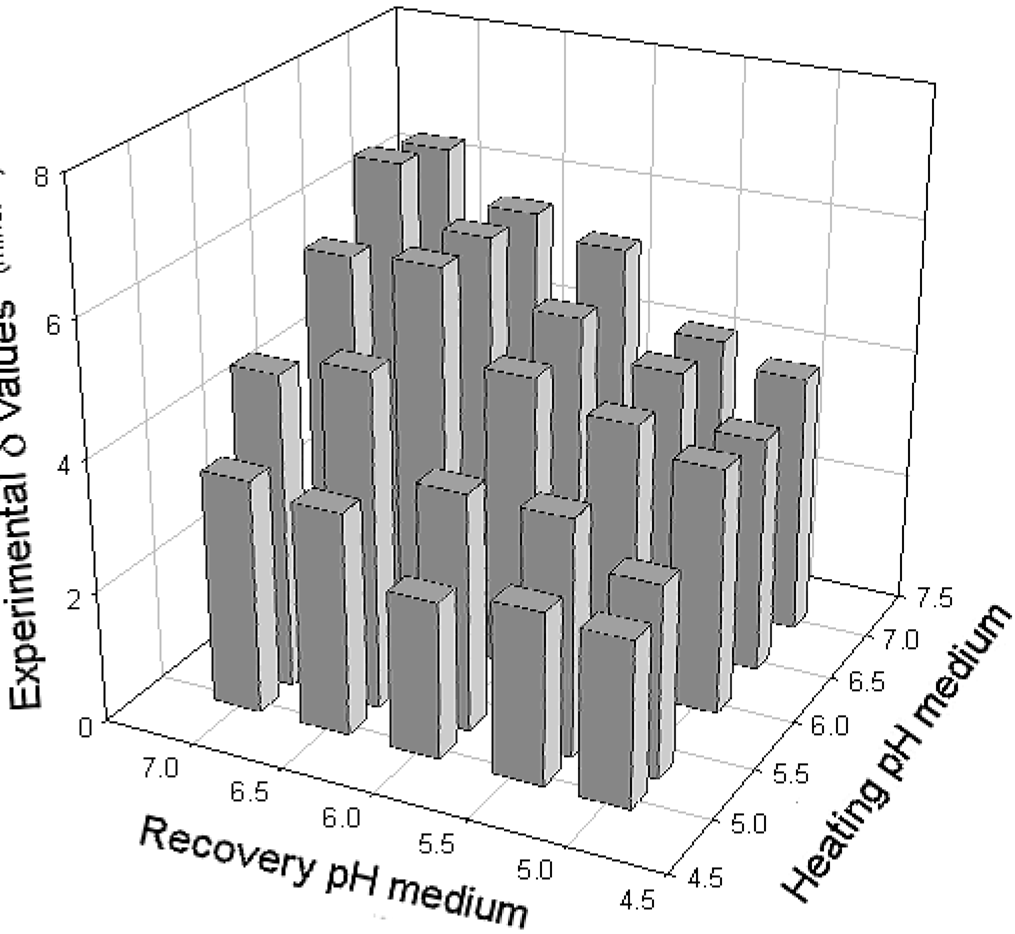
Experimental δ values (minutes)
0
2
4
6
8
Recovery pH medium
7.0
6.5
6.0
5.5
5.0
4.5
Heating pH medium
4.5
5.0
5.5
6.0
6.5
7.0
7.5

