CHAPTER 3:

Sous-Vide time-temperature profiles

3.1 INTRODUCTION

There is a paucity of data with regards to the survival of *C. jejuni* and *C. coli* in response to cooking in vacuum conditions at low temperatures as in commercial sous vide cooking. In addition, empirical evidence has shown that strains of *C. jejuni* and *C. coli* also differ in their underlying response to stress, whereby some strains and respective genotypes demonstrate greater resistance to heat. The aim of this work is to determine the survival of a range of strains of different genetic backgrounds to exposure under vacuum in a commercial sous vide machine at a range of temperatures and the upper temperature limit at which these strains can grow.

Table 383. Strains, sequence types and source of *C. jejuni* and *C. coli* species used during experimental simulations. Strains in bold are those which were used during sous vide simulations.

Strain ID	Sequence type	Species	Source
11253	(ST-825, CC-828)	*C. coli*	Human/HPA(VTRI Original)
11368	(ST-574, CC-574)	*C. jejuni*	Human/HPA(VTRI Original)
11762	(ST-829, CC-828)	*C. coli*	Human/HPA(VTRI Original)
12610	(ST-825, CC828)	*C. coli*	Poultry/HPA(VTRI Original)
12628	**(ST-1773, CC-828)**	***C. coli***	**Poultry/HPA(VTRI Original)**
12645	(ST-51, CC-443)	*C. jejuni*	Poultry/HPA(VTRI Original)
12662	**(ST-257, CC-257)**	***C. jejuni***	**Poultry/HPA(VTRI Original)**
12720	(ST-51, CC-443)	*C. jejuni*	Poultry/HPA(VTRI Original)
12745	(ST-257, CC-257)	*C. jejuni*	Poultry/HPA(VTRI Original)
13121	(ST-45, CC45)	*C. jejuni*	Poultry/HPA(VTRI Original)
13126	(ST-21, CC21)	*C. jejuni*	Poultry/HPA(VTRI Original)
13136	**(ST-45, CC-45)**	***C. jejuni***	**Poultry/HPA(VTRI Original)**
13163	(ST-21, CC-21)	*C. jejuni*	Poultry/HPA(VTRI Original)

Experimental protocols were developed to determine the growth limit of 14 strains of *C. jejuni* and *C. coli* over a range of temperatures from 37^0C – 48^0C. Furthermore, the survival of two *C. jejuni* and one *C. coli* strains at low temperatures in laboratory media using sous vide cooking over a range of temperatures. In addition, we undertake simulations to determine the survival of these strains following inoculation with *Campylobacter* on the surface of food matrices and from the interiors of food matrices at a range of temperatures, namely 52^0C – 56^0C and 50^0C – 56^0C respectively. The response and survival of all strains examined during experimental simulations were

assessed using predictive models. Models were generated using GInaFiT (1.6) (Geeraerd 2005). GInaFiT is freely available software that offers a flexible user-friendly approach to generating models suitable for use by food production and processing industry.

3.2 METHODOLOGY

Campylobacter was obtained from a culture collection (CC) stock and a strain isolate selected and plated upon Columbia Agar base containing 5% defibrinated horse blood and *Campylobacter* growth supplement FBP). Plates were incubated at 37°C under micro-aerobic conditions for between 48 – 72 hours. The growth of *Campylobacter* colonies was checked periodically to ensure that there was no contamination. A subculture of each colony was obtained and a new CAB-FBP plate inoculated and incubated at 37°C under micro-aerobic conditions for 24 hours.

The bacterial inoculum was prepared by suspending the *Campylobacter* culture in MRD (Maximum Recovery Diluent; 3ml in 7ml bijoux) and the OD_{600} (spectrometer wavelength pre-set to 600nm) checked and adjusted. A sample of 5.5 ml *Campylobacter* Enrichment Broth (CEB) was inoculated with 110µl of the prepared inoculum in a 7ml Bijoux and incubated for 18 hours.

For the temperature growth-limit experiments, *Campylobacter* isolates were grown as described above, with three replicates of each isolate prepared for each temperature tested. Each strain was tested at 37°C, 41°C, 44°C and 45°C, with a reduced set consisting of *C. jejuni* 12662 (ST-257, CC-257) and 13136 (ST-45, CC-45) and *C. coli* 12628 (ST-1773, CC-828) also tested at 46°C, 47°C and 48°C. During all experiments, isolates were re-tested at the standard 37°C to ensure that growth at the standard incubation temperature was consistent.

Whole chicken fillets were partially frozen for approximately 3 hours at -20^{0}C in order to score the surface tissue area using a tissue borer to indicate the area that each fillet would be subsequently inoculated with *Campylobacter* and then stored at 4°C.

