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

4

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12 Abstract

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

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

24 **Nomenclature**

25  $N_0$ : initial number of cells .

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

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

28  $p$ : shape parameter.

29  $T$ : heating temperature.

30  $pH$ : heating medium pH.

31  $pH'$ : recovery medium pH.

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

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

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

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

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

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

42  $\delta^*$  :estimated  $\delta$  value corresponding to  $T^*$ ,  $pH^*$  and  $pH'_{opt}$  conditions.

43

44

45 **Introduction**

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

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

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

71

## 72 **Materials and methods**

### 73 *Strain and culturing conditions*

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

76 The basic heating medium was tryptone salt broth (10g/l tryptone USP (Biokar Diagnostics,  
77 A1401HA) and 10g/l NaCl). The pH was adjusted with addition of H<sub>2</sub>SO<sub>4</sub> and sterilised by  
78 filtration through 0.22µm porosity filter.

79 The recovery medium was nutrient agar (Biokar Diagnostics, BK021HA). The pH were  
80 adjusted with H<sub>2</sub>SO<sub>4</sub> prior autoclaving at 121°C for 15 minutes. The pH values were checked  
81 after autoclaving.

### 82 *Preparation of cells suspension*

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

### 87 *Thermal treatment of bacterial suspension and recovery conditions*

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

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

#### 95 *Experimental design*

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

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

#### 103 *Data from literature*

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

#### 106 *Primary and secondary models*

107 Different authors considered the survival curve as a cumulative form of temporary  
108 distribution of lethality event frequency (Cunhan, Oliveira & Oliveira, 1998; Peleg & Cole,  
109 1998; Fernandez, Salmeron, Fernandez & Martinez, 1999). In 2002, Mafart, Couvert,  
110 Gaillard, and Leguerinel proposed a new presentation of the Weibull frequency distribution  
111 model.

$$112 \log N = \log N_0 - \left( \frac{t}{\delta} \right)^p \text{ Eq1}$$

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

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

119 The influences of environmental factors on the Weibull model parameter estimated from  
120 survival kinetics related to bacterial spores or vegetative cells, were studied (Fernandez et al.,  
121 1999; Fernandez, Collado, Cunhan, Ocio & Martinez, 2002; van Boekel, 2002; Couvert,  
122 Gaillard, Savy, Mafart & Leguerinel 2005). These studies showed that the shape parameter  
123 was practically independent of the heating temperature and the pH of the heating medium.  
124 The conclusion of these studies leads us to determine a single average shape parameter value  
125 for a set of kinetics.

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

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

$$132 \log \delta = \log \delta^* - \left| \frac{pH - pH^*}{z_{pH}} \right| - \left( \frac{pH' - pH'_{opt}}{z'_{pH}} \right)^2 \text{ Eq 2}$$

133 *Curve fitting.*

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

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

139

140 Bigelow parameter  $z_T$  was estimated from scale parameter  $\delta$  values obtained from the  
141 temperature mono factorial design. Equation 2 parameter values:  $z_{pH}$  and  $z'_{pH}$  were estimated  
142 from scale parameter  $\delta$  values obtained from pH factorial design.

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

145

## 146 **Results and discussion**

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

151 The influences of the heating temperature, the pH of the heating and of the recovery medium  
152 on the shape parameter  $p$  are shown Figure 1.  $p$  values appear to be not clearly influenced by  
153 these environmental factors. This observation is in agreement with those of Fernandez *et al.*  
154 (2002), Collado, Fernandez, Rodrigo, Camats and Martinez Lopez. (2003) regarding *Bacillus*  
155 *cereus*, Couvert *et al.* (2005) ,and *Bacillus pumilus* and van Boekel's (2002). These authors  
156 did not observe any significant influence of the temperature on the shape parameter  $p$ . The  
157 presented results (Figure 1) show no clear influence of the heating medium or the recovery pH  
158 on estimates of  $p$  for the lower pH. However structural correlation between parameters  $p$  and  
159  $\delta$  could explain the variability of  $p$  values ( Couvert *et al.*, 2005).

