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Modelling the effect of pH, sodium chloride and sodium pyrophosphate on the thermal resistance of *Escherichia coli* O157:H7 in ground beef☆

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ABSTRACT

The objective of this study was to assess the combined effects of temperature, pH, sodium chloride (NaCl), and sodium pyrophosphate (SPP) on the heat resistance of *Escherichia coli* O157:H7 in minced beef meat. A fractional factorial design consisted of four internal temperatures (55.0, 57.5, 60.0 and 62.5 °C), five concentrations of NaCl (0.0, 1.5, 3.0, 4.5 and 6.0 wt/wt.%) and SPP (0.0, 0.1, 0.15, 0.2 and 0.3 wt/wt.%), and five levels of pH (4.0, 5.0, 6.0, 7.0 and 8.0). The 38 variable combinations were replicated twice to provide a total of 76 survivor curves, which were modelled by a modified three-parameter Weibull function as primary model. The polynomial secondary models, developed to estimate the time to achieve a 3-log and a 5-log reduction, enabled the estimation of *critical* pH, NaCl and SPP concentrations, which are values at which the thermo-tolerance of *E. coli* O157:H7 reaches it maximum. The addition up to a certain critical concentration of NaCl (~2.7–4.7%) or SPP (~0.16%) acts independently to increase the heat resistance of *E. coli* O157:H7. Beyond such critical concentrations, the thermo-resistance of *E. coli* O157:H7 will progressively diminish. A similar pattern was found for pH with a critical value between 6.0 and 6.7, depending upon temperature and NaCl concentration. A mixed-effects omnibus regression model further revealed that the acidity of the matrix and NaCl concentration had a greater impact on the inactivation kinetics of *E. coli* O157:H7 in minced beef than SPP, and both are responsible for the concavity/convexity of the curves. When pH, SPP or NaCl concentration is far above or below from its critical value, the temperatures needed to reduce *E. coli* O157:H7 up to a certain log level are much lower than those required when any other environmental condition is at its critical value. Meat processors can use the model to design lethality treatments in order to achieve specific log reductions of *E. coli* O157:H7 in ready-to-eat beef products.

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1. Introduction

Escherichia coli O157:H7 is a widely known foodborne pathogen, which was first identified to be associated with two outbreaks of haemorrhagic colitis in Oregon and Michigan (Riley et al., 1983). Since then, the pathogen has been a focus of numerous studies and continues to be a pathogen of primary concern for meat processors, consumers and regulatory agencies. The outbreaks caused by *E. coli* O157:H7, in both homes and commercial food service establishments, have been frequently linked to the consumption of inadequately cooked contaminated beef; i.e., ground beef or whole muscle beef (blade-tenderized, marinated, frozen steaks, tri-tip or bottom sirloin beef, and roast beef; Armstrong, Hollingsworth, & Morris, 1996; BCPHD, 2008a,b; Laine et al., 2005; Rangel, Sparling, Crowe, Griffin, & Swerdlow, 2005). Thus, the public health consequences of consuming ground beef contaminated with *E. coli* O157:H7 can be severe. The Center for Disease Control and Prevention has estimated that foodborne diseases caused by Shiga toxin-producing *E. coli* O157:H7 (O157 STEC) account for 63,153 cases of illnesses, 2138 hospitalizations and 20 deaths in the US each year (Scallan et al., 2011).

Ground beef is the most popular beef product used for human consumption in the United States. Since asymptomatic cattle are the primary reservoirs of this pathogen (Zhao, Doyle, Shere, & Garber, 1995), contamination of meat may occur during slaughtering operations. The pathogen can be mixed to the interior of the product when meat is ground. *E. coli* O157:H7 can survive in ground beef stored at −20 °C for several months without a significant increase in population densities (Doyle & Schoeni, 1984). One effective means of eliminating *E. coli* O157:H7 from beef is the application of adequate heat treatment, a critical control point in the preparation of thermally processed foods. Inactivation of pathogens during thermal treatment depends

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on treatment temperature and time to achieve expected lethality. Several studies (Ahmed & Conner, 1995; Jackson, Hardin, & Acuff, 1996; Juneja, Snyder, & Marmer, 1997; Kotrola & Conner, 1997) have shown that the organism does not exhibit high heat resistance; and hence, it is practically feasible to inactivate the pathogen during heating. However, sensitivity or resistance of *E. coli* O157:H7 to heat is known to be influenced by many factors, including variation among isolates of *E. coli* O157:H7, growth phase, growth medium, storage temperature before heating, heat shock, heating rate, acid shock, recovery medium, and the composition/physical characteristic of the foods (Ahmed & Conner, 1995; Czechowicz, Santos, & Zottola, 1996; George, Richardson, Pol, & Peck, 1998; Jackson et al., 1996; Kaur, Ledward, Park, & Robson, 1998; Williams & Ingham, 1996). A key to designing optimal cooking regimes that ensure the safety of cooked products is specifically defining the heat resistance of the pathogen as affected by changes in multiple food formulation variables. In a study by Juneja and Novak (2003), when ground beef inoculated with *E. coli* O157:H7 was cooked in a water bath to an internal temperature of 55 to 62.5 °C for up to 1 h, the D-values at all temperatures were significantly lower ($p < 0.05$) in ground beef adjusted with acetic acid to pH 4.5 than samples with pH 5.5. Addition of plant-derived antimicrobials, carvacrol or cinnamaldehyde, also decreases the heat resistance of *E. coli* O157:H7 in sous vide processed ground beef (Juneja & Friedman, 2008). Thus, optimal or reduced heat-treatment processes can be designed to destroy pathogens and produce microbiologically-safe cooked foods while maintaining desirable food quality characteristics.

Predictive models provide an increased understanding on how changes in food formulation parameters influence the heat resistance of pathogens. These models enable food processors to estimate the log reductions of the contaminating pathogens; and thereby, assist in complying with regulatory lethality performance standards (FDA, 2013; USDA-FSIS, 1999). Juneja, Marmer, and Eblen (1999) quantitatively assessed the effects and interactions of temperature, pH, NaCl and sodium pyrophosphate, and then, using a biphasic logistic equation on the non-linear survival curves, found that the thermal inactivation of *E. coli* O157:H7 in beef gravy could be lowered by combining these intrinsic factors. This study provided some characterisation on the heat resistance of *E. coli* O157:H7 in liquid food. To extend these findings in beef, the present study was conducted to quantitatively assess the relative effects and interactions of temperature, pH, NaCl and SPP concentrations on the thermal inactivation of *E. coli* O157:H7 in 75% lean ground beef. The model presented should assist the food industry in product formulation and to design a commercial thermal process in order to estimate lethality treatment, i.e., the processing times and temperatures required to achieve specific log reductions of the pathogen, thus developing safe cooking processes to guard against *E. coli* O157:H7 in ground beef and ready-to-eat products prepared thereof.

2. Materials and methods

2.1. Bacterial culture preparation

The four strains of *E. coli* O157:H7 used in this study: 45753-35, 933, A9218 C1 and ent C9490 (the latter from Jack-in-the-Box), were obtained from the USDA in-house culture collection. Strains 45753-35 (meat isolate) and 933 (kidney isolate) were originally obtained from the Food Safety and Inspection Service, USDA, Beltsville, MD. The two other strains, strains A9218-C1 and ent C9490, are clinical isolates and were originally obtained from the Center for Disease Control and Prevention (CDC), Atlanta, GA. The strains were stored in vials at −80 °C in a mixture (85:15; v/v) of brain heart infusion broth (BHI; Becton, Dickinson & Co., Sparks, MD) and glycerol (Sigma-Aldrich Co., St. Louis, MO). During the course of the study, individual stock cultures were maintained on BHI agar slants at 4 °C with monthly transfers to maintain their viability. Working cultures were prepared and maintained as previously described (Juneja & Friedman, 2008). Each inoculum was enumerated by spiral plating (Autoplate 4000 Spiral Plater, Spiral Biotech, Gaithersburg, MD, USA), making appropriate dilutions in peptone water (0.1%, wt/v; PW) in duplicate, onto Tryptic Soy Agar (TSA; Teknova, Hollister, CA) plates to obtain the initial population densities. Plates were incubated at 37 °C for 24 h before enumeration. Equivalent proportions (2 ml) of each isolate were combined in a sterile conical vial, vortexed for 1 min to obtain a four-strain mixture (ca. 9 $\log_{10}$ CFU/ml) of *E. coli* O157:H7, and this cocktail of strains was used to inoculate the ground meat.

2.2. Ground beef sample preparation and inoculation

Raw 75% lean ground beef obtained from a local grocery store was used as the heating menstruum. The meat was separated into 300 g batches for different treatments. The pH of the meat was adjusted to range from 4 to 8 using 50% NaOH (Avantor Performance Materials, Inc., Phillipsburg, NJ) or 85% lactic acid, and then, salt (NaCl; 0–6%, wt/wt) and sodium pyrophosphate (SPP; 0–0.3%, wt/wt) were added. Lactic acid (85%), NaCl and SPP were obtained from Sigma-Aldrich Co., St. Louis, MO. The treated meat was thoroughly mixed for 2 min in a KitchenAid mixer (model no. K45SS, KitchenAid Inc., Greenville, OH), placed in bags (75 g meat/bag), vacuum sealed and stored frozen (−5 °C) until use within approximately 60 days.

On the day of the experiment, the cocktail inocula (0.15 ml) of four strains of *E. coli* O157:H7 were added to 75 g of thawed (over a period of 24 h in a refrigerator at 4 °C) beef, to obtain a final concentration of cells of approximately 8 $\log_{10}$ CFU/g. Each bag of meat was massaged manually with fingers and then, pummelled with a Seward Laboratory Stomacher 400 (UK) for 2 min, to ensure homogeneous distribution of the organisms in the respective menstruum, as confirmed in preliminary studies. Duplicate meat samples (5 g) were then weighed aseptically into 9.5 × 18 cm sterile filtered stomacher bags (BagPage^{+}, Interscience Laboratories Inc., Rockland, MA). Filter bags containing meat samples inoculated with 0.15 ml of 0.1% (wt/v) sterile peptone water served as negative controls. To ensure even heat transfer, the bags were massaged manually and then, firmly pressed against a flat surface into a thin layer of about 1 mm thickness, thereby excluding as much air as possible as well as eliminating possible air pockets. Finally, the bags were heat-sealed.

2.3. Experimental design

A factorial design was used to assess the effects and interactions of heating temperature (55.0, 57.5, 60.0 and 62.5 °C), salt (NaCl) concentration (0.0, 1.5, 3.0, 4.5 and 6.0 wt/wt.%), sodium pyrophosphate (SPP) concentration (0.0, 0.1, 0.15, 0.2 and 0.3 wt/wt.%) and pH (4.0, 5.0, 6.0, 7.0 and 8.0). Because of the many factors and levels within each factor, a fractional factorial design was used that optimises the amount of information from the experimental region in 38 runs. The design was constructed in a way that it provided as much orthogonality as possible between the columns of the design matrix (Montgomery, 2012). The 38 variable combinations, which will be referred to as environmental or experimental conditions, are shown in Table 1, and they were produced in duplicate to yield a total of 76 experimental survivor curves. A combination was designated by identifying the four-tuple set of values of temperature, NaCl, SPP and pH. Models were developed to describe the combined effect of these factors on the heat resistance of *E. coli* O157:H7 cells inoculated in minced beef.

2.4. Thermal inactivation and enumeration of surviving bacteria

Meat bags were placed in a basket and then fully submerged in a temperature-controlled water bath (Neslab RTE 17 Digital One,

Table 1
Significance of *log* N_{res} in the four-parameter modified Weibull model and its comparison with the three-parameter variant by an F-test and BIC criterion difference for each of the 76 survival curves. Parameter estimates and standard errors of the three-parameter modified Weibull model are also shown.

