Thermal resistance of *Salmonella enterica, Escherichia coli* and *Staphylococcus aureus* isolated from vegetable feed ingredients

Isabel R. Amado[ab*], Jose A. Vázquez[b], Nelson P. Guerra[a] and Lorenzo Pastrana[a]

[a]Departamento de Química Analítica y Alimentaria, Facultad de Ciencias de Ourense (Universidad de Vigo), Campus As Lagoas s/n, Ourense, Spain

[b]Grupo de Reciclado y Valorización de Materiales Residuales (REVAL), Instituto de Investigaciones Marinas (IIM-CSIC), Eduardo Cabello, 6. Vigo (Pontevedra), Spain

[*]Corresponding author: Isabel Rodríguez Amado
Tel.:+34986214469/31930; fax:+34986292762
E-mail address: sabelara@uvigo.es (Isabel Rodríguez Amado)
Present address: [a]Departamento de Química Analítica y Alimentaria, Facultad de Ciencias de Ourense (Universidad de Vigo), Campus As Lagoas s/n, Ourense, Spain

Running title: Foodborne pathogen inactivation in feed

Abstract

BACKGROUND: Cattle feed is at the beginning of the food chain in the "farm-to-fork" model and might serve as a source of contamination with pathogenic bacteria. Heat treatments are one of the most effective methods utilized to ensure the microbial safety of feeds. In this work, the thermal resistance of *Salmonella enterica*, *Escherichia coli* and *Staphylococcus aureus* isolated from vegetable feed ingredients was investigated in phosphate buffer saline (PBS) and in cattle feed.

RESULTS: Mean *D* values calculated in PBS ranged from 34.08 to 5.70 min at 55ºC decreasing to 0.37 and 0.22 min at 65ºC for *E. coli* and *S. enterica*, respectively. No relationship was found between thermoresistance and source of isolation. *D* values in feed were calculated from the adjustment of two nonlinear models to the inactivation data. Thermal resistance of *E. coli* and *S. enterica* in cattle feed showed similar results to liquid medium however, a 5-fold increment of *S. aureus* thermoresistance in feed was observed. Our results also revealed an increase of microbial thermoresistance with the mean feed particle diameter.

CONCLUSION: These results provide relevant information for the improvement in the safety of cattle feed regarding its process conditions (*i.e.* time, temperature and particle size).

Keywords: food safety; thermal processing; foodborne microorganism; cattle feed; mathematical modeling.

Abbreviations: AIC, Akaike's information criterion; LIA, Lysine Iron Agar; PBS, phosphate buffer saline; TSB, tryptic soy broth; TSI, Triple Sugar Iron Agar; XLD, xylose lysine deoxycholate.

INTRODUCTION

Despite the development of new food processing technologies, microbial contamination of feed continues to be a global concern since it is at the beginning of the food chain in the "farm-to-fork" model. [1] Contamination with pathogenic bacteria in the animal production industry has been linked to the consumption of contaminated feed, being considered a vehicle for the transmission of pathogens, some of great health significance for humans such as *Salmonella enterica* or *Escherichia coli*, including *E. coli* O157:H7. [2] One of the sources of such contamination is feed ingredients, which are susceptible to contamination by pathogens at several stages from the growth and harvesting to transport and storage. [3]

Among the wide number of preservation methods available to reduce the microbial contamination of feeds, heat treatments are one of the most effective methods utilized. Although the effectiveness of heat treatments is usually high, the resulting pelleted feeds are sensitive to post-processing recontamination. [4] In this regard, Bucher et al. [5] suggested that the most thermoresistant *Salmonella* strains could survive the heating process during feed pelleting. Besides, several studies have reported increased microbial thermal resistance due to adverse environmental conditions such as low a_w, [6,7] acidity [8] and even food structure. [9]

Therefore, a critical point to ensure the microbial safety of feeds is defining a heat treatment designed to achieve a specific lethality of target microorganisms. Nevertheless, few published papers deal with microbial heat resistance in animal feed,

105 [7,10-12] since its low a_w limits the proliferation of remaining bacteria after heat treatment.
106 [12]