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



165  $1.648 \pm 0.313$  (Jäckle *et al.*,1987),  $1.538 \pm 0.187$  (Garibaldi *et al.*, 1969),  $1.429 \pm 0.295$   
166 (Mackey & Derrick, 1986).  $\log N_0$  , the scale parameters  $\delta$  and their confidence interval  
167 coefficients as functions of different pH and temperature conditions, are presented in Table 1  
168 and 2. The scale parameter  $\delta$  values are influenced by environmental factors: heating  
169 temperature, pH of the heating and the recovery medium. Within the investigated temperature  
170 range (53°C -59°C) the classical Bigelow relationship was kept to quantify the effect of  
171 temperature on  $\delta$  value. The corresponding  $z_T$  value 4.64°C (Table 3) is lower than  $z_T$  values  
172 determined from Jäckle *et al.* (1987) data for the same bacterial strain but are in agreement  
173 with  $z_T$  value estimated from Mackey and Derrick data (1986) and with other values found in  
174 literature. Doyle and Mazzotta (2000) reviewed  $z_T$  values concerning different *Salmonella*  
175 *typhimurium* strains heated in different media. These  $z_T$  values, ranging from 3.24°C to 9.5°C  
176 with a mean of 5.56°C, illustrates the large variability of  $z_T$  values reported in literature. For  
177 the same *Salmonella typhimurium* strain ATCC13311 Casadei *et al.* (2001) reported a  $z_T$ -  
178 value of 4.6°C (Table 4).

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

186 Equation 2 was used to describe the effect of the heating medium pH and the recovery  
187 medium pH on  $\delta$  values. This model does not take interactions between these two  
188 environmental factors into account. To estimate the weight of the possible interaction, a  
189 variance analysis was performed. For the studied pH range, the weight of heating and

190 recovery medium pH represent 62.9% and 34.7% respectively, while the unexplained  
 191 variance comprising interactions represented only 2.4% of the total variability. This  
 192 observation concerning *Salmonella typhimurium* is in agreement with Couvert et al. (1999)  
 193 results related to *Bacillus cereus* spores. Such observations led us to neglect interactions and  
 194 to retain the simple Equation 2 without crossed term. Model parameters (Eq 2) determined for  
 195 *Salmonella typhimurium* (Table 5) were estimated from the whole set of  $\delta$  values, according  
 196 to factorial design.

197 The determination coefficient between experimental and calculated values ( $R^2$ :0.958) and  
 198 Figures 3a and 3b illustrate the goodness of fit of the model. The high  $z_{pH}$  parameter value  
 199 8.25, indicates a poor influence of the heating medium pH on the heat resistance for this  
 200 *Salmonella* strain. For other salmonella strains few data allow to evaluate this  $z_{pH}$  parameter.  
 201 From Blackburn et al. (1997), Casadei et al. (2001) and Mañas, Pagán, Raso and Condón  
 202 (2003) data, an optimal pH value appears to be variable and often lower than 7. This  
 203 observation leads to replace a fixed reference  $pH^*$ , equal to 7, by a variable optimal pH  
 204 parameter ( $pH_{opt}$ ) to be estimated Eq 3.

$$205 \quad \log \delta = \log \delta_{opt} - \left( \frac{T - T^*}{z_T} \right) - \left| \frac{pH - pH_{opt}}{z_{pH}} \right| \quad \text{Eq 3}$$

206 Parameters  $z_{pH}$ ,  $pH_{opt}$  and  $z_T$  estimated from published values are shown Table 4. Regarding  
 207 the studied bacterial strain and different bacterial species,  $z_{pH}$  values estimated from data of  
 208 literature and our own data are lower than  $z_{pH}$  obtained in this work, even for the same  
 209 *Salmonella typhimurium* strain ATCC 11331. This difference could be explained by errors on  
 210  $D$  value estimates due to the fitting of concave curves by linear regressions or growth  
 211 condition and physiology state of bacteria which can have produced stress protein.  
 212 Concerning the effect of the pH of the recovery medium, the  $z'_{pH}$  value (3.6) indicates a  
 213 higher influence of this factor than that of the heating medium pH. It is generally accepted

214 that the pH of the recovery medium exerts a large influence on the apparent heat resistance of  
215 spores:  $D$ -values decrease as pH is reduced (Cook and Brown, 1965; Yokoya and York, 1965;  
216 Cook and Gilbert, 1968; Mallidis and Scholefield, 1986; Santos and Zarzo, 1996; Lopez et al.,  
217 1997). Observed  $z'_{pH}$  values, which characterized the influence of the recovery medium pH,  
218 for *Salmonella typhimurium* cannot be compared with values regarding other *Salmonella*  
219 strains or species. As far as we know, no data which could be compared to our results, are  
220 available from literature.