Expt. cond. (Rep)	Temp (°C)	Salt (%)	SPP (%)	pH	Log N_{res} (p-val)	p-val from F-test	ΔBIC[a]	Three-parameter model		
								Log N_0 (st. error)	χ (st. error)	β (st. error)
1(1)	55.0	0.0	0.0	4.0	−0.233 (0.87)	0.72[ns]	1.31	4.187 (0.394)	0.627 (0.043)	3.964 (2.100)
1(2)					−0.617 (0.80)	0.60[ns]	0.75	4.800 (0.594)	0.652 (0.064)	3.156 (1.939)
2(1)	55.0	0.0	0.3	4.0	−0.003 (0.99)	0.99[ns]	1.60	3.440 (0.183)	0.535 (0.119)	9.152 (2.488)
2(2)					−0.003 (0.80)	0.71[ns]	0.59	3.402 (0.008)	0.544 (0.002)	9.250 (0.511)
3(1)	55.0	6.0	0.0	4.0	−9.470 (0.36)	0.02[*]	7.84	5.565 (0.212)	1.168 (0.072)	0.969 (0.105)
3(2)					−0.297 (0.84)	0.60[ns]	1.65	5.849 (0.483)	0.798 (0.140)	0.759 (0.171)
4(1)	55.0	3.0	0.15	6.0	−7.416 (0.75)	0.59[ns]	1.45	7.283 (0.390)	236.2 (48.59)	0.661 (0.168)
4(2)					1.670 (0.14)	0.44[ns]	0.71	7.318 (0.216)	118.3 (6.620)	0.904 (0.099)
5(1)	55.0	0.0	0.0	8.0	−0.833 (0.56)	0.26[ns]	0.32	7.385 (0.297)	31.59 (2.107)	1.314 (0.155)
5(2)					−0.984 (0.44)	0.19[ns]	−0.44	7.061 (0.277)	29.70 (1.750)	1.529 (0.189)
6(1)	55.0	0.0	0.3	8.0	−0.849 (0.67)	0.36[ns]	1.00	7.236 (0.434)	37.07 (3.642)	1.332 (0.203)
6(2)					−0.703 (0.46)	0.15[ns]	1.01	7.317 (0.254)	30.27 (1.686)	1.307 (0.133)
7(1)	55.0	6.0	0.0	8.0	0.293 (0.85)	0.87[ns]	1.87	5.519 (0.247)	14.595 (0.738)	1.501 (1.170)
7(2)					1.088 (0.08)	0.25[ns]	−1.72	5.822 (0.141)	15.20 (0.648)	1.222 (0.101)
8(1)	57.5	4.5	0.1	5.0	−11.44 (0.27)	0.03[*]	−5.70	7.183 (0.154)	54.13 (2.385)	0.857 (0.063)
8(2)					−14.00 (0.50)	0.09[ns]	−2.72	7.075 (0.284)	54.41 (4.362)	0.843 (0.121)
9(1)	57.5	4.5	0.2	5.0	2.170 (0.02)	0.26[ns]	0.00	7.329 (0.297)	49.92 (4.433)	0.810 (0.106)
9(2)					−8.240 (0.69)	0.30[ns]	0.39	7.331 (0.213)	45.52 (3.010)	0.714 (0.064)
10(1)	57.5	1.5	0.1	5.0	−2.002 (0.23)	0.03[*]	−4.45	7.101 (0.256)	30.11 (0.953)	2.746 (0.378)
10(2)					−2.670 (0.43)	0.16[ns]	−2.39	7.112 (0.440)	29.71 (1.466)	2.644 (0.595)
11(1)	57.5	1.5	0.2	5.0	−7.183 (0.66)	0.19[ns]	0.14	7.317 (0.176)	37.75 (0.943)	1.863 (0.162)
11(2)					−2.909 (0.67)	0.40[ns]	1.06	7.153 (0.210)	36.79 (0.879)	2.260 (0.232)
12(1)	57.5	3.0	0.15	6.0	−28.92 (0.69)	0.20[ns]	0.38	5.919 (0.433)	90.65 (9.220)	1.377 (0.496)
12(2)					−8.750 (0.54)	0.19[ns]	−0.76	6.012 (0.239)	94.37 (7.910)	0.817 (0.124)
13(1)	57.5	4.5	0.1	7.0	−0.413 (0.89)	0.69[ns]	2.13	5.449 (0.733)	43.120 (7.020)	1.291 (0.581)
13(2)					−8.400 (0.48)	0.19[ns]	−0.42	6.923 (0.300)	50.19 (5.295)	0.629 (0.091)
14(1)	57.5	4.5	0.2	7.0	−8.520 (0.66)	0.39[ns]	0.40	6.637 (0.826)	24.25 (8.767)	0.537 (0.201)
14(2)					−0.643 (0.75)	0.46[ns]	1.13	5.859 (0.805)	32.22 (6.869)	1.003 (0.411)
15(1)	57.5	1.5	0.1	7.0	−0.007 (0.99)	0.95[ns]	2.56	7.126 (0.719)	45.91 (8.19)	0.784 (0.219)
15(2)					−1.410 (0.62)	0.32[ns]	0.51	6.275 (0.706)	45.44 (6.386)	1.301 (0.511)
16(1)	57.5	1.5	0.2	7.0	−11.53 (0.58)	0.28[ns]	0.56	6.789 (0.328)	43.50 (5.808)	0.752 (0.153)
16(2)					−7.055 (0.65)	0.44[ns]	0.75	6.141 (0.365)	40.02 (6.678)	0.689 (0.167)
17(1)	60.0	3.0	0.15	4.0	−0.772 (0.32)	0.09[ns]	−3.28	6.705 (0.363)	0.831 (0.066)	1.291 (0.175)
17(2)					−1.085 (0.51)	0.23[ns]	−0.71	6.643 (0.474)	0.971 (0.108)	1.083 (0.202)
18(1)	60.0	3.0	0.15	5.0	−2.031 (0.21)	0.02[*]	−4.67	6.949 (0.248)	13.43 (0.644)	1.466 (0.172)
18(2)					−1.316 (0.37)	0.12[ns]	−2.16	7.090 (0.323)	13.21 (0.698)	1.658 (0.238)

(*continued on next page*)

Table 1 (*continued*)

Expt. cond. (Rep)	Temp (°C)	Salt (%)	SPP (%)	pH	Log N_{res} (p-val)	p-val from F-test	ΔBIC[a]	Three-parameter model		
								Log N_0 (st. error)	χ (st. error)	β (st. error)
19(1)	60.0	6.0	0.15	6.0	−11.69 (0.68)	0.16[ns]	−0.92	6.884 (0.381)	23.66 (2.404)	0.872 (0.138)
19(2)					0.115 (0.97)	0.98[ns]	2.19	7.316 (0.165)	43.89 (3.047)	0.738 (0.064)
20(1)	60.0	4.5	0.15	6.0	0.036 (0.99)	0.99[ns]	2.48	7.462 (0.206)	40.00 (2.320)	0.904 (0.082)
20(2)					−0.760 (0.68)	0.34[ns]	1.05	6.576 (0.540)	30.31 (3.750)	1.067 (0.273)
21(1)	60.0	3.0	0.0	6.0	−10.91 (0.74)	0.52[ns]	1.38	7.208 (0.809)	17.23 (4.382)	0.690 (0.278)
21(2)					−5.102 (0.70)	0.58[ns]	1.59	7.085 (0.324)	24.07 (4.399)	0.500 (0.098)
22(1)	60.0	3.0	0.1	6.0	−12.07 (0.78)	0.33[ns]	0.09	7.533 (0.364)	15.40 (1.530)	0.807 (0.201)
22(2)					1.115 (0.45)	0.65[ns]	1.607	7.104 (0.530)	13.19 (1.946)	0.862 (0.178)
23(1)	60.0	3.0	0.15	6.0	−25.21 (0.85)	0.57[ns]	1.56	6.218 (0.296)	45.25 (3.154)	2.243 (0.569)
23(2)					−28.75 (0.73)	0.30[ns]	0.05	6.488 (0.268)	44.51 (2.714)	2.087 (0.507)
24(1)	60.0	3.0	0.2	6.0	−8.100 (0.77)	0.57[ns]	1.09	6.625 (0.448)	52.73 (18.48)	0.803 (0.376)
24(2)					−3.513 (0.79)	0.69[ns]	1.72	6.532 (0.261)	95.29 (32.48)	0.485 (0.107)
25(1)	60.0	3.0	0.3	6.0	−18.47 (0.60)	0.19[ns]	−0.78	6.632 (0.402)	20.18 (2.392)	1.026 (0.294)
25(2)					0.160 (0.97)	0.98[ns]	2.30	7.117 (0.275)	15.68 (1.883)	0.541 (0.066)
26(1)	60.0	1.5	0.15	6.0	−28.90 (0.44)	0.01*	−9.35	6.798 (0.257)	15.17 (0.640)	2.065 (0.335)
26(2)					−0.003 (0.81)	0.19[ns]	−0.85	6.652 (0.382)	14.75 (0.848)	2.420 (0.641)
27(1)	60.0	0.0	0.15	6.0	1.078 (0.30)	0.46[ns]	0.48	7.380 (0.169)	8.168 (0.241)	1.699 (0.141)
27(2)					1.081 (0.23)	0.40[ns]	−0.02	7.436 (0.178)	7.839 (0.239)	1.655 (0.138)
28(1)	60.0	3.0	0.15	7.0	−5.166 (0.76)	0.42[ns]	1.78	6.961 (0.211)	59.84 (2.912)	1.229 (0.131)
28(2)					0.466 (0.67)	0.73[ns]	1.95	6.275 (0.127)	66.52 (2.168)	1.196 (0.086)
29(1)	60.0	3.0	0.15	8.0	1.134 (0.35)	0.62[ns]	1.53	5.822 (0.383)	4.287 (0.561)	0.805 (0.162)
29(2)					−0.081 (0.96)	0.84[ns]	1.98	6.401 (0.501)	3.066 (0.466)	0.859 (0.184)
30(1)	62.5	4.5	0.1	5.0	2.557 (0.00)	0.08[ns]	−2.32	7.520 (0.199)	5.818 (0.293)	0.971 (0.091)
30(2)					3.268 (0.00)	0.12[ns]	−1.31	7.246 (0.244)	7.219 (0.585)	1.084 (0.159)
31(1)	62.5	4.5	0.2	5.0	−7.569 (0.76)	0.43[ns]	1.48	6.979 (0.158)	6.169 (0.254)	1.107 (0.101)
31(2)					3.079 (0.00)	0.01*	−7.06	7.208 (0.177)	6.714 (0.357)	1.120 (0.113)
32(1)	62.5	1.5	0.1	5.0	2.355 (0.07)	0.37[ns]	0.31	7.492 (0.208)	3.432 (0.127)	2.179 (0.293)
32(2)					−19.26 (0.47)	0.01*	−10.9	7.300 (0.086)	3.242 (0.041)	2.464 (0.144)
33(1)	62.5	1.5	0.2	5.0	−3.521 (0.89)	0.66[ns]	1.64	7.518 (0.255)	3.019 (0.108)	2.274 (0.328)
33(2)					−31.94 (0.83)	0.35[ns]	0.13	7.442 (0.271)	3.208 (0.136)	1.911 (0.309)
34(1)	62.5	3.0	0.15	6.0	−5.035 (0.88)	0.77[ns]	2.44	7.352 (0.323)	11.08 (1.624)	0.783 (0.149)
34(2)					2.737 (0.04)	0.40[ns]	0.80	7.393 (0.284)	11.02 (1.333)	0.707 (0.106)
35(1)	62.5	4.5	0.1	7.0	−4.025 (0.87)	0.73[ns]	1.96	4.870 (0.325)	6.008 (0.936)	1.294 (0.577)
35(2)					−1.645 (0.82)	0.73[ns]	2.20	6.325 (0.311)	3.722 (0.276)	1.026 (0.152)
36(1)	62.5	4.5	0.2	7.0	−3.439 (0.86)	0.73[ns]	2.19	5.862 (0.265)	5.473 (0.519)	0.767 (0.164)
36(2)					−4.819 (0.80)	0.73[ns]	2.20	6.790 (0.339)	5.294 (0.665)	0.647 (0.112)
37(1)	62.5	1.5	0.1	7.0	−7.25 (0.71)	0.54[ns]	1.64	6.662 (0.189)	10.27 (1.513)	0.849 (0.139)

Table 1 (*continued*)

Expt. cond. (Rep)	Temp (°C)	Salt (%)	SPP (%)	pH	Log N_{res} (p-val)	p-val from F-test	ΔBIC[a]	Three-parameter model		
								Log N_0 (st. error)	χ (st. error)	β (st. error)
37(2)					−15.70 (0.60)	0.20[ns]	−0.34	6.918 (0.191)	5.916 (0.332)	1.054 (0.128)
38(1)	62.5	1.5	0.2	7.0	0.841 (0.12)	0.29[ns]	0.58	6.050 (0.239)	3.660 (0.140)	2.524 (0.368)
38(2)					−0.007 (0.99)	0.93[ns]	2.38	5.533 (0.236)	3.944 (0.134)	3.958 (0.779)

[a] ΔBIC was estimated as BIC from the four-parameter model minus BIC from the three-parameter model.
[ns] ns : non-significant.
* Significant P < 0.05l.