107 Finally, different experimental conditions referred in the literature to quantify decimal
108 reduction times make it difficult to compare the effectiveness of heat treatments,
109 particularly because frequent deviations from the classical semi-logarithmic linear
110 behavior (presence of shoulders and tail-effects) are widely reported in the literature. [13]
111 The aim of this study was to characterize the thermal inactivation of *Salmonella*, *E. coli*
112 and *S. aureus* isolated from cereals and vegetable thermally-treated feed ingredients in
113 feed. For this purpose, thermal inactivation kinetics of 21 isolates of *Salmonella*, *E. coli*
114 and *S. aureus* were carried out in liquid medium (PBS). The effect of feed matrix was
115 also studied with selected isolates. The linear model and two nonlinear models (biphasic
116 linear and biphasic logistic) were fitted to survival curves, comparing their goodness-of-
117 fit and predicted parameters.
118

119 # MATERIALS AND METHODS

120 **Bacterial isolates and culture conditions**

121 Bacteria were isolated in our laboratory from vegetable feed ingredients (Table 1).
122 Detection and isolation were performed using the ISO methods for *Salmonella* spp.
123 (ISO 6579: 2002), *E. coli* (ISO 4831:2006 and ISO 4832:2006) and coagulase-positive
124 *Staphylococcus* (ISO 6888-1:1999) detection in food and animal feed. Biochemical
125 confirmative tests were performed following preliminary identification based on colony
126 morphology on selective media. Isolates were preserved as frozen stocks at −80°C in
127 Tryptic Soy Broth (Cultimed Panreac Química S.A., Barcelona, Spain), containing 300
128 μL ml^{-1} of glycerol, and propagated twice in appropriate media before use. All cultures
129 were grown in 250 ml Erlenmeyer flasks containing 50 ml of TSB on a rotary shaker, at

37°C for 24 h.

Thermal inactivation in liquid medium

Cells were harvested by centrifugation (13000 *g*, 10 min, 4ºC), washed twice in 8 g L^{-1} sterile buffered saline solution (PBS) and suspended in 5 mL of PBS. A sample of working bacterial suspension (50 µl) was dispensed in glass capillary tubes (Micro haematocrit capillary 1.15×75mm, BRAND GmBH, Germany) in duplicate. Tubes were heat sealed and immediately incubated in a thermostatically controlled water bath at 55, 57.5, 60, 62.5 and 65ºC. At each sampling time, samples were removed, immediately cooled and sanitized with 100 mL L^{-1} sodium hypochlorite. After rinsing, the content of each capillary tube was diluted with PBS, obtaining the count suspension (S_c). Then, 0.1 mL of appropriate dilutions of S_c was plated, in duplicate, using the following media: Levine (*E. coli*), Xylose lysine deoxycholate (*Salmonella*) and Baird-Parker (*S. aureus*), purchased from Cultimed Panreac Química S.A. (Barcelona, Spain). Plates were aerobically incubated at 37ºC for 48 h, and colonies were counted and recorded as numbers of cfu mL^{-1}.

Preparation of contaminated feed

The composition of the antibiotic and acid-free pelleted cattle feed utilized is shown in Table 2. The feed was previously milled using a laboratory batch mill (IKA-Werke GmbH & Co. KG, Staufen, Germany) and sterilized by autoclaving. Cultures were centrifuged (13000 *g*, 10 min, 4 ºC), cells resuspended in PBS and added (20 mL kg^{-1}) to the feed at a concentration of approximately 1×10^5 cfu g^{-1} in case of *Salmonella* and 1×10^7 cfu g^{-1} for *E. coli* and *S. aureus* isolates. Cultures were sprayed and then agitated end-over-end in a 1.5 L plastic beaker for 4 minutes, as previously optimized in

155 our laboratory.

156

157 **Thermal inactivation in cattle feed**

158 For thermal inactivation experiments, one gram of acidified feed was used to fill
159 devices specifically designed to perform the kinetics. [14] These devices (3 mm thick and
160 45 mm of internal diameter) consisting of a flat rubber O-ring completely sealing two
161 aluminum layers, were submerged in a thermostatically controlled water bath at the
162 same temperatures assayed in PBS. After heat challenges, the procedure was identical to
163 that described in the previous section, determining the number of surviving bacteria (cfu
164 g^{-1}) in the contaminated feed after incubation at 37ºC for 48 h. Experiments were
165 performed in triplicate.