Campylobacter growth in CEB was checked at late log-phase following incubation for 18 hours. Each fillet was injected with 100µl of culture broth at the marked points using three replicates, and each fillet was placed individually in to a Sous Vide cooking bag (180mm x 120mm) which was subsequently vacuum sealed. The fillets were incubated at 37°C for one hour prior to overnight storage at 4°C.

Experimental simulations were then undertaken covering a range of potentially inadequate heating temperatures (50°C-56°C). Observations were taken at 0, 20, 40 and 60 minutes. For simulations undertaken at 50°C and 52°C the duration of the experiment was three hours and two hours respectively. Each fillet was placed in the pre-heated sous vide water bath directly from

storage at 4°C to ensure consistent starting temperature conditions. Fillets were removed at given time points and allowed to cool at room temperature prior to removal of the pre-marked meat pieces. These were then added individually to 10ml of CEB and were placed into stomacher bags and treated for 90 seconds in total (60 seconds before mixing and a further 30 second treatment). Serial dilutions were obtained for each replicate at each time point, and plated on CAB-FBP plates (Miles-Misra method) then incubated under micro-aerobic conditions for 48 hours.

The heat resistance of *Campylobacter* strains *C. jejuni* 12662 (ST-257) and 13136 (ST-45) and *C. coli* 12628 in CEB 18-hour culture was tested at 52°C and 56°C under commercial sous vide conditions by adding 2ml of standard overnight broth to a 80mm x 60mm bag prior to sealing. Samples were stored on ice before immersion in the pre-heated Sous Vide water bath.

In addition, the heat resistance of *Campylobacter* isolates *C. jejuni* 12662 (ST-257, CC-257) and 13136 (ST-45, CC-45) and *C. coli* 12628 (ST-1773, CC-828) within small (2g) meat pieces was tested at 52°C and 56°C by inoculating individual pieces with 100µl of standard 18h overnight culture and vacuum sealing in 80mm x 60mm sous vide bags. After sealing, the meat pieces were incubated for 1h at 37°C before overnight storage at 4°C prior to heating at the appropriate temperature in a pre-heated sous vide water bath.

3.3   RESULTS

3.3.1   Growth-Limit Time-Temperature Simulations:

The growth-limit of *Campylobacter* strains (Table 383) to various temperatures was analysed using a linear mixed-effects model. The model assessed differences in the recovery of cells between strains at five temperatures (37^{0}C, 41^{0}C, 44^{0}C, 45^{0}C and 46^{0}C). The global mean number of cells recovered at 37°C is quantified by the Intercept (8.933, P-value = 0.000), whereas estimates for each time-temperature profile are also presented (Table 384). In each instance, an increase in temperature results in a decrease in the numbers of cells recovered (Figure 394). For example, at 41^{0}C the global mean numbers of cells recovered decreases, however, this effect is not statistically significant (-0.431, P-value = 0.866). In contrast, further increases in temperature resulted in significant decreases in the numbers of cells recovered (Figure 394 and Table 384). The numbers of cells recovered at 44^{0}C decreased by an estimated -0.736 (P-value = 0.004). Decreases in numbers were also observed for 45^{0}C and 46^{0}C, -2.385 and -5.825 respectively (P-value = 0.000) (Table 384).

Random-effects components were specified as individual strains. In each case, the random-effects were used to generate best linear unbiased predictions (BLUP). Such values are used to determine the degree to which predictions for an individual random-effect (strain) differs from the global mean (intercept). For instance, BLUP for strain 11762 (ST-1773, CC-828) is 0.756 and indicates

that greater than average numbers of cells were recovered of this strain. When added to the intercept 8.933, BLUP provides an estimate of the numbers of cells recovered for this strain 9.690 (Table 385). In contrast, BLUP for strain 12745 (ST-257, CC-257) is -0.597, indicating that the numbers of cells recovered from this strain are predicted to be lower than average. Similarly, when added to the intercept, BLUP produces a corresponding estimate of 8.336 (Table 385). The rank-order of each strain as determined by BLUP is shown in Figure 395.

Table 384. Linear mixed-effects model for upper-growth limits of *Campylobacter* at different temperatures (R^2 = 0.818).

Estimate	Value	Standard Error	t-value	P-value
(Intercept)	8.933	0.219	40.76	0.000
Temperature 41^{0}C	-0.431	0.248	0.170	0.866
Temperature 44^{0}C	-0.736	0.248	-2.970	0.004
Temperature 45^{0}C	-2.385	0.254	-9.400	0.000
Temperature 46^{0}C	-5.825	0.270	-21.550	0.000

Table 385. Random-effects estimates for each strain corresponding to the Best Linear Unbiased Predictions (BLUP). Strains are ranked according to the combined coefficient estimate.