Thermo Electron Corp, Newington, NH, USA) maintained at 55.0, 57.5, 60.0 and 62.5 °C as described previously (Juneja, Marks, & Mohr, 2003). Recorded come-up times (<30 s) were negligible and therefore, were included in the total heating time for determining surviving microbial population after heat treatment. Sampling frequency varied and was based on the heating temperature, pH, and concentrations of both NaCl and SPP. Total heating times ranged from 0.83 to 190 min at 55 °C; 40 to 90 min at 57.5 °C; 2 to 90 min at 60 °C; and 3.5 to 9 min at 62.5 °C. For enumerating surviving cells, after bags were removed from the water bath and plunged in an ice-water bath, meat samples were aseptically opened; PW was added to obtain 1:1 (wt/v) slurry and pummelled for 2 min with a stomacher. Thereafter, decimal serial dilutions were prepared in 0.1% PW and appropriate dilutions were spiral plated, in duplicate, onto TSA. Samples not inoculated with the *E. coli* O157:H7 were plated as controls. In addition, 0.1 and 1.0 ml volumes of the first dilution were surface plated, when a low number of surviving cells was expected for longer sampling times. After resuscitation for 2h at room temperature to facilitate recovery of heat-injured cells, 10 ml of pre-tempered to 47 °C Sorbitol MacConkey agar (Becton Dickinson & Co., Sparks, MD) was overlaid on the surface plated TSA. Thereafter, the plates were allowed to dry at room temperature before incubating at 30 °C. After 48 h of incubation, colonies were enumerated manually as the number of survivors. The data were converted to log CFU/g and represented surviving cells recovered on four plates at each sampling time since each replicate experiment was performed in duplicate.

2.5. Statistical analyses

The first step towards the goal of developing a primary kinetics model for the inactivation of *E. coli* O157:H7 in minced beef was to examine, graphically, the shape of the 78 experimental curves to determine the most appropriate equation of population decay. It was observed that in all cases the experimental curves were non-log-linear, exhibiting either upward or downward concavity or sigmoidal shape. As in some curves, the tailing phenomenon was noticed, a number of flexible non-log-linear models capable of describing shoulder/tail and concavity/convexity were first evaluated: a biphasic model (Cerf, 1977), an empirical sigmoidal model (Augustin, Carlier, & Rozier, 1998), a log-logistic model (Cole, Davies, Munro, Holyoak, & Kilsby, 1993), a double Weibull curve (Coroller, Leguerinel, Mettler, Savy, & Mafar, 2006), and a Weibull-type inactivation model which was proposed in this research (Eq. (1)). Pooled variances and pooled Bayesian Information Criterion (BIC) were calculated as an average of variances and BIC of the individually fitted curves, respectively, weighed by the number of observations of each survival curve. After comparing the pooled variances and BIC for each of the five models considered, it was concluded that, with the same number of parameters, the modified Weibull described most of the curves better than the other models. Thus, it was the model chosen for further analysis and, next, it was compared with a simpler three-parameter modified Weibull.

2.5.1. Primary modelling

2.5.1.1. The Weibull model. The Weibull distribution function is used extensively in reliability engineering, and, as such, many parameterisations exist (Murthy, Xie, & Jiang, 2004; Seber & Wild, 2003). In predictive microbiology, the Weibull models have been increasingly used because of its simplicity, ease of fit, and capability of modelling concave and convex curves (Couvert, Gaillard, Savy, Mafart, & Leguerinel, 2005). In a simple two-parameter Weibull model, the survival function $S(t) = \log N(t)/N_0$ is defined as,

$$S(t) = \exp\left(-\left(\frac{t}{\chi}\right)^{\beta}\right). \quad (1)$$

The scale and shape parameters of the underlying Weibull distribution are χ and β, respectively. Although the Weibull model is basically of empirical nature, van Boekel (2002) suggested that $\beta < 1$ (i.e., concave curves) presumes that the surviving microorganisms at any point in the inactivation curve have the capacity to adapt to the applied stress, whereas $\beta > 1$ (convex curves) indicates that the remaining cells become increasingly susceptible to heat.

In order to make the model flexible enough to describe the different shapes observed in the experimental curves (i.e., downward concavity with shoulder, upward concavity with tail and sigmoid-like shape), the survival function $S(t)$ was re-defined as:

$$S(t) = \frac{\log N(t) - \log N_{res}}{\log N_0 - \log N_{res}} \quad (2)$$

which produced a modified four-parameter Weibull model,

$$\log N(t) = (\log N_0 - \log N_{res}) \exp\left(-\left(\frac{t}{\chi}\right)^{\beta}\right) + \log N_{res}. \quad (3)$$

The dependent variable $\log N(t)$ used is the logarithm base 10 (log) of the number of cells at time t. N_{res} is the residual number of microorganisms, which is in fact a parameter associated with the tailing effect, and N_0 is the initial number of microorganisms. N_0 was considered to be a model parameter and not the first observation when $t = 0$. Likewise, Geeraerd, Herremans, and Van Impe (2000) support the concept that a model should describe the absolute population and not the population relative to the initial population. In addition, the model presented in Eq. (3) possesses the structural requirements indicated by Geeraerd et al. (2000). The model of Geeraerd, Vladramidis, and Van Impe (2005) was not tested in our curves as it cannot describe upward concavity.

2.5.1.2. Fitting of the primary model. The primary model was separately fitted to each of the 76 survival curves. The following model's parameters were extracted: $\log N_0$, $\log N_{res}$ and the scale χ and shape parameter β, along with their standard errors. As in most curves, it was observed that $\log N_{res}$ was not significantly different from zero;

a simplified three-parameter Weibull model was fitted to each of the curves,

$$\log N(t) = \log N_0 \exp\left(-\left(\frac{t}{\chi}\right)^{\beta}\right). \quad (4)$$

For each of the experimental curves, the residuals of the nested models (3) and (4) were compared by an F-test with a significance level $\alpha = 0.05$. In most experimental curves, the three-parameter Weibull was not different ($p > 0.05$) from the four-parameter one, reason as to why the former model was preferred.

For each of the survival curves, the lethality times needed to obtain a 3-log relative reduction (t_{3D}) and 5-log relative reduction (t_{5D}) were estimated as,

$$t_{3D} = \chi\left[Ln\frac{\log N_0}{\log N_0 - 3}\right]^{1/\beta} \quad (5)$$

$$t_{5D} = \chi\left[Ln\frac{\log N_0}{\log N_0 - 5}\right]^{1/\beta} \quad (6)$$

after replacing $\log N(t)$–$\log N_0$ by either 3 or 5, respectively, in Eq. (4). The standard errors of $Ln\ t_{3D}$ and $Ln\ t_{5D}$ were also computed.

2.5.2. Secondary modelling

Initially, the 76 estimates of the shape β and location χ parameters from the modified three-parameter Weibull model were logarithmically transformed ($Ln\ \beta$ and $Ln\ \chi$). These transformed values underwent stepwise regressions to identify the statistically-significant environmental conditions that could predict them. Thus, a second-order polynomial function was obtained separately for $Ln\ \chi$ and $Ln\ \beta$, in terms of the independent variables considered (temperature, pH, NaCl concentration and SPP concentration). In each stepwise regression, the predictors were entered as linear terms, quadratic terms and all their two-variable interactions. As there is a concern of including terms in the secondary model that do not contribute to obtaining good predictors of primary model parameter values, the stepwise regressions were performed in a systematic way. To determine the terms to be included in the model, the regressions were performed with an entry significance of 0.10 and a required significance of 0.025 to stay in the model. At each step, the studentized residuals were examined for identifying spurious data points. Following the same methodology, other stepwise regressions were conducted to find the best polynomial models that could predict $Ln\ t_{3D}$ and $Ln\ t_{5D}$ in terms of the environmental conditions.

2.5.3. Global modelling approach: omnibus model

An omnibus or a global model is a model type that fits the primary and secondary models at the same time using all the data from the experimental curves (Juneja, Gonzales-Barron, Butler, Yadav, & Friedman, 2013; Pradhan et al., 2012), and as such, they can predict survival curves for any specified value of the environmental conditions. The independent variables (i.e., temperature, pH, NaCl and SPP concentrations) and interactions predicting the model parameters were selected by the previous stepwise-regressions, and were added to the omnibus model one by one while assessing the improvement in the goodness-of-fit measures (log-likelihood, Akaike Information Criterion [AIC] and BIC) and the behaviour of the residuals.

The omnibus mixed-effects model based on the Weibull model assumed that both parameters χ and β could be expressed as a function of the environmental variables: temperature (T), pH (pH), salt percentage concentration ($NaCl$) and sodium pyrophosphate percentage concentration (SPP). The random-effects terms u and v were added to the mean of the intercepts a_1 and b_1 of the polynomial expressions predicting $Ln\ \chi$ and $Ln\ \beta$, respectively. This was done because some fraction of the variability in the scale and shape parameters could not be explained by their fixed-effects predictors. Because the initial microbial concentration $\log N_0$ was variable from condition to condition, this variability was accounted for by adding a third random-effects term w. The three random effects were assumed to follow normal distributions with means zero and covariance matrix [s^2_u, s^2_{uv}, s^2_{uw}; s^2_{uv}, s^2_v, s^2_{vw}; s^2_{uw}, s^2_{vw}, s^2_w]. Since an analysis-of-variance components test revealed that the variability within experimental conditions (replicates) was not significant in contrast to the high between-experimental condition variance, a nested error structure of random effects of replicates within experimental condition was not necessary in this case. Hence, the random effects u, v and w were assumed to take in random shifts subject to a given set j of experimental conditions. The residual error ε_{ijk} followed a normal distribution with mean zero and variance s^2. The log CFU/g concentration taken at the time k in the food sample i exposed at the environmental condition j ($j = 1, \ldots 38$) was estimated as,

$$\log N_{ijk} = \log N_{0_j} \exp\left(\frac{t}{\chi_j}\right)^{\beta_j} + \varepsilon_{ijk} \quad (6)$$

$$Ln\ \chi_j = a_1 + a_2 SPP + a_3 pH + a_4 NaCl^2 + a_5 SPP^2 + a_6 pH^2 + \ldots$$
$$= \ldots\ a_7 NaCl \times pH + a_8 NaCl \times T + a_9 pH \times T + u_j$$

$$Ln\ \beta_j = b_1 + b_2 pH + b_3 NaCl + b_4 NaCl^2 + b_5 NaCl \times pH + v_j$$

$$\log N_{0_j} = \log N_{0\text{mean}} + w_j.$$

Many other mixed-effects models were assessed. However, the model of Eq. (6) fitted significantly better than the others and is the only one presented here.