166 To analyze the effect that cattle feed structure had on bacterial survival, inactivation
167 kinetics were carried out at 60ºC, using feed with different particle diameters (mm):
168 $1<\varphi<2$, $0.5<\varphi<1$ and $\varphi<0.5$.

169

170 **Mathematical modeling**

171 *Survival kinetics in PBS*

172 Survival data were transformed onto their base-10 logarithms (log (cfu mL^{-1})) and a
173 linear equation was fitted to the time course of surviving bacteria:

174

175 $$\log N(t) = \log N_0 - \frac{t}{D} \qquad [1]$$

176

177 where, N_0 and $N(t)$ are the initial and final number of cells (cfu mL^{-1}) after a treatment
178 time of t (min), respectively. D is the decimal reduction time (min).

179 The decimal reduction temperature (z_D) or the temperature increase required to reduce

the D value in one logarithm unit, was obtained using the following linear relationship: [15]

$$\log D = \alpha - \frac{T}{z_D} \qquad [2]$$

where, α is the intercept and T is the temperature (ºC).

Survival kinetics in cattle feed

Two types of equations were used to fit the survival kinetics in cattle feed:

i) a biphasic linear model proposed by Cerf & Metro [16] considering a heat-sensitive and a heat-resistant population and formulated based on the equation of Den Besten et al.: [17]

$$\log N_T(t) = \log N_{T_0} + \log\left[\left(1-f\right)e^{\frac{-2.3t}{D_1}} + fe^{\frac{-2.3t}{D_2}}\right] \qquad [3]$$

where, N_{T0} is the initial number of cells (cfu g^{-1}), N_T is the number of survivors (cfu g^{-1}) after a treatment time of t (min) and f is the fraction of bacteria in the subpopulation –*2*–. D_1 and D_2 are the decimal reduction times (min) of the two subpopulations, respectively. When the value of log N_{T0} is reduced in one logarithmic unit, then t is equal to D and so, the D value can be estimated by means of numerical optimization, after substituting in equation [3] the values of f, D_1 and D_2 previously calculated by nonlinear regression.

ii) a biphasic logistic model, describing survival profiles of two distinct subpopulations with different specific mortality rates. The equation described for biphasic survival curves by Kamau et al. [18] was utilized in the form of Xiong et al., [19] parameterized to have explicit D_1 and D_2:

$$\log N_T(t) = \log N_{T_0} + \log\left[\frac{2f}{1+e^{\left(\frac{2.3t}{D_2}\right)}} + \frac{2(1-f)}{1+e^{\left(\frac{2.3t}{D_1}\right)}}\right] \quad [4]$$

where, N_{T0} is the initial number of cells (cfu g^{-1}), N_T is the number of survivors (cfu g^{-1}) after a treatment time of t (min) and f, D_1 and D_2 have the same meaning as described above. The D_T value can be estimated by means of numerical optimization, as previously described, after substituting in equation [4] the values of f, D_1 and D_2 calculated by nonlinear regression.

Numerical and statistical analysis

Fitting procedures and parametric estimations were carried out by minimizing the sum of quadratic differences between observed and model predicted values using the nonlinear least-squares (quasi-Newton) method provided by the *Solver* macro of the Microsoft Excel 2007 spreadsheet (Microsoft, Redmond, WA). Confidence intervals from the parametric estimates (Student's *t*-test) and consistence of mathematical models (Fisher's *F* test) were evaluated using DataFit 9 (Oakdale Engineering, Oakdale, PA). Also the Akaike´s information criterion (AIC) was also used for equation comparison. [20,21]

A one-way analysis of variance (ANOVA) with the Tukey post hoc test (P = 0.05) was used to determine whether there were significant differences between *D* and *zD* mean