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

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

233 This work confirms the impact of low recovery medium pH on the apparent heat resistance.  
234 This influence allows reducing heat treatments with the same safety objective to keep better  
235 nutritional and sensory quality of foods. This approach could be extended to other vegetative  
236 strains and species which would require further data:  $p$  and  $z$  parameter values, related to  
237 heating temperatures and heating and recovery medium pH. From these data, standard values  
238 of  $z_{pH}$  and  $z'_{pH}$  could be defined according to a similar approach to the one that was

239 implemented for the standard  $z_T$  value equal to 7°C for most resistant vegetative cells and  
240 input in pasteurisation process calculations.

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339 **Table legends**

340 Table 1

341  $\log N_0$  and  $\delta$  estimates and their confidence interval coefficients as functions of the heating  
342 temperature.

343

344 Table 2

345  $\log N_0$  and  $\delta$  estimates and their confidence interval coefficients as functions of the heating  
346 and the recovery medium pH.

347

348 Table 3

349 Parameters  $z_T$  estimated (from  $\log \delta$  values determined) from our own (data) and published  
350 data for different *Salmonella* strains

351

352 Table 4

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

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355 Table 5

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

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358 **Figure legends**

359 Figure 1

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

362

363 Figure 2

364 Survival kinetics experimental data and fitted curves, with p-value equal 1.67, at different  
365 heating medium pH for different recovery pH (pH 7 ●, pH 6.5 □, pH 6 ▲, pH 5.5 ▽, pH 5 ◆)  
366 and at different temperatures (59°C ●, 57°C □, 55°C ▲, 53°C ▽) heating and recovery pH  
367 fixed at 7

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369 Figure 3a 3b

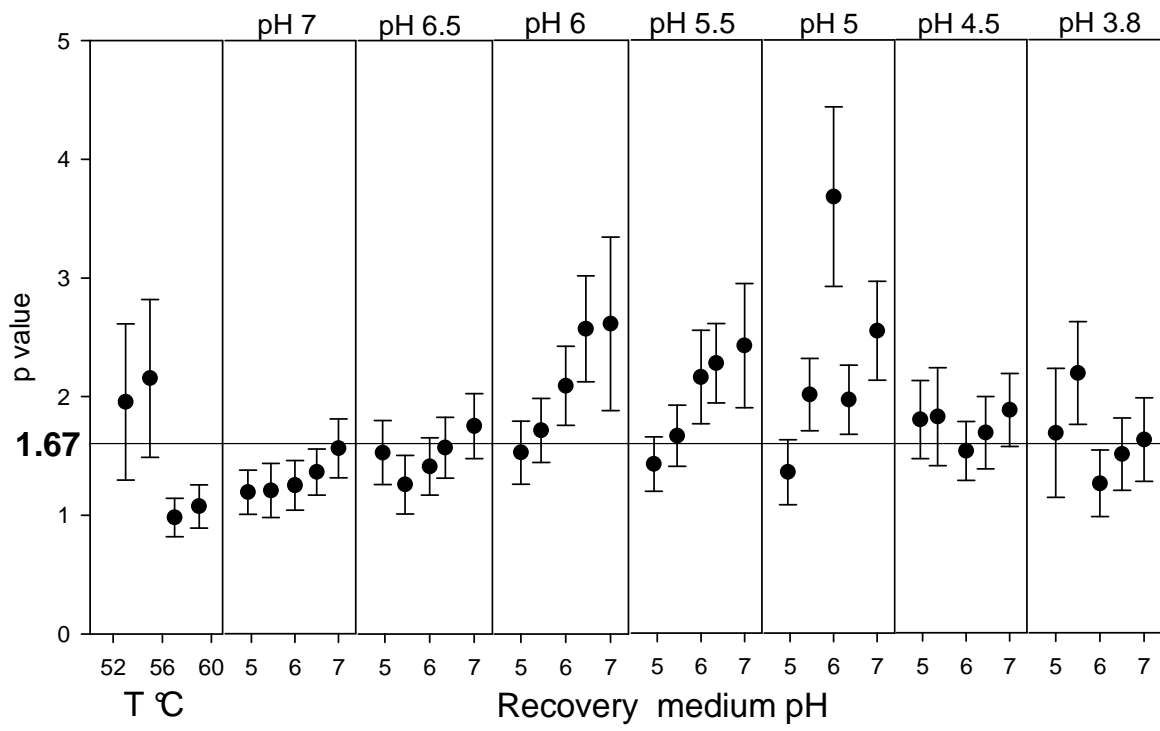
370 Observed and calculated  $\log \delta$  values for different conditions of heating and recovery medium  
371 pH