figure 3a

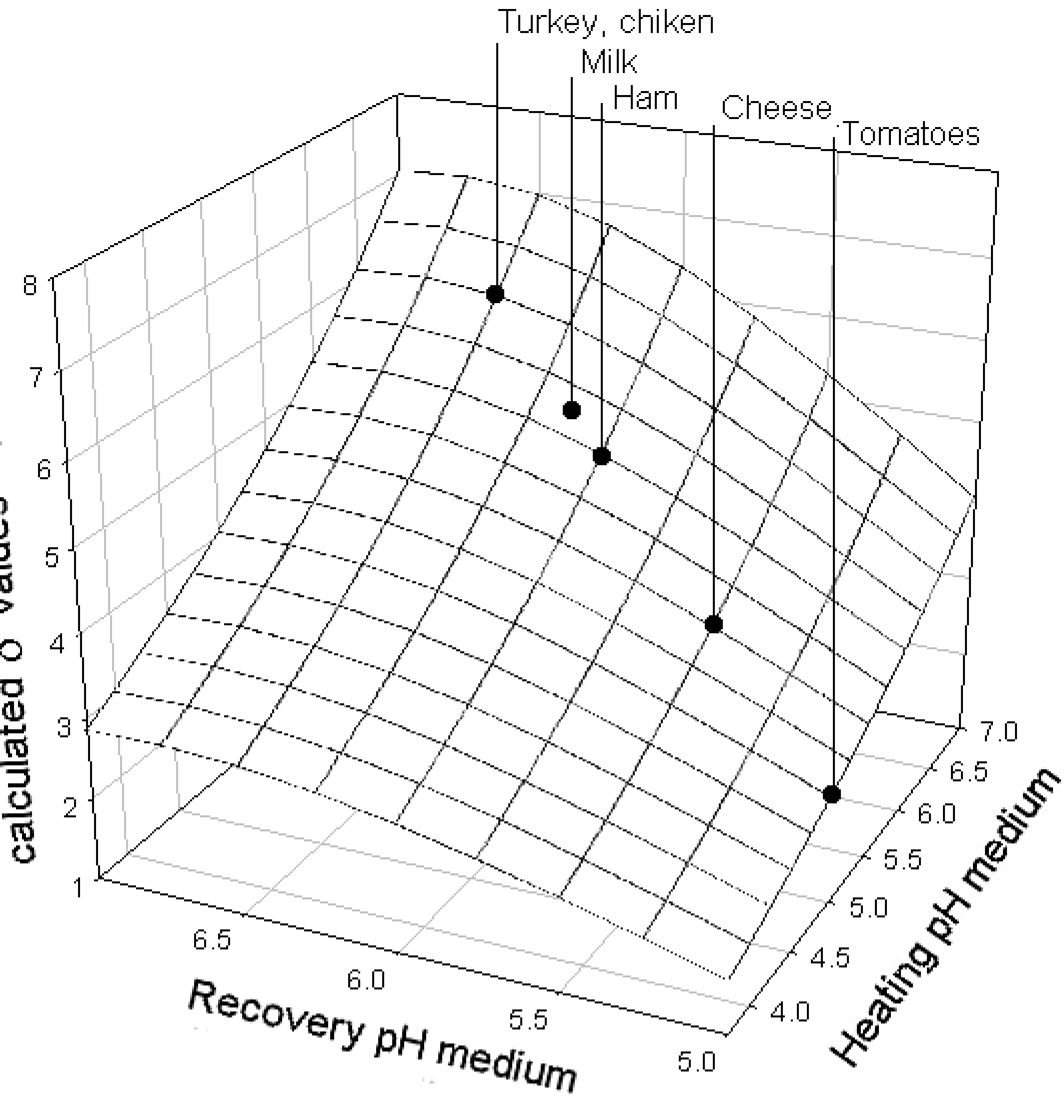
Turkey, chiken
Milk
Ham
Cheese
Tomatoes
calculated δ values
(minutes)
8
7
6
5
4
3
2
1
6.5
6.0
5.5
5.0
Recovery pH medium
7.0
6.5
6.0
5.5
5.0
4.5
4.0
Heating pH medium

Figure 3 b

Heating temperature °C	$\log N_0$	CI $_{95\%}$	δ minutes	CI $_{95\%}$
59	7.730	± 0.385	0.619	± 0.047
57	7.522	± 0.352	2.495	± 0.182
55	7.837	± 0.297	5.846	± 0.690
53	8.077	± 0.315	12.741	± 1.547