227 values. Statistical analysis was performed using the general linear model (GLM)
228 procedure of the software package IBM® SPSS® Statistics 20 for Windows (Release
229 20.0.0, IBM SPSS Inc., Armonk, NY, 2011).
230
231 # RESULTS
232 **Thermal inactivation in liquid medium**
233 Survival curves of *Salmonella*, *E. coli* and *S. aureus* in PBS at different temperatures
234 are shown in Figure 1. Due to the linear behavior of the logarithmic representation of
235 the counts, equation [1] acceptably fitted the data (R^2>0.9). As expected, *D* values
236 decreased with increasing temperature (Table 3). Microbial viability fell at temperatures
237 above 57.5°C, however after 2 min of heat treatment, drops of viability varied from 1
238 log-unit at 55°C and 57.5ºC to reductions of 4-5 log-units (*Salmonella*) and 2-3 log-
239 units (*E. coli* and *S. aureus)* at the highest temperatures assayed.
240
241 **Thermal inactivation in cattle feed**
242 Isolates showing the highest *D* values among assayed temperatures (*slSAL-1*, *ecSJ4-2*
243 and *stSAL-7*) were selected to carry out survival kinetics in cattle feed. Equation [1] was
244 used for modeling the semi-logarithmic plots of the counts (Figure 2). Results showed
245 thermal resistance decreased in the order *S. aureus* > *E. coli* > *Salmonella*, although the
246 time required for reducing the viability equivalently was rather different. After 5 min of
247 heating at 55°C and 57.5ºC, *Salmonella* and *E. coli* counts were reduced in 1 log-unit,
248 while 2 and 1 h were necessary to ensure consistent reductions of *S. aureus* counts at
249 those temperatures. At 65ºC, heating for 2 or 5 min resulted in around 2 log-units
250 reductions in *Salmonella* and *E. coli* numbers, respectively. By contrast, at this
251 temperature, 30 min of heat treatment were required to achieve reductions of 4 log-units

252 in *S. aureus* counts. Decimal reduction times (*D*) from [1] were calculated using the
253 linear portion of the inactivation curves (Figure 2). Generally, *D* values were higher
254 than those observed in PBS, with differences particularly relevant for *stSAL-7*.
255 Although calculating *D* values from the linear portion of the semi-logarithmic plots of
256 survival curves is a common practice in thermobacteriology, non-linear models must be
257 applied to correctly describe tailing curves. In the present study two equations
258 commonly used to describe biphasic profiles, the Cerf model [3] [16] and the Kamau
259 model [4], [18] were compared using the logarithmic counts as survival response. Figure 3
260 shows the experimental results and descriptions according to both equations. Parameter
261 estimates and statistical analysis are also listed in Table 4. The results showed that both
262 equations were statistically robust ($p < 0.01$ from Fisher's F test) and parameter
263 estimations were almost always significant (Student's t test, $\alpha = 0.05$). Besides, all the
264 adjusted coefficients of multiple determination between predicted and observed values
265 were higher than 0.97. Comparison of the r^2 and Akaike's information criterion (data
266 not shown) indicated that both models adequately described the inactivation data in
267 cattle feed, though differences were found for each species. For most of the
268 experimental conditions, the Kamau model was most likely to be correct for fitting
269 experimental data (probability higher than 65%) of *Salmonella* and *S. aureus* isolates.
270 While for *E. coli* isolate, the Cerf model described better the inactivation data, with a
271 probability higher than 65% at all temperatures tested.
272 As can be seen in Table 4, *D* values calculated from equations [3] and [4] show very
273 close values due to the suitability of both models to describe the experimental data. In
274 addition, thermoresistance of *stSAL-7* was clearly higher than that observed for *E. coli*
275 and *Salmonella* isolates. Specifically, $D_{55.0}$ values increased from 12 min in PBS to
276 more than 2 h for the thermoresistant subpopulation, *i.e.* nearly a 9-fold increment of

277 microbial viability in cattle feed.