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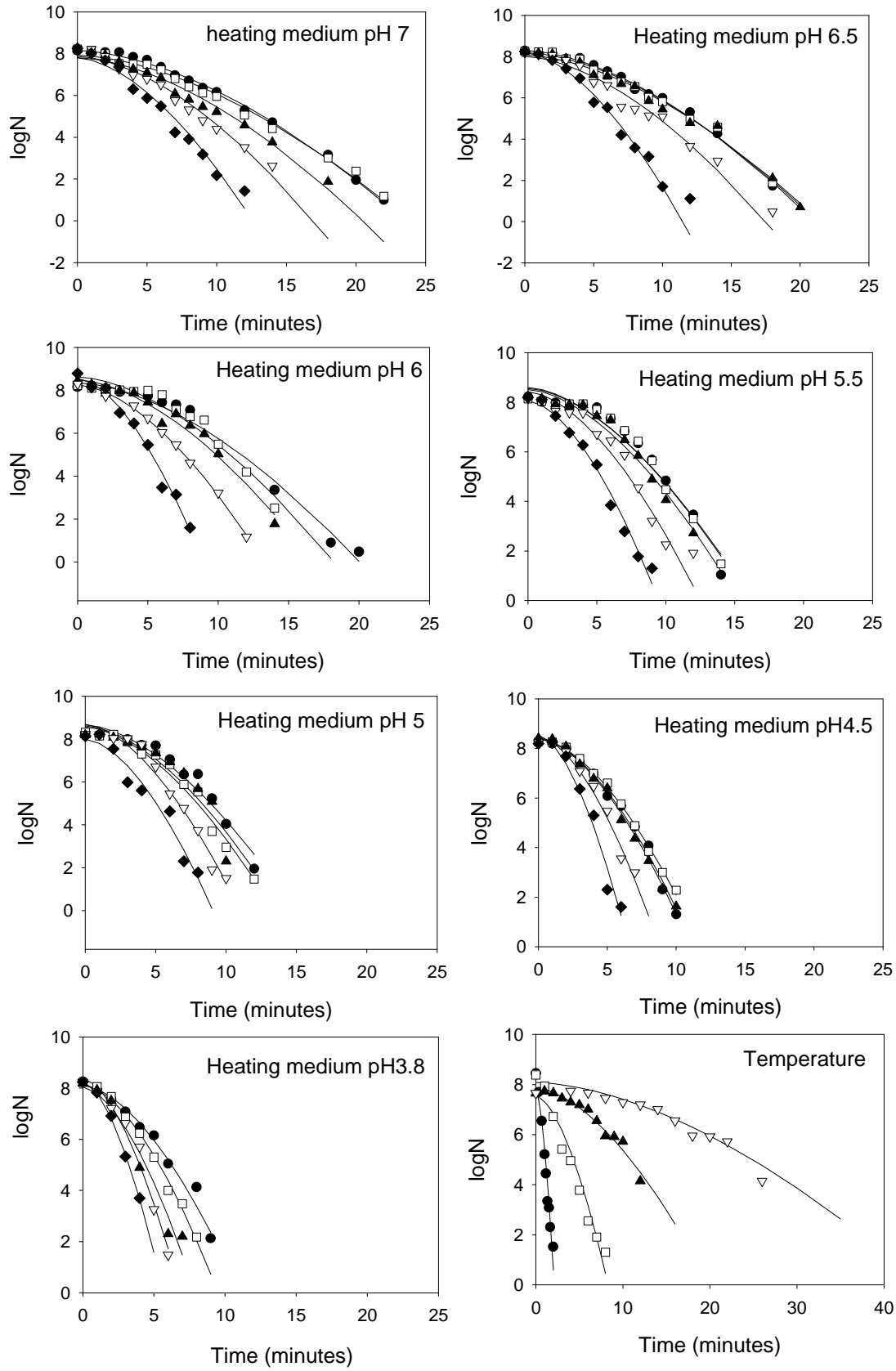