2.5.4. Model validation

The model was validated by the leave-one-out method or internal validation (Schvartzman, Gonzales-Barron, Butler, & Jordan, 2014). The procedure is described as follows: the inactivation data of one environmental condition including the two replicates was randomly selected and removed from the whole data set. Eq. (6) was then re-fitted to the remaining data; and, using the new model parameters, the mean bacterial concentrations and prediction intervals along time were estimated for the environmental condition that was removed. Such *predicted* inactivation curve was then contrasted with the observed values. This was repeated thirty eight times, each time removing one environmental condition. Finally, for the evaluation of the performance of the model, two statistical validation indices proposed by Ross (1996), the bias factor (Bf) and the accuracy factor (Af), were computed from the observed and predicted values. All independent non-linear regressions, stepwise regressions, and non-linear mixed-effects models were adjusted in R version 2.14.2 (R Development Core Team) using the 'MASS' and 'nlme' packages.

3. Results and discussion

In the present study, *E. coli* O157:H7 did not follow log-linear inactivation curves in any environmental condition. Only a few (18%) inactivation curves presented a shoulder (i.e., conditions #1, 2, 10, 11, 32, 33 and 38) while 92% of the curves displayed either sigmoid shapes (i.e., #1, 2, 5, 6, 7, 10, 11, 18, 26, 27, 28, 31, 35 and 38) or upward concavity and tail (i.e., #3, 4, 8, 9, 12–17, 19–22, 24, 25, 29, 30, 34, 36 and 37) (Figs. 1–4). This concave shape is evidence that sensitive members of the bacterial population perish rapidly, leaving behind progressively more resistant microorganisms (Mattick, Legan, Humphrey, & Peleg, 2001). In only three environmental conditions (# 23, 32 and 33), downward concavity was verified, suggesting the opposite: this is, that there is cumulative damage occurring, making it increasingly difficult for the cells to survive. Thus, the primary model to be chosen had to be capable of describing the distinct shapes

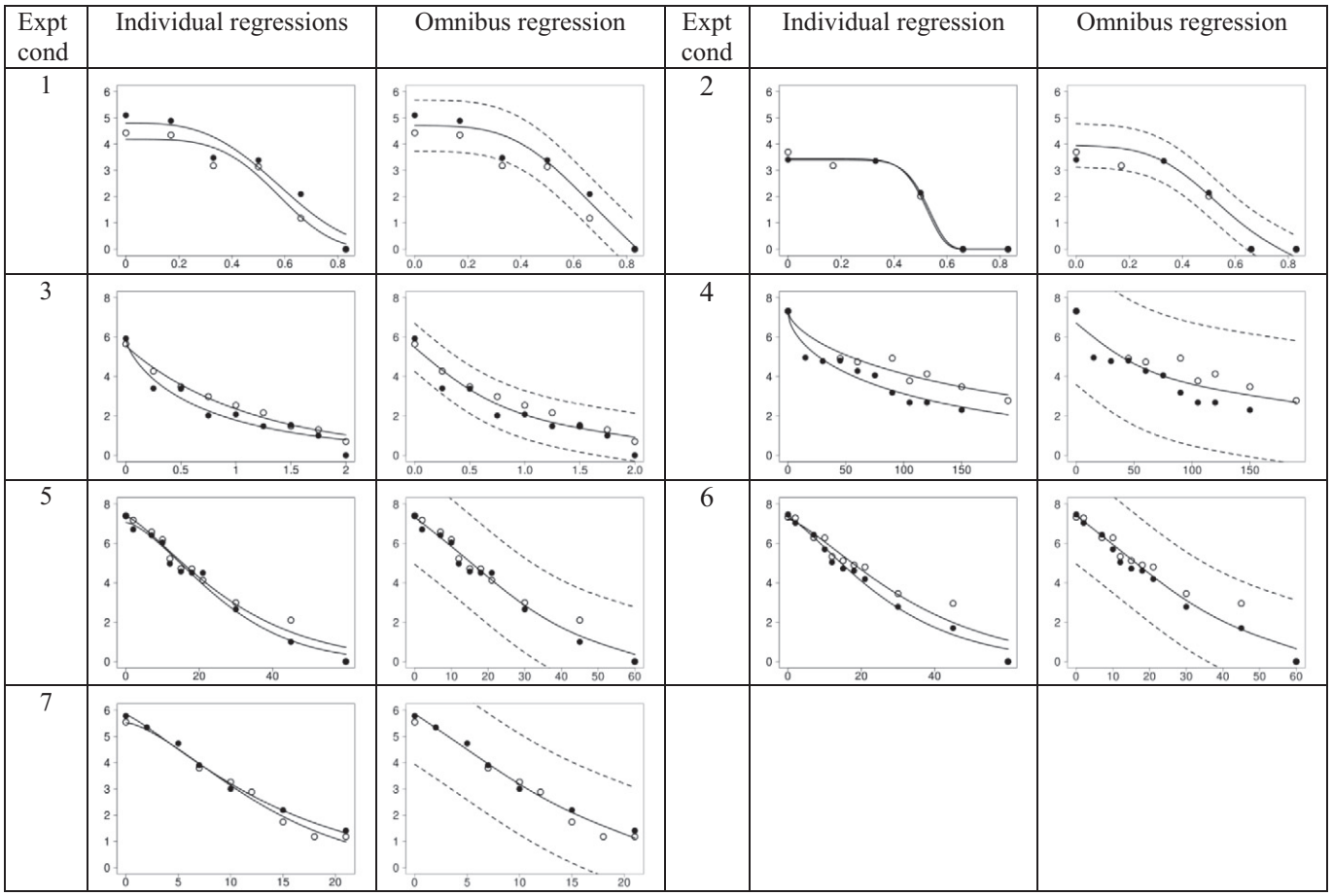

Fig. 1. Survival curves of *E. coli* O157:H7 in ground beef for the different combinations of values of pH, salt and SPP concentrations for temperature = 55.0 °C, as modelled by individual regressions and by a mixed-effects omnibus regression for the modified three-parameter Weibull inactivation model. Mean predicted values and 95% prediction intervals are shown for the omnibus regression. Same markers represent observations from the same experiment.

discerned in this study. For this reason, the survival function of the Weibull model (Eq. (2)) was modified in order to make the model more flexible to represent all the survival curve shapes observed.

3.1. Comparison of the four-parameter with the three-parameter modified Weibull primary model

For each of the individual curves, a three-parameter modified Weibull model ($\log N_0$, χ, β) was compared against a four-parameter modified Weibull model ($\log N_0$, χ, β, $\log N_{res}$). Table 1 presents, for each combination of experimental conditions: (i) the value of the $\log N_{res}$ parameter and its significance as fitted by the four-parameter model; (ii) the p-value of an F-test for comparison of nested models to assess whether one model gives a significantly better fit to the data; and (iii) the difference in BIC between the four-parameter model and the three-parameter model. Although the ΔBIC was generally small across the individual curves, most of them (54 out of 76) presented a positive value, suggesting that in the majority of curves the three-parameter model could describe the data slightly better than the four-parameter model. From the 22 individual curves whose ΔBIC was negative (i.e., a possibility that the four-parameter model could be slightly better), 16 curves yielded non-significant F-values, indicating that differences in BIC were not enough to cause statistical significance, and hence the four-parameter model did not represent the data better than the three-parameter model. Nonetheless, in either case, whether the F-value was significant (curves #9(1), 11(1), 19(1), 27(1), 33(2)) or not (curves #6(2), 8(2), 9(2), 11(2), 13(2), 14(2), 18(1), 18(2), 19(2), 20(1), 26(1), 27(2), 28(2), 38(2)), given a negative ΔBIC, the additional parameter $\log N_{res}$ was not significantly different from zero in most of those 22 individual curves except for three curves: #30(1), 30(2) and 31(2) (Table 1). These curves with a significant $\log N_{res}$ shared all the same feature: the $\log N_{res}$ values were relatively high (i.e., 2.5, 3.3, and 3.1 log CFU/g, respectively) in comparison to the others whereby $\log N_{res}$ took even very low negative values. Notice in Fig. 4 that curves #30(1), 30(2) and 31(2), having a high $\log N_{res}$ value, present a shape of either sigmoid or upward concavity, yet in any case they tail at a high microbial concentration. Nevertheless, from the 76 individual curves, only one curve (#31(2)) was significantly better described by the four-parameter model, as attested by the three tests (i.e., significant $\log N_{res}$, significant F-test and a relatively high negative ΔBIC). Hence, after this careful analysis, it was concluded that the simpler three-parameter model was preferable because, apart from being parsimonious (i.e., having still a good fit with a manageable number of parameters), it was a model sufficiently flexible to characterise all the different decay shapes (Figs. 1–4). Having chosen a non-log-linear equation to describe the inactivation kinetics of *E. coli* O157:H7 in ground beef, our model cannot furnish the common D values but other useful measurements of lethality such as 3D and 5D.

Visual examination of the individual fitted curves (Figs. 1–4) also suggested that, in general, there was little variation among replicates, which can be also attested by the estimates of the model parameters (Table 1). The individual curves exhibited the same shape or trend between replicates for most of the conditions, except for the pairs #12, 13, 14 (Fig. 2) and #37 (Fig. 4). Such differences

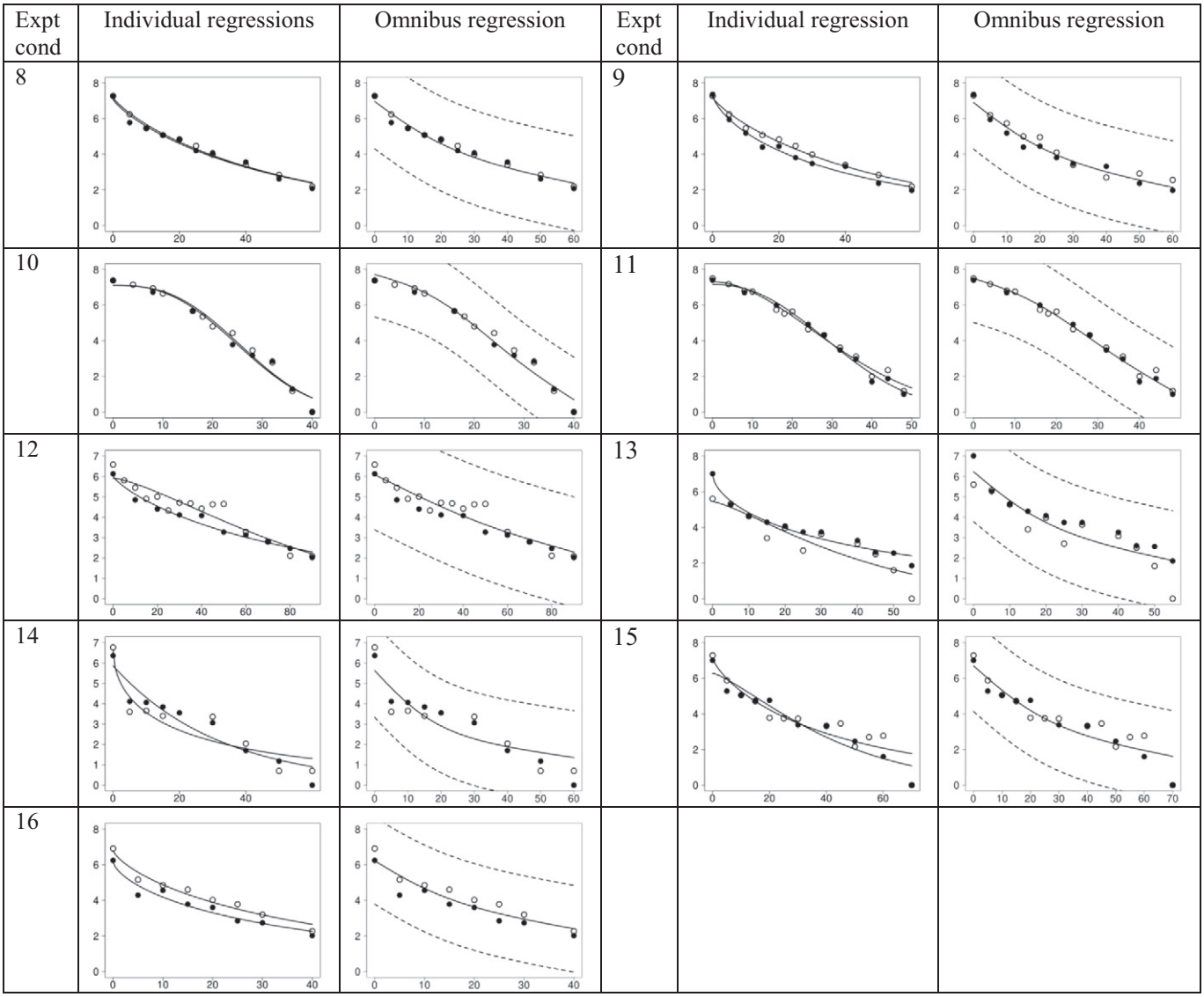

Fig. 2. Survival curves of *E. coli* O157:H7 in ground beef for the different combinations of values of pH, salt and SPP concentrations for temperature = 57.5 °C, as modelled by individual regressions and by a mixed-effects omnibus regression for the modified three-parameter Weibull inactivation model. Mean predicted values and 95% prediction intervals are shown for the omnibus regression. Same markers represent observations from the same experiment.

reflected a greater proportional variation in the shape factor β (defining degree of convexity or concavity) between replicates (Table 1).