278

279 **Effect of feed structure in thermal inactivation**

280 To assess the effect of cattle feed structure on bacterial survival, inactivation kinetics
281 were carried out at 60°C using feed with different particle size (mm): $1<\varphi<2$, $0.5<\varphi<1$
282 and $\varphi<0.5$. Our results showed that particle size influenced the specific mortality rate
283 with an increase of microbial thermoresistance (*D* values) with the mean feed particle
284 diameter (Figure 4). *E. coli* $D_{60.0}$ values increased from 4 min in fine feed particles
285 (<0.5 mm) to 10 min in coarser feed ($1<\varphi<2$ mm), *i.e*. a 2.5-fold increment of microbial
286 viability. A lesser effect was observed for *Salmonella* and *S. aureus*, showing in both
287 cases a 1.6-fold greater $D_{60.0}$ values in feed with larger particle size.

288

289 # DISCUSSION

290

291 Bacteria isolation sources shown in Table 1 include cereals and thermally treated
292 ingredients (soybean meals, wheat bran and corn distillers dried grains with solubles).
293 This selection followed a double goal, to include microbial indicators of good
294 manufacturing practices and to investigate whether heat treatment influenced the
295 thermoresistance of isolates from processed ingredients.

296 The average *D* values of *Salmonella* isolates in PBS (Table 3) were similar to
297 previously reported for multiantimicrobial-resistant strains in TSB [22] and slightly higher
298 than those of *Salmonella* Enteritidis and Typhimurium in PBS. [23] Lower $D_{55.0}$ values
299 were obtained than those reported by Stopforth et al. [24] in peptone water, while higher
300 thermoresistances at 60 and 65ºC were observed in the present study. Otherwise, the
301 resistance of *E. coli* isolates used in this work (57.5ºC) was similar to that described by
302 Buchanan & Edelson [25] for three strains of *E. coli* O157:H7 in TSB at 58ºC. Gabriel &

303 Nakano [26] reported significantly lower $D_{55.0}$ values for *E. coli* O157:H7 and *E. coli* K-
304 12 in PBS. Except for *ecSJ4-2*, $D_{55.0}$ values were among those reported (2.6 and 21.5
305 min) for 17 different strains of *E. coli* O157:H7 in BHI broth. [27] Nevertheless, at 60ºC,
306 higher thermoresistance was observed in the isolates assayed in the present work, since
307 the highest $D_{60.0}$ value reported by these authors was 2.1 min. Thermal resistance data of
308 *S. aureus* available in the bibliography are not as abundant as *D* values of *Salmonella*
309 and *E. coli*. In general, *D* values obtained in this study were lower than those previously
310 reported for *S. aureus* in TSB [28]. These authors reported D_{55} and D_{60} values ranged from
311 13.7 to 21 min and 4.8 to 6.5 min after direct selective plating onto Baird-Parker agar.
312 The average z_D values obtained in this study ranged from 7ºC to 14ºC (Table 3), being
313 greater than those reported by Juneja & Eblen [29] for *Salmonella* in chicken broth at
314 temperatures ranging from 58 to 62ºC and by Bacon et al. [22] in TSB. On the other hand,
315 Buchanan & Edelson [25] reported a decimal reduction temperature of 4.3ºC for *E. coli*
316 O157:H7 in TSB (56-62ºC).
317 Differences in *D* and z_D values reported in this study compared to those described in the
318 literature can be due to variations in experimental conditions, both in terms of strain and
319 medium in which the thermoresistance is studied (*i.e.*, pH, a_w), conditions of microbial
320 growth, etc. [30] Besides, from our results no relationship was found between
321 thermoresistance and source of isolation. So, we cannot conclude that isolates from
322 thermally processed ingredients are a selection of the most heat-resistant
323 microorganisms, as suggested by some authors. [5,13]
324 Thermal inactivation curves of isolates showing the highest *D* values in PBS (slSAL-1,
325 ecSJ4-2 and stSAL-7) had a tailing effect in cattle feed at all temperatures (Figure 2).
326 Profiles with tailing effects and lag phases have been widely reported for thermal
327 inactivation kinetics. [31,32] Different causes can explain non-linear kinetic data, including