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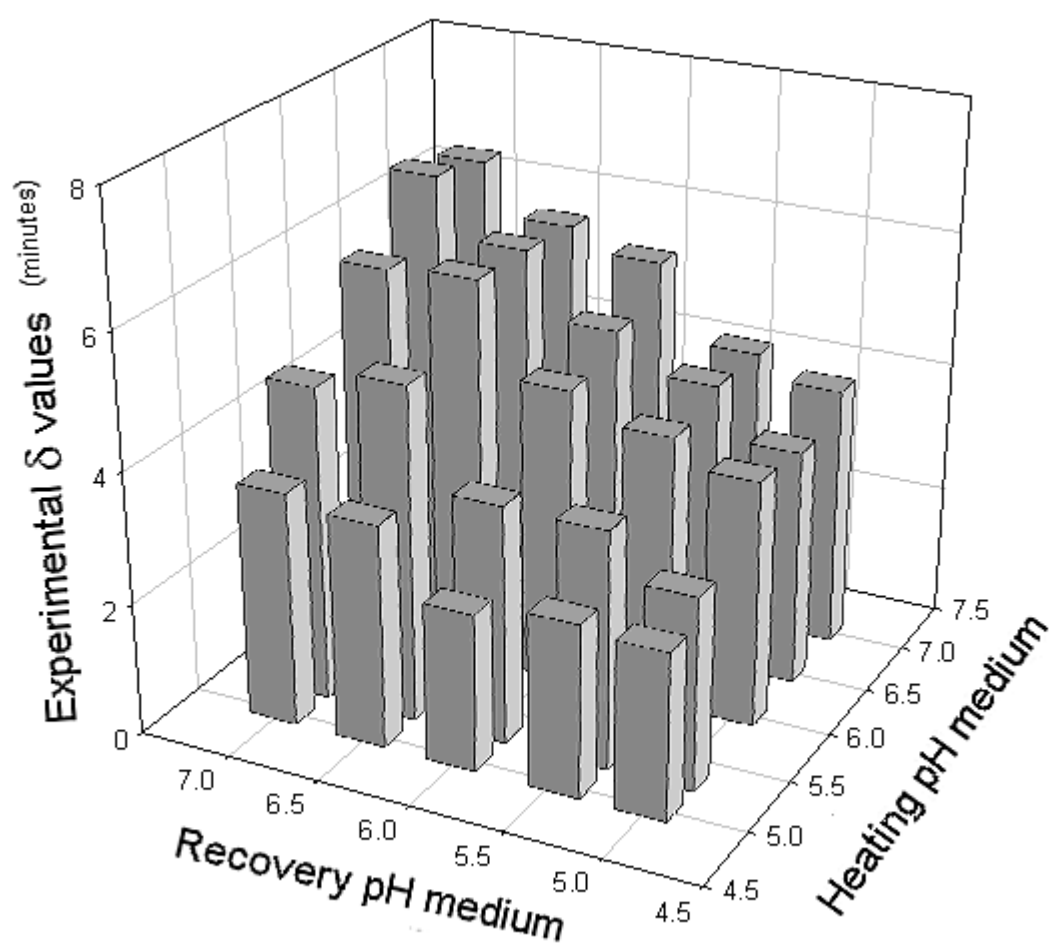
378 Figure 1