Table 2 summarises the means and the standard deviations of the estimated values of $Ln\ t_{3D}$ from the derived 76 individual regressions. Included in these tables are the geometric mean of the t_{3D} values (min), the mean of the $Ln\ t_{3D}$, the standard deviation of these between the replicate experiments for each experimental condition, and the pooled standard error of these estimates owing to regression, computed by taking the square root of the sum of the weighted variances for the individual estimates $Ln\ t_{3D}$, with weight equal to the degrees of freedom of the individual regression. Because there was an absence of correlation between the standard deviations with the mean values of $Ln\ t_{3D}$ ($r = 0.022$) as well as between the pooled standard errors with the means of $Ln\ t_{3D}$ ($r = 0.028$), pooling variances over the row entries of Table 2 can provide a rough summary of the goodness-of-fit of Eq. (4) for individual survival curves. The last row of the table includes the pooled standard deviation and error weighted by the number of observations minus one. The pooled between-experiment standard deviation of 0.165 (Table 2) can be used to approximate a confidence interval (CI) for $Ln\ t_{3D}$ estimated from a single experiment. For instance, suppose that from a single experiment, a t_{3D} value of 10 min is obtained. Then, a 95% CI for $Ln\ t_{3D} = 2.30$ can be computed as $2.30 \pm 1.96 \times 0.165$. However, a great range of this confidence interval [1.98–2.60] or [4.74–13.46] min is due to the error of the regression. Using the pooled standard error of 0.123 (Table 2), the range of the error due to regression for $Ln\ t_{3D} = 2.30$ can be calculated as [2.05–2.54] or [7.77–12.68] min. Likewise, Table 3 compiles the means and the standard deviations of $Ln\ t_{5D}$ from the derived 76 individual regressions. Notice that the pooled between-experiment standard deviation (0.167) and the pooled standard error due to regression (0.119) for $Ln\ t_{3D}$ are numerically close to those for $Ln\ t_{5D}$, which is due to the high correlation between these two lethality time estimates ($r = 0.96$).

3.2. Secondary models for the Weibull parameters and lethality times

Results from Tables 2 and 3 show that the addition of SPP in ground beef can either increase or decrease the heat resistance of *E. coli* O157:H7 depending on the matrix acidity and on the concentration of salt. Likewise, the addition of salt in ground beef can either increase or decrease the thermo-tolerance of *E. coli* O157:H7 subject to pH and the concentration of SPP. With aims to elucidate such

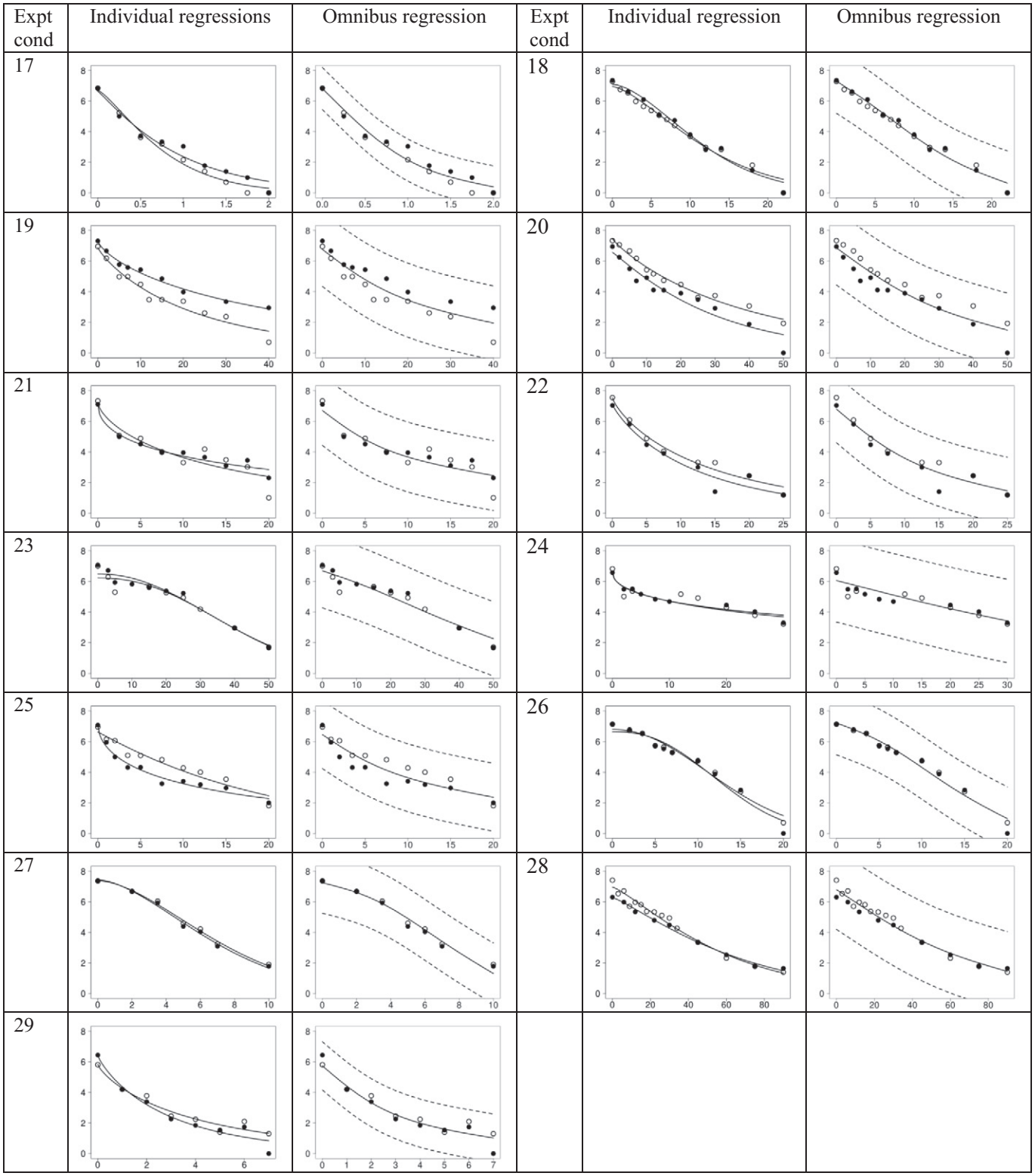

Fig. 3. Survival curves of *E. coli* O157:H7 in ground beef for the different combinations of values of pH, salt and SPP concentrations for temperature = 60.0 °C, as modelled by individual regressions and by a mixed-effects omnibus regression for the modified three-parameter Weibull inactivation model. Mean predicted values and 95% prediction intervals are shown for the omnibus regression. Same markers represent observations from the same experiment.

intricate combined effects of pH, salt and SPP on the lethality times, scatter plots between the environmental variables and *Ln* t_{3D} or *Ln* t_{5D} were initially explored; and subsequently, secondary models predicting lethality times were built. Bear in mind that the curves displayed per temperature in Fig. 5A–F are not lines fitted from the secondary models for lethality times, but only trend lines superimposed to facilitate viewing. The influence of pH on both lethality times was of a quadratic nature (Fig. 5A and D). Such scatter plots suggested that in high acidic media (pH = 4), increasing the temperature seemingly does not have a marked effect on bringing down the lethality times to reach a certain

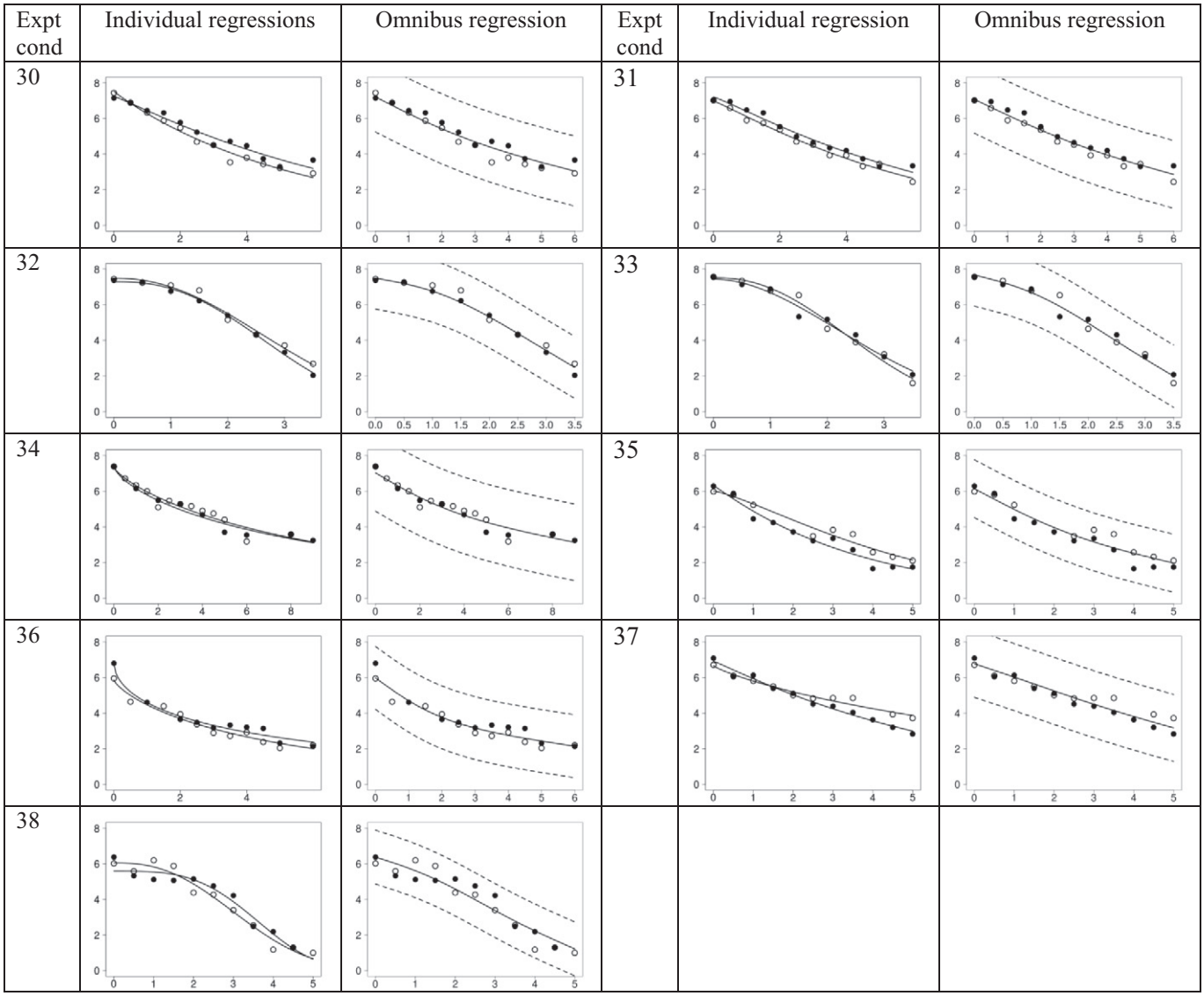

Fig. 4. Survival curves of *E. coli* O157:H7 in ground beef for the different combinations of values of pH, salt and SPP concentrations for temperature = 62.5 °C, as modelled by individual regressions and by a mixed-effects omnibus regression for the modified three-parameter Weibull inactivation model. Mean predicted values and 95% prediction intervals are shown for the omnibus regression. Same markers represent observations from the same experiment.