328 the need of certain damage before inactivation follows a first order kinetics [33] or the
329 presence of subpopulations with different death mechanisms or different sensitivities to
330 heat. [16] Also this tailing effect was reported to be a consequence of using dry heat in
331 thermal inactivation studies of *Escherichia coli* O157:H7 in cattle feeds. [12]
332 In these cases, *D* values were obtained from the adjustment of two nonlinear models
333 (biphasic linear and biphasic logistic) to the inactivation data (Figure 3). Both biphasic
334 equations accurately described the tailing-survival curves (Table 4), suggesting the
335 existence of two subpopulations with different thermoresistance. In fact, *S. aureus*
336 isolate showed a markedly tailing behavior at 57.5, 60 and 62.5ºC (Figure 3), indicating
337 the presence of a highly heat-resistant subpopulation. Although this group of cells is a
338 minor fraction of the population (Table 4), might be responsible of the enhanced
339 thermoresistance observed in cattle feed. *D* values calculated using this approach were
340 comparable to results reported by Hutchison et al., [12] who found reductions of 2 log
341 units of a mixture of *E. coli* O157 after thermal treatment at 70ºC for 2 min in cattle
342 feed.
343 As mentioned in the introduction, other factors like a_w, acidity and structure of foods
344 influence the heat resistance of foodborne microorganisms. In regards to feed, Liu et al.
345 [7] reported greater thermal resistance (52-85ºC) of *Salmonella* Senftenberg 775W in dry
346 feeds with lower moisture content. However, despite being particle size a relevant
347 variable in feed pelletization technology and on the thermal resistance of foodborne
348 microorganisms, [9] to our knowledge, its effect on microbial heat inactivation parameters
349 has not been investigated.
350 Laroche et al. [6] observed a significant effect of food powders size on the heat resistance
351 of *Saccharomycces cerevisiae*. These authors attributed the higher thermal resistance to
352 an increase in the time required for the diffusion of heat into the food particles, reducing

353 the temperature at which cells are exposed and increasing the time or temperature
354 needed to achieve an equivalent level of decontamination. Likewise, the protective
355 effect of feed observed on the thermal resistance of *Salmonella*, *E. coli* and *S. aureus*
356 can be due the lower heat conductivity into the feed particle when the mean diameter is
357 increased. In general, larger particle size has yielded higher microbial heat resistance in
358 solid food matrices as diverse as wheat flour [34] and meats such as beef [35] and turkey. [9]
359

360 ## CONCLUSIONS

361 This study focused on the characterization of *Salmonella enterica*, *Escherichia coli* and
362 *Staphylococcus aureus* thermal resistance in liquid medium (PBS) and in cattle feed.
363 The bacteria utilized in the present work were isolated in our laboratory from cereals
364 and thermally treated ingredients. Mean *D* values calculated in PBS ranged from 34.08
365 to 5.70 min at 55ºC decreasing to 0.37 and 0.22 min at 65ºC for *E. coli* and *S. enterica*,
366 respectively. Furthermore, from our results we found no association between the
367 thermoresistance and the source of isolation, suggesting that isolates from thermally
368 processed ingredients are not a selection of the most heat-resistant microorganisms.
369 Thermal inactivation curves of isolates showing the highest *D* values in PBS had a
370 tailing effect in cattle feed at all temperatures and so, *D* values were calculated from the
371 adjustment of two nonlinear models to the inactivation data. According to this approach,
372 thermal resistance of *E. coli* and *S. enterica* in cattle feed showed similar results to PBS,
373 however, a 5-fold increment was observed for *S. aureus D* values. Our results also
374 revealed an increase of microbial thermoresistance with the mean feed particle diameter.
375 Overall, these results provide relevant information for the improvement in the safety of
376 cattle feed regarding its process conditions (*i.e.* time, temperature and particle size).
377

ACKNOWLEDGMENTS

This research was financially supported by the Xunta de Galicia, Spain (project PGIDIT03TAL07E). Isabel Rodríguez was funded by a PhD fellowship from the Plan Galego de Investigación, Desenvolvemento e Innovación Tecnolóxica-Incite (2006-2010) (Xunta de Galicia). We thank COREN, S.C.L. for their collaboration in the elaboration of this work.

REFERENCES

1. Crump JA, Griffin PM and Angulo FJ, Bacterial contamination of animal feed and its relationship to human foodborne illness. *Clin Infect Dis* **35**:859-865 (2002).