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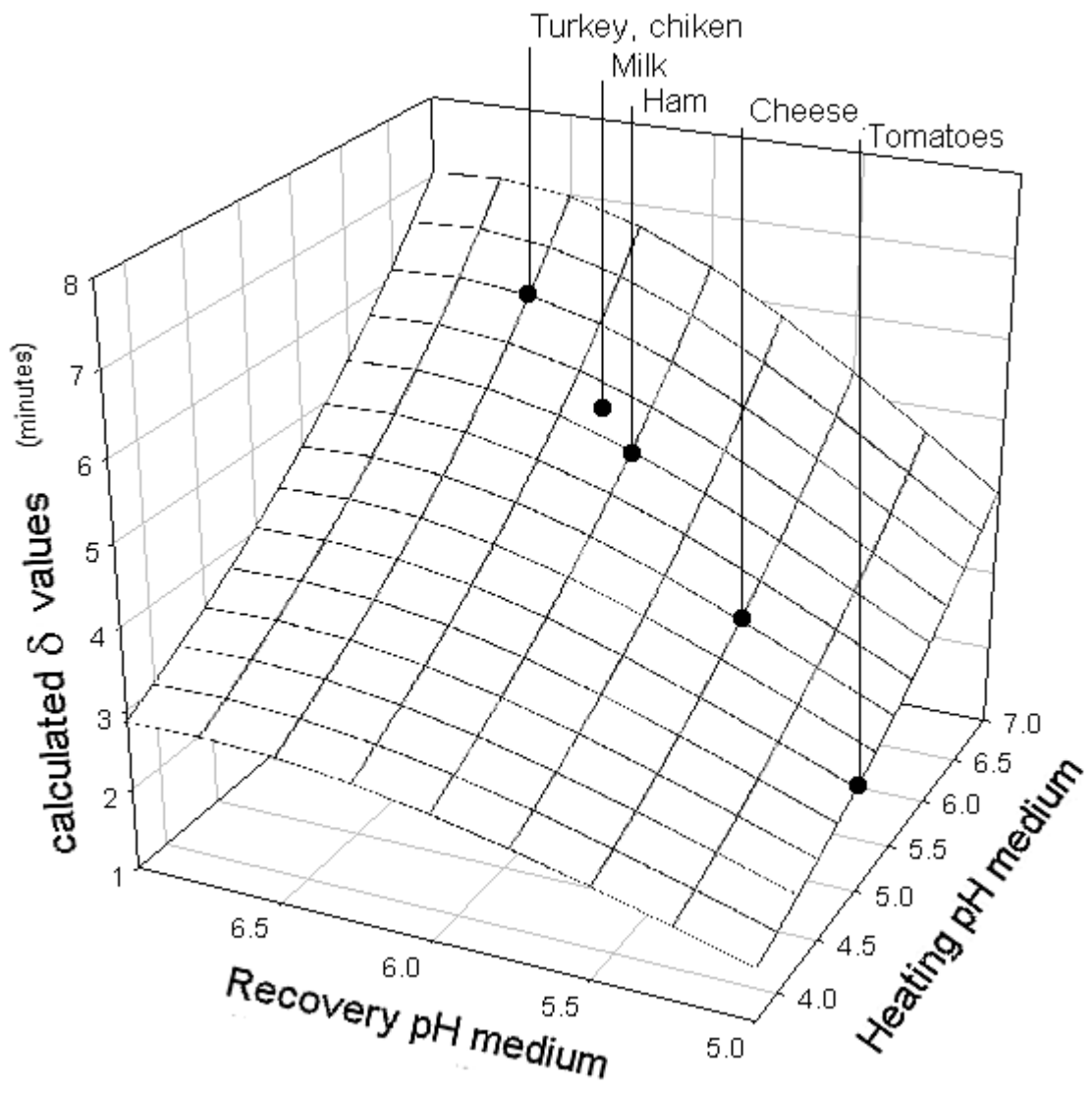