Table 1

		Global p value		1.677	±0.065
Heating pH	Recovery pH	logN_0	CI $_{95\%}$	δ minutes	CI $_{95\%}$
7.02	7	8.155	±0.244	6.734	±0.475
7.02	6.5	7.925	±0.244	6.922	±0.499
7.02	6	7.812	±0.257	6.010	±0.447
7.02	5.45	7.872	±0.281	4.949	±0.380
7.02	4.92	7.808	±0.302	3.696	±0.258
6.5	7	8.295	±0.257	5.956	±0.440
6.5	6.35	8.197	±0.257	6.059	±0.454
6.5	6	8.067	±0.257	6.178	±0.472
6.5	5.45	7.998	±0.281	5.063	±0.397
6.5	4.95	8.191	±0.321	3.276	±0.236
6	7	8.356	±0.270	5.657	±0.370
6	6.45	8.626	±0.281	5.041	±0.394
6	6	8.489	±0.284	4.683	±0.367
6	5.45	8.261	±0.323	3.760	±0.277
6	5	8.570	±0.352	2.483	±0.181
5.3	7	8.599	±0.283	4.458	±0.314
5.3	6.35	8.559	±0.282	4.506	±0.319
5.3	6	8.514	±0.299	4.273	±0.335
5.3	5.45	8.425	±0.320	3.513	±0.268
5.3	4.92	8.066	±0.336	2.731	±0.191
5	7	8.659	±0.300	4.107	±0.310
5	6.35	8.568	±0.302	3.728	±0.261
5	6	8.613	±0.319	3.857	±0.324
5	5.45	8.684	±0.322	3.047	±0.209
5	4.95	7.990	±0.356	2.628	±0.201
4.4	7	8.414	±0.321	3.171	±0.223
4.4	6.45	8.392	±0.321	3.338	±0.244
4.4	6	8.400	±0.322	3.114	±0.217
4.4	5.35	8.488	±0.352	2.458	±0.178
4.4	4.95	8.548	±0.394	1.836	±0.141
3.8	7	8.117	±0.322	3.170	±0.226
3.8	6.5	8.152	±0.351	2.722	±0.214
3.8	6	8.045	±0.398	2.281	±0.182
3.8	5.5	8.402	±0.394	1.935	±0.155
3.8	5	8.264	±0.455	1.611	±0.183

Table2

Bacterial strain		Heating medium	n*	T°C range	z_T°C	CI95 %	R^2
Salmonella typhimurium ATCC13311		Tryptone salt broth	4	51-59	4.64	±0.877	0.989
Salmonella typhimurium ATCC13311	Jäckle *et al*. 1987	Tryptone salt broth	3	58.5-61.5	8.55	±1.796	1.000
Salmonella typhimurium NCBI 10248	Mackey & Derrick 1986	Tryptone salt broth	5	50-59	3.44	±0.510	0.994

* n: data number

Table3

Bacterial strain		Heating medium	n	T°C range	z_T°C	CI 95%	pH range	z_{pH}	CI 95%	pH_{opt}	R^2
Salmonella typhimurium ATCC13311	Manas *et al*. 2003	Citrate buffer	8				4-7.7	3.27	0.969	5.75	0.939
Salmonella enteritidis P167807	Blackburn *et al*. 1997	Tryptone salt broth + HCl /NaOH	8				4.3-9.5	3.92	2.261	6.00	0.724
Salmonella typhimurium NCTC 74	Casadei *et al.* 2001	Nutrient broth + Citric acid	8	48-54	4.61	1.204	7 & 3	2.17	0.203	ND Fixed at 7	0.994

* n: data number

Table 4

	Parameter values	CI 95%
log $\delta^*_{55°C,\ pH7,\ pH'opt}$	0.851	±0.053
z_{pH}	8.254	±1.572
z'_{pH}	3.655	±1.349
pH'_{opt}	6.805	±0.656
R^2	0.958	

Table 5