log-reduction. As the minced beef becomes less acidic, the bacterial thermal resistance tends to increase (notice the increase in lethality times up to a pH of approximately 6.0 in Fig. 5A and D); although increasing the temperature at a constant pH will bring about larger reductions in lethality times. However, as the beef matrix becomes more alkaline (pH > 6), there is a tendency for the thermo-tolerance to decrease, and hence increasing the temperature has again a lesser effect on the required lethality times of *E. coli* O157:H7. Because of the strong effect of pH on lethality, in the stepwise regression analyses for both $Ln\ t_{3D}$ and $Ln\ t_{5D}$, pH and pH^2 were the first variables selected with a high significance (Tables 4 and 5). Similar quadratic effects were found for SPP and NaCl as predictors of the lethality times $Ln\ t_{3D}$ and $Ln\ t_{5D}$. As suggested by Fig. 5B, C, E and F, such quadratic relationships were more dispersed than those of pH (Fig. 5A, D). Once again, the quadratic terms indicate that, as the concentration of SPP or NaCl increases from zero, *E. coli* O157:H7 cells in minced beef "tend" to acquire more heat resistance. The fact that the addition of salt to the food matrix increases bacterial thermo-tolerance has also been supported by the experimental results of Mañus et al. (2001) and Juneja et al. (2003). According to Fig. 5B and E, once a maximum lethality time was reached at a SPP concentration of ~0.15% or at a NaCl concentration of ~3% (Fig. 5C and F), increments in SPP or NaCl only undermined progressively the heat resistance (i.e., shorter lethality times). In both regression analyses, the interaction terms of temperature (NaCl × Temperature and pH × Temperature) entered the model with higher significance than the interaction term pH × NaCl (Tables 4 and 5). The interaction of salt and pH with temperature can be visually assessed in the scatter plots of Fig. 5A, C and F. Notice that there was no linear effect of temperature on the lethality times, and furthermore, for both $Ln\ t_{3D}$ and $Ln\ t_{5D}$, the predictors selected by the stepwise regressions were exactly the same (Tables 4 and 5). Once again, this can be explained by the high correlation between both lethality times. The polynomial equations predicting $Ln\ t_{3D}$ or $Ln\ t_{5D}$ were, hence, of the form,

$$Ln\ t_{3/5D_{ij}} = a_1 + a_2 SPP + a_3 pH + a_4 NaCl \times T + a_5 T \times pH + a_6 pH \times NaCl + \ldots = \ldots + a_7 pH^2 + a_8 NaCl^2 + a_9 SPP^2 + \varepsilon_{ij} \quad (7)$$

but with different fitted parameters, as listed in Tables 4 and 5. During the stepwise regressions of $Ln\ t_{3D}$ and $Ln\ t_{5D}$, no influential data points

Table 2
Natural logarithm of the time needed to achieve a 3-log reduction ($t_{3.0}$) of *E. coli* O157:H7 in ground beef from the individual regressions for the 38 experimental conditions.

Expt. cond.	Temp (°C)	pH	Salt (%)	SPP (%)	$t_{3.0}$ (min) (geom. mean)	Mean Ln $t_{3.0}$	Between-expt. St. dev (Ln $t_{3.0}$)	Pooled St. error (Ln $t_{3.0}$)
1	55.0	4.0	0.0	0.00	0.656	−0.421	0.018	0.085
2	55.0	4.0	0.0	0.30	0.584	−0.537	0.013	0.336
3	55.0	4.0	6.0	0.00	0.681	−0.384	0.390	0.180
4	55.0	6.0	3.0	0.15	72.73	4.287	0.312	0.149
5	55.0	8.0	0.0	0.00	19.70	2.981	0.033	0.087
6	55.0	8.0	0.0	0.30	20.74	3.032	0.158	0.115
7	55.0	8.0	6.0	0.00	12.04	2.488	0.043	0.053
8	57.5	5.0	4.5	0.10	33.51	3.512	0.311	0.088
9	57.5	5.0	4.5	0.20	20.47	3.019	0.140	0.115
10	57.5	5.0	1.5	0.10	23.94	3.175	0.016	0.065
11	57.5	5.0	1.5	0.20	27.44	3.312	0.034	0.041
12	57.5	6.0	3.0	0.15	65.05	4.175	0.113	0.093
13	57.5	7.0	4.5	0.10	27.22	3.304	0.406	0.181
14	57.5	7.0	4.5	0.20	14.77	2.692	0.635	0.464
15	57.5	7.0	1.5	0.10	26.34	3.271	0.304	0.064
16	57.5	7.0	1.5	0.20	21.81	3.082	0.038	0.158
17	60.0	4.0	3.0	0.15	0.580	−0.545	0.064	0.151
18	60.0	5.0	3.0	0.15	9.155	2.214	0.009	0.078
19	60.0	6.0	6.0	0.15	15.18	2.720	0.277	0.130
20	60.0	6.0	4.5	0.15	19.11	2.950	0.004	0.152
21	60.0	6.0	3.0	0.00	7.156	1.968	0.028	0.335
22	60.0	6.0	3.0	0.10	6.615	1.889	0.008	0.087
23	60.0	6.0	3.0	0.15	36.48	3.597	0.042	0.072
24	60.0	6.0	3.0	0.20	31.33	3.445	0.155	0.198
25	60.0	6.0	3.0	0.30	7.958	2.074	0.616	0.155
26	60.0	6.0	1.5	0.15	11.81	2.469	0.016	0.076
27	60.0	6.0	0.0	0.15	5.412	1.689	0.040	0.143
28	60.0	7.0	3.0	0.15	41.75	3.732	0.150	0.063
29	60.0	8.0	3.0	0.15	2.272	0.821	0.331	0.142
30	62.5	5.0	4.5	0.10	3.429	1.232	0.235	0.074
31	62.5	5.0	4.5	0.20	3.762	1.325	0.037	0.065
32	62.5	5.0	1.5	0.10	2.514	0.922	0.005	0.033
33	62.5	5.0	1.5	0.20	2.256	0.814	0.008	0.059
34	62.5	6.0	3.0	0.15	4.611	1.528	0.074	0.069
35	62.5	7.0	4.5	0.10	2.978	1.091	0.293	0.053
36	62.5	7.0	4.5	0.20	2.856	1.049	0.306	0.044
37	62.5	7.0	1.5	0.10	4.407	1.483	0.341	0.074
38	62.5	7.0	1.5	0.20	3.417	1.229	0.115	0.042
Pooled SD							0.165	0.123

were identified and all studentized residuals fell within −2 and 2. In both cases, the residuals could be assumed to distribute as a normal (p-values of Shapiro test = 0.33 and 0.14) and their Q–Q plots were close to normality.

The fitted parameters and terms selected for the secondary models predicting the lethality times allowed understanding the separate and combined effects of the environmental conditions on the heat resistance of *E. coli* O157:H7 in minced beef. At a constant temperature, independent of the level of SPP, increasing the pH increases the thermo-tolerance of *E. coli* O157:H7 up to a maximum at a critical pH that lies between 6.0 and 6.7 (critical pH values were calculated from the partial derivatives of the polynomial equation of $Ln\ t_{5D}$ with respect to pH). The exact critical pH value will depend on the temperature and salt concentration as pH has significant interactions with both temperature and salt (Tables 4 and 5). From this critical pH, further increments in pH will only decrease the heat resistance as indicated by the negative quadratic effect of pH. The negative interaction of temperature and pH indicates that the lower the temperature, the higher the pH at which the heat resistance of *E. coli* O157:H7 reaches its maximum (critical pH along an isotherm). For instance, at a temperature of 55 °C and 6% NaCl, the critical pH is 6.45 while at a higher temperature of 62.5 °C with the same salt concentration, the critical pH decreases at 6.0 Likewise, the negative interaction between salt and pH suggests that the lower the salt content, the higher the critical pH. For example, at a temperature of 55 °C and a lower salt concentration of 0%, the critical value increases from 6.45 (previous example) to 6.7.

A similar analysis can be done for NaCl, since regardless of the level of SPP, the addition of salt boosts the heat resistance of *E. coli* O157:H7. Within an isotherm, the maximum thermo-tolerance of the pathogen will be reached at a critical NaCl concentration (2.7–4.7%) that depends on pH and temperature (see interaction terms in Tables 4 and 5), from which lower or higher salt concentrations will progressively undermine the thermo-tolerance. The positive interaction of temperature and salt suggests that, as temperature increases, the salt concentration needs to be higher to become critical. For example, for a constant pH of 4, at a temperature of 55 °C, the critical salt concentration is 4.0%, and increasing the temperature to 62.5 °C raises the critical salt concentration to 4.8%. Contrarily, the negative interaction between pH and salt suggests that the lower the pH, the higher the concentration of salt at which the heat resistance is the highest. For example, at a constant temperature of 62.5 °C, the critical salt concentration decreases from 4.8%, when pH = 4, to 3.4% when pH = 8.

The fact that SPP has a quadratic effect signifies that its addition also boosts the thermo-tolerance of *E. coli* O157:H7, yet the concentration at which the lethality time achieves its maximum is independent of the pH or salt concentration (as indicated by the absence of interaction terms with SPP) and such a value is 0.16% (as determined by the partial derivative of $Ln\ t_{5D}$ with respect to SPP using the polynomial model of Table 5). Salt and SPP were not identified to have an antagonistic influence on the heat resistance of *E. coli* O157:H7 (i.e., no interaction term between them). On the contrary, they acted separately in a similar fashion, both increasing the lethality times up to a maximum — depending on pH and temperature in the case of salt.

Means and prediction intervals for the lethality times can be computed from Tables 4 and 5, as follows. For instance, for minced beef of pH 5.5 treated at 60 °C with 0.05% SPP concentration and no salt

Table 3
Natural logarithm of the time needed to achieve a 5-log reduction ($t_{5.0}$) of *E. coli* O157:H7 in ground beef from the individual regressions for the 38 experimental conditions.