2. Davis MA, Hancock DD, Rice DH, Call DR, DiGiacomo R., Samadpour M, *et al*, Feedstuffs as a vehicle of cattle exposure to *Escherichia coli* O157:H7 and *Salmonella* enterica. *Vet Microbiol* **95**:199-210 (2003).

3. Wales AD, Allen VM and Davies RH, Chemical treatment of animal feed and water for the control of *Salmonella*. *Foodborne Pathog Dis* **7**:3-15 (2010).

4. Maciorowski KG, Herrera P, Jones F, Pillai SD and Ricke SC, Cultural and immunological detection methods for *Salmonella* spp. in animal feeds- a review. *Vet Res Comm* **3**:127-137 (2006).

5. Bucher O, D'Aoust J and Holley RA, Thermal resistance of *Salmonella* serovars isolated from raw, frozen chicken nuggets/strips, nugget meat and pelleted broiler feed. *Int J Food Microbiol* **124**:195–198 (2008).

6. Laroche C, Fine F and Gervais P, Water activity affects heat resistance of microorganisms in food powders. *Int J Food Microbiol* **97**:307–315 (2005).

7. Liu TS, Snoeyenbos GH and Carlson VL, Thermal resistance of *Salmonella Senftenberg* 775W in dry animal feeds. *Avian Dis* **13**:611–631 (1969).

403 8. Leyer GJ and Johnson EA, Acid adaptation induces cross-protection against
404 environmental stresses in *Salmonella* typhimurium. *Appl Environ Microbiol* **59**:1842–
405 1847 (1993).
406 9. Tuntivanich V, Orta-Ramirez A, Marks BP, Ryser ET and Booren AM, Thermal
407 inactivation of *Salmonella* in whole muscle and ground turkey breast. *J Food Prot*
408 **71**:2548–2551 (2008).
409 10. Blank G, Savoie S and Campbell LD, Microbiological decontamination of poultry
410 feed - Evaluation of steam conditioners. *J Sci Food Agric* **72**:299–305 (1996).
411 11. Himathongkham S, Pereira MDG and Riemann H, Heat destruction of *Salmonella* in
412 poultry feed: Effect of time, temperature, and moisture. *Avian Dis* **40**:72–77 (1996).
413 12. Hutchison ML, Thomas DJI and Avery SM, Thermal death of *Escherichia coli*
414 O157:H7 in cattle feeds. *Lett Appl Microbiol* **44**:357–363 (2007).
415 13. Skandamis PN, Yoon Y, Stopforth JD, Kendall PA and Sofos JN. Heat and acid
416 tolerance of *Listeria monocytogenes* after exposure to single and multiple sublethal
417 stresses. *Food Microbiol* **25**:294–303 (2008).
418 14. Amado IR, Vázquez JA, Fuciños P, Méndez J, Pastrana L, Optimization of
419 antimicrobial combined effect of organic acids and temperature on foodborne
420 *Salmonella* and *Escherichia coli* in cattle feed by response surface methodology.
421 *Foodborne Pathog Dis* **10**:1030–1036 (2013).
422 15. Humpheson L, Adams MR, Anderson WA and Cole MB, Biphasic thermal
423 inactivation kinetics in *Salmonella* enteritidis PT4. *Appl Environ Microbiol* **64**:459–464
424 (1998).
425 16. Cerf O and Metro F, Tailing of survival curves of bacterial spores. *J Appl Bacteriol*
426 **42:**1-19 (1977).
427 17. Den Besten HMW, Mataragas M, Moezelaar R, Abee T and Zwietering MH,