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381 Figure2



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383 figure 3a  
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 387 Figure 3 b  
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Heating temperature °C	$\log N_0$	CI <sub>95%</sub>	$\delta$ minutes	CI <sub>95%</sub>
59	7.730	$\pm 0.385$	0.619	$\pm 0.047$
57	7.522	$\pm 0.352$	2.495	$\pm 0.182$
55	7.837	$\pm 0.297$	5.846	$\pm 0.690$
53	8.077	$\pm 0.315$	12.741	$\pm 1.547$

392 Table 1

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Heating pH	Recovery pH	Global $p$ value		1.677 $\pm$ 0.065	
		$\log N_0$	CI <sub>95%</sub>	$\delta$ minutes	CI <sub>95%</sub>
7.02	7	8.155	$\pm$ 0.244	6.734	$\pm$ 0.475
7.02	6.5	7.925	$\pm$ 0.244	6.922	$\pm$ 0.499
7.02	6	7.812	$\pm$ 0.257	6.010	$\pm$ 0.447
7.02	5.45	7.872	$\pm$ 0.281	4.949	$\pm$ 0.380
7.02	4.92	7.808	$\pm$ 0.302	3.696	$\pm$ 0.258
6.5	7	8.295	$\pm$ 0.257	5.956	$\pm$ 0.440
6.5	6.35	8.197	$\pm$ 0.257	6.059	$\pm$ 0.454
6.5	6	8.067	$\pm$ 0.257	6.178	$\pm$ 0.472
6.5	5.45	7.998	$\pm$ 0.281	5.063	$\pm$ 0.397
6.5	4.95	8.191	$\pm$ 0.321	3.276	$\pm$ 0.236
6	7	8.356	$\pm$ 0.270	5.657	$\pm$ 0.370
6	6.45	8.626	$\pm$ 0.281	5.041	$\pm$ 0.394
6	6	8.489	$\pm$ 0.284	4.683	$\pm$ 0.367
6	5.45	8.261	$\pm$ 0.323	3.760	$\pm$ 0.277
6	5	8.570	$\pm$ 0.352	2.483	$\pm$ 0.181
5.3	7	8.599	$\pm$ 0.283	4.458	$\pm$ 0.314
5.3	6.35	8.559	$\pm$ 0.282	4.506	$\pm$ 0.319
5.3	6	8.514	$\pm$ 0.299	4.273	$\pm$ 0.335
5.3	5.45	8.425	$\pm$ 0.320	3.513	$\pm$ 0.268
5.3	4.92	8.066	$\pm$ 0.336	2.731	$\pm$ 0.191
5	7	8.659	$\pm$ 0.300	4.107	$\pm$ 0.310
5	6.35	8.568	$\pm$ 0.302	3.728	$\pm$ 0.261
5	6	8.613	$\pm$ 0.319	3.857	$\pm$ 0.324
5	5.45	8.684	$\pm$ 0.322	3.047	$\pm$ 0.209
5	4.95	7.990	$\pm$ 0.356	2.628	$\pm$ 0.201
4.4	7	8.414	$\pm$ 0.321	3.171	$\pm$ 0.223
4.4	6.45	8.392	$\pm$ 0.321	3.338	$\pm$ 0.244
4.4	6	8.400	$\pm$ 0.322	3.114	$\pm$ 0.217
4.4	5.35	8.488	$\pm$ 0.352	2.458	$\pm$ 0.178
4.4	4.95	8.548	$\pm$ 0.394	1.836	$\pm$ 0.141
3.8	7	8.117	$\pm$ 0.322	3.170	$\pm$ 0.226
3.8	6.5	8.152	$\pm$ 0.351	2.722	$\pm$ 0.214
3.8	6	8.045	$\pm$ 0.398	2.281	$\pm$ 0.182
3.8	5.5	8.402	$\pm$ 0.394	1.935	$\pm$ 0.155
3.8	5	8.264	$\pm$ 0.455	1.611	$\pm$ 0.183

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401 Table2



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

403 \* n: data number

404 Table3

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

414 \* n: data number

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417 Table 4

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

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Table 5