Expt. cond.	Temp (°C)	pH	Salt (%)	SPP (%)	$t_{5.0}$ (min) (geom. mean)	Mean Ln $t_{5.0}$	Between-expt. St. dev (Ln $t_{5.0}$)	Pooled St. error (Ln $t_{5.0}$)
1	55.0	4.0	0.0	0.0	ND	ND	ND	ND
2	55.0	4.0	0.0	0.3	ND	ND	ND	ND
3	55.0	4.0	6.0	0.0	2.281	0.825	0.261	0.142
4	55.0	6.0	3.0	0.15	202.1	5.309	0.538	0.179
5	55.0	8.0	0.0	0.0	34.35	3.537	0.013	0.063
6	55.0	8.0	0.0	0.3	37.53	3.625	0.153	0.080
7	55.0	8.0	6.0	0.0	26.11	3.262	0.012	0.075
8	57.5	5.0	4.5	0.1	67.83	4.217	0.031	0.074
9	57.5	5.0	4.5	0.2	57.04	4.044	0.050	0.083
10	57.5	5.0	1.5	0.1	32.16	3.471	0.008	0.036
11	57.5	5.0	1.5	0.2	40.29	3.696	0.014	0.024
12	57.5	6.0	3.0	0.15	165.0	5.106	0.209	0.208
13	57.5	7.0	4.5	0.1	80.70	4.391	0.115	0.236
14	57.5	7.0	4.5	0.2	52.91	3.969	0.218	0.285
15	57.5	7.0	1.5	0.1	61.68	4.122	0.075	0.064
16	57.5	7.0	1.5	0.2	73.72	4.300	0.204	0.244
17	60.0	4.0	3.0	0.15	1.184	0.169	0.157	0.081
18	60.0	5.0	3.0	0.15	15.36	2.731	0.042	0.049
19	60.0	6.0	6.0	0.15	41.11	3.716	0.361	0.093
20	60.0	6.0	4.5	0.15	43.58	3.775	0.040	0.098
21	60.0	6.0	3.0	0.0	28.13	3.337	0.349	0.258
22	60.0	6.0	3.0	0.1	16.85	2.824	0.024	0.087
23	60.0	6.0	3.0	0.15	54.90	4.006	0.035	0.093
24	60.0	6.0	3.0	0.2	128.5	4.856	0.660	0.507
25	60.0	6.0	3.0	0.3	25.06	3.221	0.159	0.159
26	60.0	6.0	1.5	0.15	17.17	2.843	0.021	0.054
27	60.0	6.0	0.0	0.15	8.578	2.149	0.034	0.143
28	60.0	7.0	3.0	0.15	84.42	4.436	0.214	0.049
29	60.0	8.0	3.0	0.15	7.022	1.949	0.483	0.142
30	62.5	5.0	4.5	0.1	7.298	1.988	0.191	0.077
31	62.5	5.0	4.5	0.2	7.702	2.041	0.018	0.065
32	62.5	5.0	1.5	0.1	3.511	1.256	0.030	0.030
33	62.5	5.0	1.5	0.2	3.265	1.183	0.055	0.041
34	62.5	6.0	3.0	0.15	13.08	2.571	0.001	0.069
35	62.5	7.0	4.5	0.1	6.618	1.890	0.199	0.053
36	62.5	7.0	4.5	0.2	10.28	2.330	0.309	0.044
37	62.5	7.0	1.5	0.1	10.64	2.365	0.497	0.149
38	62.5	7.0	1.5	0.2	4.727	1.553	0.048	0.049
Pooled SD							0.167	0.119

added, the estimated mean value of *Ln* t_{3D} is 1.49 or 4.4 min. The 95% prediction band is calculated using the mean value in the logarithmic scale, and using the standard deviation of $(0.266)^{1/2} = 0.515$. This leads to [0.48–2.50] or [1.62–12.2] min back-transforming to the time scale. The polynomial equations for predicting lethality times can be alternatively derived using the estimates of *Ln* t_{3D} and *Ln* t_{5D} produced by omnibus modelling. Although the estimated lethality times compared reasonably well with those obtained directly from the individual regressions, the standard errors of predictions from the omnibus model were even larger than those already obtained by the secondary models based on the individual regressions (results not shown). For this reason, the approach used in this study was to fit the polynomial equations using the dependent variables, *Ln* t_{3D} and *Ln* t_{5D}, fitted from the 76 individual regressions. The secondary models predicted acceptably the *Ln* t_{3D} and *Ln* t_{5D}, as nearly all 'observations' (values taken from the individual regressions) were within the 95% prediction bands in both cases (Fig. 6A, B). However, a slightly better precision about the predictions of the times to achieve a 5-log reduction is expected. The regression lines of the residuals versus the predicted values of both lethality times were virtually flat while the spread of the residuals over the fitted values appeared nearly homogenous (graphs not shown).

Prior to fitting the omnibus model, the relationships between the primary model parameters (*Ln* χ and *Ln* β) and the environmental conditions were explored by scatter plots and separate stepwise regression analyses. Quadratic effects on *Ln* χ were identified for salt, SPP and pH (see the effects of salt and SPP in Fig. 7A, B). The tendencies were much alike those for the lethality times (Fig. 5B, D). Therefore, the interpretations of the quadratic effects of salt, SPP and pH and their interactions on the lethality times, which have been discussed above, can be extended to the parameter *Ln* χ. This correspondence was not a coincidence since the Weibull parameter χ represents by itself another lethality parameter, related to the time to achieve the first decimal reduction. In fact, the stepwise regression for *Ln* χ ($r^2 = 90\%$) selected the same predictors (i.e., SPP, pH, pH^2, $NaCl^2$, SPP^2, Temp × NaCl, pH × Temp and pH × NaCl with $p < 0.05$; Table 6) as those selected by the stepwise regressions for the lethality times to achieve a 3D and 5D reduction (Tables 4 and 5). This coincidence provided the authors with evidence of the neatness of the models and fitting procedures. Graphically, a certain interaction was found between salt concentration and temperature (Fig. 7A) and between SPP and temperature (Fig. 7B), although the stepwise model only admitted the interaction NaCl × Temperature as significant. The parameter *Ln* β is also influenced by the environmental conditions ($r^2 = 90\%$). In this model, the selected significant predictors were SPP, pH, NaCl, $NaCl^2$, NaCl × SPP, NaCl × pH and SPP × pH. Although in the scatter plot, temperature appeared to have some effect on *Ln* β (Fig. 7D), it was not chosen as a significant variable during the stepwise regression analysis. This was not investigated further. The concentration of salt and pH had the strongest impact on the concavity (*Ln* β) of the inactivation curves. As the salt content increases, the *E. coli* O157:H7 cells become less susceptible, adapting better to the stress (i.e., notice in Fig. 7C that the higher the NaCl content, the lower the values of *Ln* β representing increments in upward concavity). However, increasing the salt content beyond a certain critical concentration, whose value depends on the pH of the matrix, will produce a decrease in the resistance of the cells (i.e., downward concavity). As for the acidity of the matrix, cells tend to adapt better to stress as the pH becomes

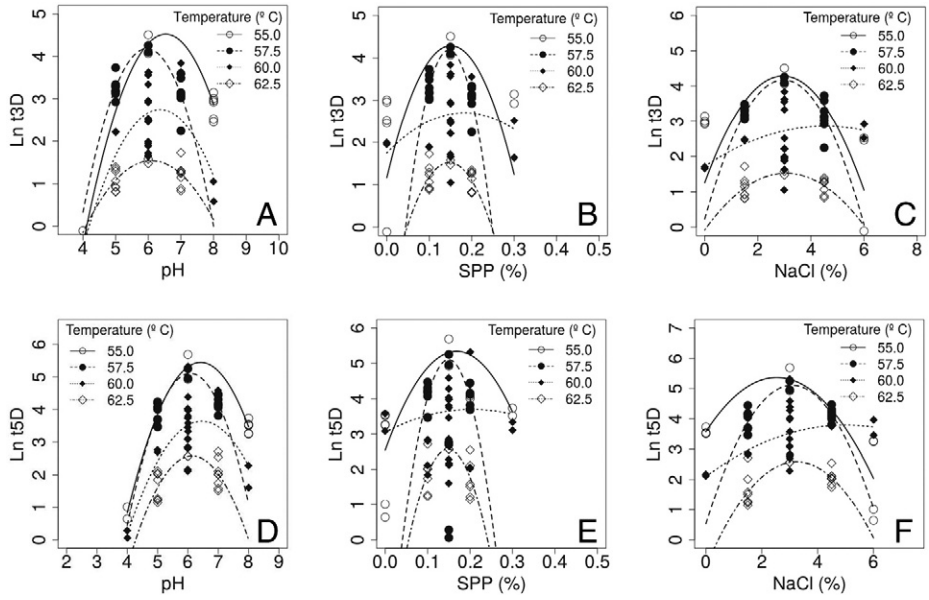

Fig. 5. Scatter plots of estimated values of the lethality times *Ln* t_{3D} and *Ln* t_{5D} from the 76 individual regressions versus environmental conditions.

less acid, producing inactivation curves of upward concavity (scatter plot not shown). Both effects of salt and pH on the concavity of the inactivation curves harmonize well with the influence of salt and pH on lethality times, as previously discussed.

3.3. Omnibus model and validation

In the simultaneous fitting of the primary and secondary models (i.e., omnibus modelling), some of the previously selected predictors turned out to be non-significant ($p > 0.05$). These terms (i.e., SPP, SPP × NaCl, SPP × pH) were all from the linear equation of *Ln* β, and were removed one at a time, while assessing the improvement in log-likelihood, BIC, the histogram of residuals and the residuals versus fitted plot. For the linear equation of *Ln* χ, all the terms remained significant when entered into the omnibus model except for the interaction term pH × NaCl ($p = 0.0878$ in Table 6). Given the importance of this interaction in the calculation of the lethality times (as explained above), it was decided to maintain it in the omnibus model. Thus, the final omnibus model presented a total of twenty-two parameters (Eq. (6)), from which fifteen were fixed effects or predictors of *Ln* χ, *Ln* β and log N_0, and seven were variances of the random effects, their covariance and the residual error. The omnibus model described well all the types of inactivation curves that arouse from the combinations of environmental conditions. Notice in Figs. 1–4 that the omnibus model provided a good coverage of the observed data points as most of the observations lay well within the 95% prediction bands.

Table 6 compiles the parameter estimates for the omnibus model. Analysing the environmental predictors and their p-values, it can be stated that both pH and salt had a larger impact on the inactivation kinetics of *E. coli* O157:H7 than temperature itself, contrarily to what can be expected (i.e., notice that temperature only entered the model as interactions of salt and pH predicting *Ln* χ). The combined effect of salt and pH on the inactivation of *E. coli* O157:H7 may surmount the effect of temperature, at least for the temperature range under study. On the other hand, the effect of SPP appears to be much less pronounced than the effects of salt and pH, as only two SPP terms entered the omnibus model and with higher p-values than those of salt and pH (Table 6). The linear function of *Ln* β did not have temperature as a significant predictor. This is in agreement with the findings of van Boekel (2002), in his assessment of the Weibull model fitted to fifty-five bacterial inactivation cases taken from the literature, who observed that in the majority of cases (48), the shape parameter β was independent of temperature. With regards to the model's random effects, the three variances were all significant ($p < 0.05$) and the correlation coefficients were higher for the random effects of log N_0 with the random effects of the intercept of the linear predictor of *Ln* χ ($r = 0.43$) and with that of *Ln* β ($r = -0.73$) than between the random effects placed on both intercepts ($r = -0.01$) (Table 6). This outcome originated from the large variation in the inoculum size used among experimental

Table 4
Parameter estimates of the polynomial secondary model predicting the natural logarithm of the time to reach a 3-log reduction of *E. coli* O157:H7 in ground beef as a function of temperature, salt concentration, SPP concentration and pH.

Parameters	Mean	Standard error	Pr > |t|	AIC/BIC
Predictors of Ln ($t_{3.0}$)				
a_1 (Intercept)	−23.18	1.873	<.0001	125/149
a_2 (SPP)	10.74	2.934	0.0005	
a_3 (pH)	12.21	0.764	<.0001	
a_4 (NaCl × Temperature)	0.013	0.003	0.0005	
a_5 (pH × Temperature)	−0.074	0.005	<.0001	
a_6 (pH × NaCl)	−0.049	0.023	0.0334	
a_7 (pH^2)	−0.612	0.055	<.0001	
a_8 ($NaCl^2$)	−0.067	0.024	0.0076	
a_9 (SPP^2)	−35.43	9.576	0.0004	
Variance				
s^2 (residual)	0.266		Adj. R^2	0.848

Table 5
Parameter estimates of the polynomial secondary model predicting the natural logarithm of the time to reach a 5-log reduction of *E. coli* O157:H7 in ground beef as a function of temperature, salt concentration, SPP concentration and pH.