428 Quantification of the effect of salt stress and physiological state on thermotolerance of
429 *Bacillus cereus* ATCC 10987 and ATCC 14579. *Appl Environ Microbiol* **72**:5884–5894
430 (2006).
431 18. Kamau D, Doores S and Pruitt K, Enhanced thermal destruction of *Listeria*
432 *monocytogenes* and *Staphylococcus aureus* by the lactoperoxidase system. *Appl*
433 *Environ Microbiol* **56**:2711–2716 (1990).
434 19. Xiong R, Xie G, Edmondson AE and Sheard MA, A mathematical model for
435 bacterial inactivation. *Int J Food Microbiol* **46**:45–55 (1999).
436 20. Burnham KP and Anderson DR, *Model selection and multimodel inference: A*
437 *practical information-theoretic approach*. Springer-Verlag, New York (2002).
438 21. Murado MA, Vázquez JA, Rial D and Beiras R, Dose-response modelling with two
439 agents: Application to the bioassay of oil and shoreline cleaning agents. *J Hazard Mat*
440 **185**:807–817 (2011).
441 22. Bacon RT, Ransom JR, Sofos JN, Kendall PA, Belk KE and Smith, GC, Thermal
442 inactivation of susceptible and multiantimicrobial-resistant *Salmonella* strains grown in
443 the absence or presence of glucose. *Appl Environ Microbiol* **69**:4123–4128 (2003).
444 23. Breeuwer P, Lardeau A, Peterz M and Joosten H, Desiccation and heat tolerance of
445 *Enterobacter sakazakii*. *J Appl Microbiol* **95**:967–973 (2003).
446 24. Stopforth JD, Suhalim R, Kottapalli B, Hill WE and Samadpour M, Thermal
447 inactivation *D*- and *z*-values of multidrug-resistant and non-multidrug-resistant
448 *Salmonella* serotypes and survival in ground beef exposed to consumer-style cooking. *J*
449 *Food Prot* **71**:509–515 (2008).
450 25. Buchanan RL and Edelson SG, Effect of pH-dependent, stationary phase acid
451 resistance on the thermal tolerance of *Escherichia coli* O157:H7. *Food Microbiol*
452 **16**:447–458 (1999).

453 26. Gabriel AA and Nakano H, Inactivation of *Salmonella*, *E. coli* and *Listeria*
454 *monocytogenes* in phosphate-buffered saline and apple juice by ultraviolet and heat
455 treatments. *Food Control* **20**:443–446 (2009).
456 27. Whiting RC and Golden MH, Variation among *Escherichia coli* O157:H7 strains
457 relative to their growth, survival, thermal inactivation, and toxin production in broth. *Int*
458 *J Food Microbiol* **75**:127–133 (2002).
459 28. Kennedy J, Blair IS, McDowell DA and Bolton DJ. An investigation of the thermal
460 inactivation of *Staphylococcus aureus* and the potential for increased thermotolerance as
461 a result of chilled storage. *J Appl Microbiol* **99**: 1229–1235 (2005).
462 29. Juneja VK and Eblen BS, Heat inactivation of *Salmonella* typhimurum DT104 in
463 beef as affected by fat content. *Lett Appl Microbiol* **30**:461–467 (2000).
464 30. Jung Y and Beuchat LR, Sensitivity of multidrug-resistant *Salmonella* typhimurium
465 DT104 to organic acids and thermal inactivation in liquid egg products. *Food Microbiol*
466 **17**:63–71 (2000).
467 31. Juneja VK, Eblen BS, Marks HM, Modeling non-linear survival curves to calculate
468 thermal inactivation of *Salmonella* in poultry of different fat levels. *Int J Food*
469 *Microbiol* **70**:37–51 (2001).
470 32. Juneja VK and Marks HM, Heat resistance kinetics variation among various
471 isolates of *Escherichia coli*. *Inn Food Sci Emerg Technol* **6**:155–161 (2005).
472 33. Geeraerd AH, Herremans CH, Van Impe JF, Structural model requirements to
473 describe microbial inactivation during a mild heat treatment. *Int J Food Microbiol*
474 **59**:185–209 (2000).
475 34. Fine F, Ferret E, Gervais P, Thermal properties and granulometry of dried powders
476 strongly influence the effectiveness of heat treatment for microbial destruction. *J Food*
477 *Prot* **68**:1041–1046 (2005).

35. Mogollón MA, Marks BP, Booren AM, Orta-Ramirez A, Ryser ET, Effect of beef product physical structure on *Salmonella* thermal inactivation. *J Food Sci* **74**:M347–M351 (2009).