Parameters	Mean	Standard error	Pr > |t|	AIC/BIC
Predictors of Ln ($t_{3.0}$)				
a_1 (Intercept)	−24.75	2.002	<.0001	105/128
a_2 (SPP)	7.220	2.761	0.0112	
a_3 (pH)	13.05	0.759	<.0001	
a_4 (NaCl × Temperature)	0.019	0.004	<.0001	
a_5 (pH × Temperature)	−0.078	0.005	<.0001	
a_6 (pH × NaCl)	−0.064	0.026	0.0166	
a_7 (pH^2)	−0.649	0.052	<.0001	
a_8 ($NaCl^2$)	−0.098	0.022	<.0001	
a_9 (SPP^2)	−22.22	9.140	0.0179	
Variance				
s^2 (residual)	0.218		Adj. R^2	0.860

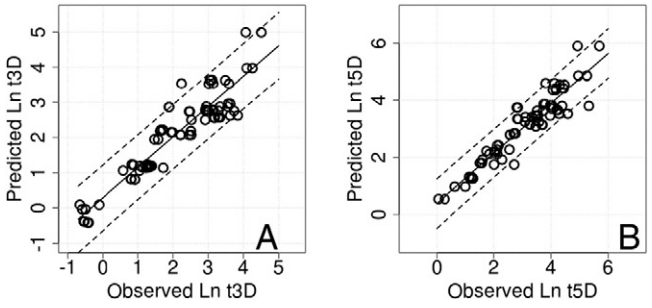

Fig. 6. Scatter plots of the natural logarithm of the time needed to reach a 3-log (A) and a 5-log reduction (B) derived from the individual regressions, and predicted values and 95% probability bounds estimated using the polynomial secondary models in Tables 4 and 5, respectively.

conditions. Hence, the shifts in log N_0 were compensated by shifts in either *Ln* χ or *Ln* β. Interestingly, the intercepts for *Ln* χ and *Ln* β were not correlated, contrarily to the significant covariance that other authors encountered between the Weibull parameters (Juneja et al., 2013; van Boekel, 2002).

Our omnibus model (Eq. (6)) was compared with a larger one comprising the same fixed effects but with a nested error structure reflecting both between-environmental condition variability and within-environmental condition variability. For this nested error model, the BIC criterion increased slightly to 1307 because of the further three nested random-effects parameters. The nested error model, although decreased the residual error from 0.258 to 0.168, was in the end not chosen because the variances of the random effects of replicates within environmental conditions were very low at 0.00045, 0.00122 and 0.00540 for the intercepts of *Ln* β, *Ln* χ and log N_0, respectively. Furthermore, the three coefficients of correlations of the random effects of replicates within environmental condition were all very high (>0.95), and when the nested error model was compared to that of Table 6, a likelihood ratio test revealed that the former did not fit to the data significantly better than the latter. Hence, we opted for the omnibus model considering the random effects only as realisations of the environmental conditions. For this omnibus model, the studentized residuals fell between −3 and 3, and according to the Shapiro–Wilk test, their distribution did not deviate from a normal distribution. Furthermore, the studentized residuals versus the fitted values (i.e., microbial concentrations in time) did not exhibit any singular pattern (graphs not shown). They were randomly spread with a coefficient of correlation of −0.085. Finally, the omnibus model was successfully validated using the leave-one-out method. Predictions for six environmental conditions (#9, 18, 21, 24, 29 and 36) are shown in Fig. 8. Notice that, although each time the omnibus model was refitted, it did not use the data from the environmental condition being tested; the agreement between the predicted survival curve for such condition and its observed data was good in all cases. Such agreement was supported by the bias factor (Bf) of 1.02. While the Bf value suggested that the model may tend to overestimate, yet only very slightly, the microbial concentrations; the accuracy factor (Af = 1.20) indicated that on average predictions are 1.2 factors of difference with respect to observations.

4. Conclusion

In minced beef, salt and SPP act independently increasing the thermo-tolerance of *E. coli* O157:H7 up to a maximum resistance reached at a certain critical concentration. For salt, such concentration is between ~2.7 and 4.7%, and depends strongly on the temperature and pH of the food matrix. At a fixed temperature, the lower the pH, the higher the critical salt concentration. For SPP, the maximum thermo-tolerance of *E. coli* O157:H7 is attained at a critical concentration of ~0.16%, independently of the temperature, the pH of the media

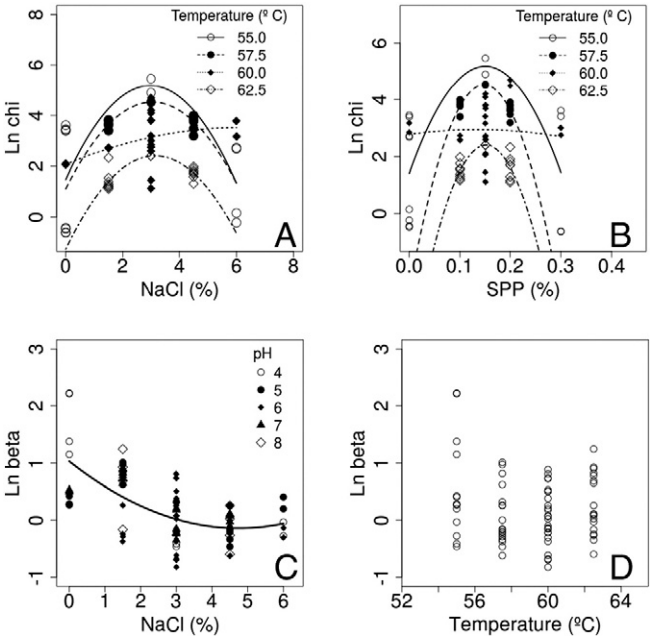

Fig. 7. Scatter plots of estimated values of the Weibull parameters *Ln* χ and *Ln* β from the 76 individual regressions versus selected environmental conditions.

or the salt content. Beyond such critical concentrations of salt and SPP, subsequent increments in concentration will progressively diminish the thermo-tolerance of *E. coli* O157:H7, decreasing the lethality times. A similar pattern was observed for the pH effect: for extreme values of pH (i.e., for instance, pH = 4 or pH = 8), the heat resistance of *E. coli* O157:H7 is lower than in low acidic matrices. Hence, higher times to reach a 3- or 5-log reduction are needed when pH is at a critical value between 6.0 and 6.7, yet this critical pH depends upon the temperature and the salt concentration.

The inactivation kinetics of *E. coli* O157:H7 in ground beef, at any combination of the environmental conditions studied, can be predicted

Table 6
Parameter estimates of the mixed-effects omnibus model predicting the non-log-linear decline of *E. coli* O157:H7 in minced beef as a function of temperature, pH, salt concentration (NaCl) and sodium pyrophosphate concentration (SPP).

Parameters	Mean	Standard error	Pr > |t|	AIC/BIC
Predictors of *Ln* χ				
a_1 (Intercept)	−23.74	1.987	<.0001	1194/1300
a_2 (SPP)	8.227	3.152	0.0092	
a_3 (pH)	12.79	0.820	<.0001	
a_4 ($NaCl^2$)	−0.086	0.026	0.0011	
a_5 (SPP^2)	−23.76	10.39	0.0225	
a_6 (pH^2)	−0.638	0.058	<.0001	
a_7 (pH × NaCl)	−0.043	0.025	0.0878	
a_8 (NaCl × Temperature)	0.014	0.003	<.0001	
a_9 (pH × Temperature)	−0.079	0.006	<.0001	
Predictors of *Ln* β				
b_1 (Intercept)	6.444	0.804	<.0001	
b_2 (pH)	−0.627	0.111	<.0001	
b_3 (NaCl)	−1.449	0.262	<.0001	
b_4 ($NaCl^2$)	0.076	0.021	0.0003	
b_5 (NaCl × pH)	0.115	0.029	0.0001	
Log N_{0mean}	6.838	0.143	<.0001	
Variances				
s^2_u (a_1)	0.174			
s^2_v (b_1)	0.195			
s^2_w (log N_{0mean})	0.652	Correlations		
s^2_{uv} (Cov(a_1, b_1))	−0.003	ρ(a_1, b_1)	−0.014	
s^2_{uw} (Cov(a_1, log N_{0mean}))	0.143	ρ(a_1, log N_{0mean})	0.425	
s^2_{vw} (Cov(b_1, log N_{0mean}))	−0.260	ρ(b_1, log N_{0mean})	−0.731	
s^2 (residual)	0.258			

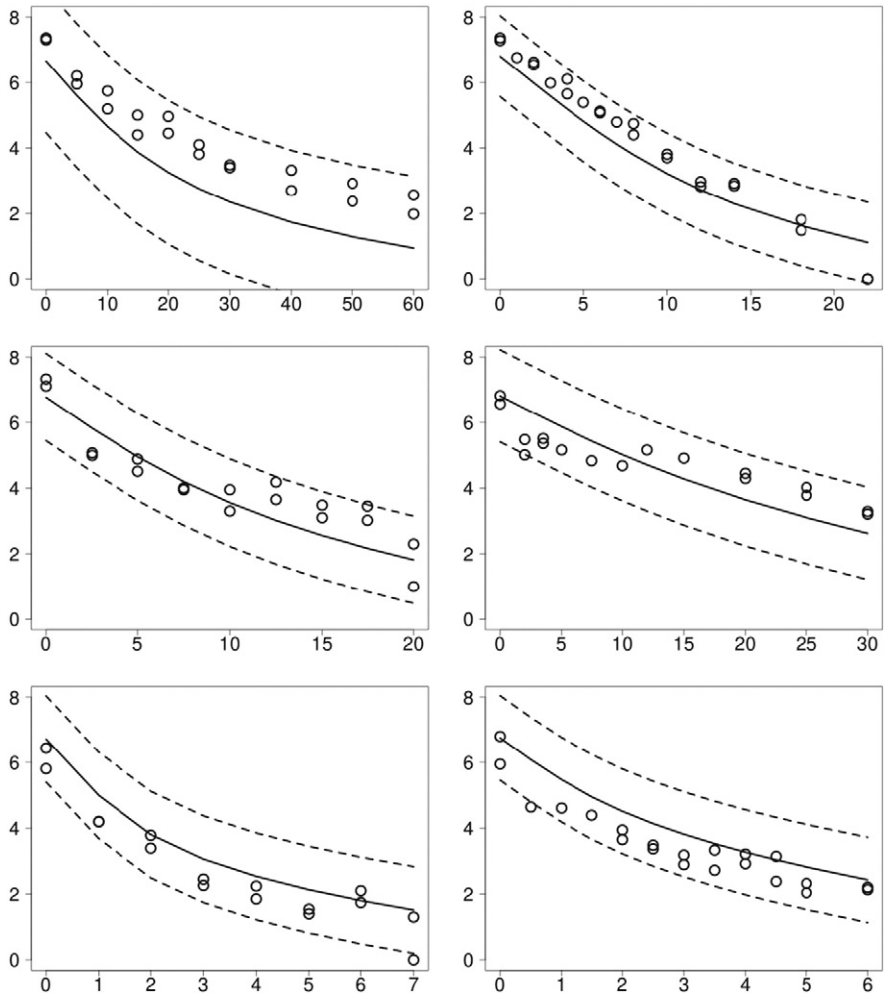

Fig. 8. Mean and 95% confidence intervals of the concentration (log CFU/g in y-axis) of *E. coli* O157:H7 in ground beef against time (min in x-axis), as predicted by the omnibus model refitted leaving out a randomly-selected environmental condition one at a time. Model validation for conditions #9, 18, 21, 24, 29 and 36 is shown (left to right, top to bottom).

by the non-linear mixed-effects omnibus model. According to such a model, the combined and separate effects of pH and salt concentration determine the inactivation dynamics of *E. coli* O157:H7 in ground beef more than SPP. The meat pH and salt content account the most for the curvature of the inactivation curve, represented by the shape parameter of the Weibull model (i.e., as pH or salt increases up to a critical value, cells tend to become less susceptible to heat adapting better to stress); and for the lethality, represented by the scale parameter (i.e., as pH or salt content increases up to a critical value, the time to reach a given lethality also comes up). Finally, the models should assist meat processors in determining the processing times and temperatures required to achieve specific log reductions of *E. coli* O157:H7 in ground beef and ready-to-eat beef products prepared thereof, formulated with a given acidity (pH) and NaCl/SPP concentrations.

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