| Strain ID	Sequence type	BLUP	Coefficient Estimate
11762	(ST-829, CC-828)	0.756	9.689
12628	(ST-1773, CC-828)	0.501	9.434
12610	(ST-825, CC-828)	0.379	9.312
11253	(ST-825, CC-828)	0.379	9.312
12662	(ST-257, CC-257)	0.119	9.052
13136	(ST-45, CC-45)	0.045	8.978
12645	(ST-51, CC-443)	0.037	8.970
12720	(ST-51, CC-443)	-0.031	8.902
13126	(ST-21, CC-21)	-0.061	8.873
13163	(ST-21, CC-21)	-0.186	8.747
13121	(ST-45, CC-45)	-0.370	8.563
12783	(ST-574, CC-574)	-0.476	8.458
11368	(ST-574, CC-574)	-0.495	8.439
12745	(ST-257, CC-257)	-0.597	8.336
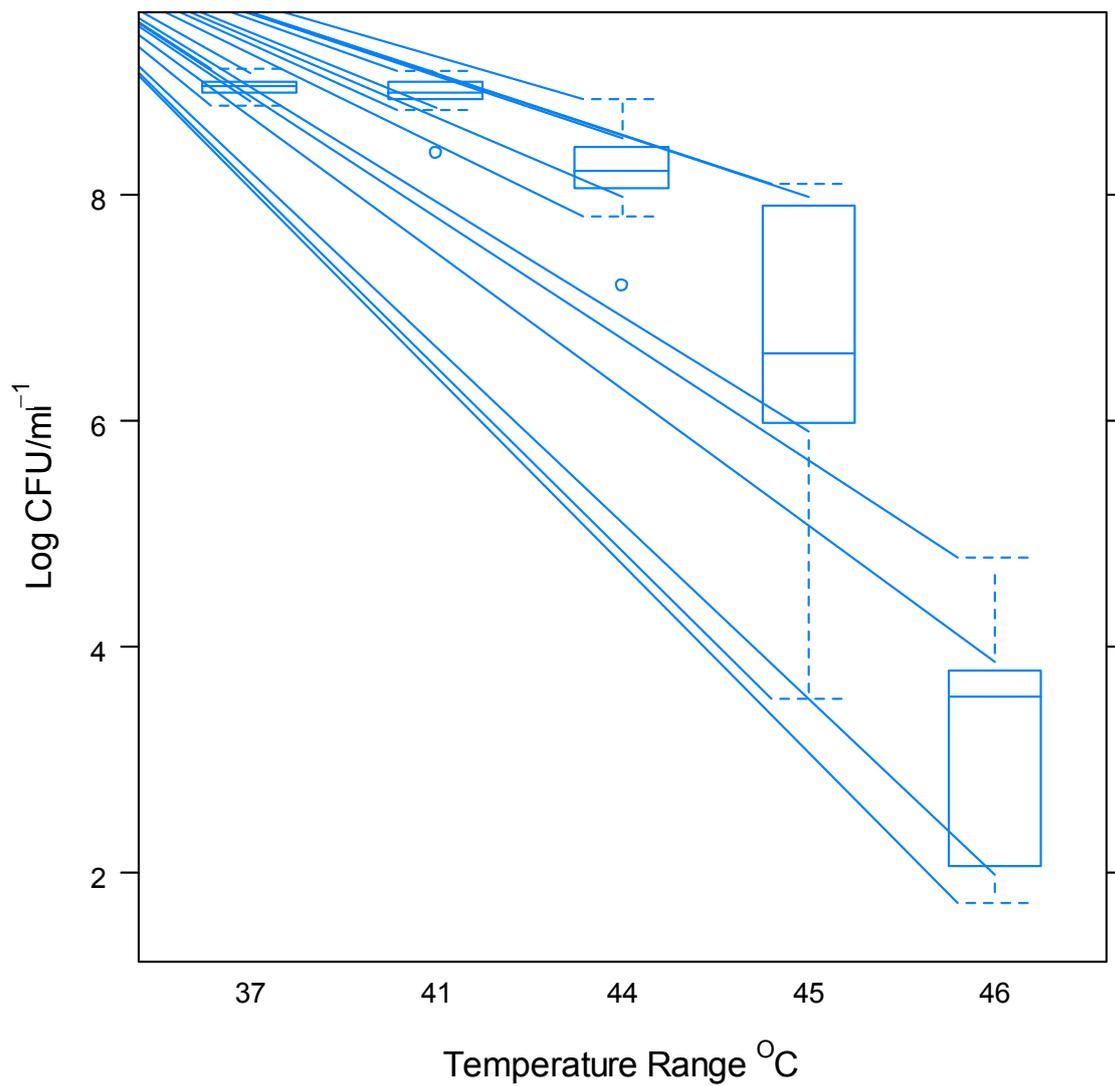
Figure 394. Box-plot illustrating the growth-limits for multiple strains of *Campylobacter* at a range of temperatures.
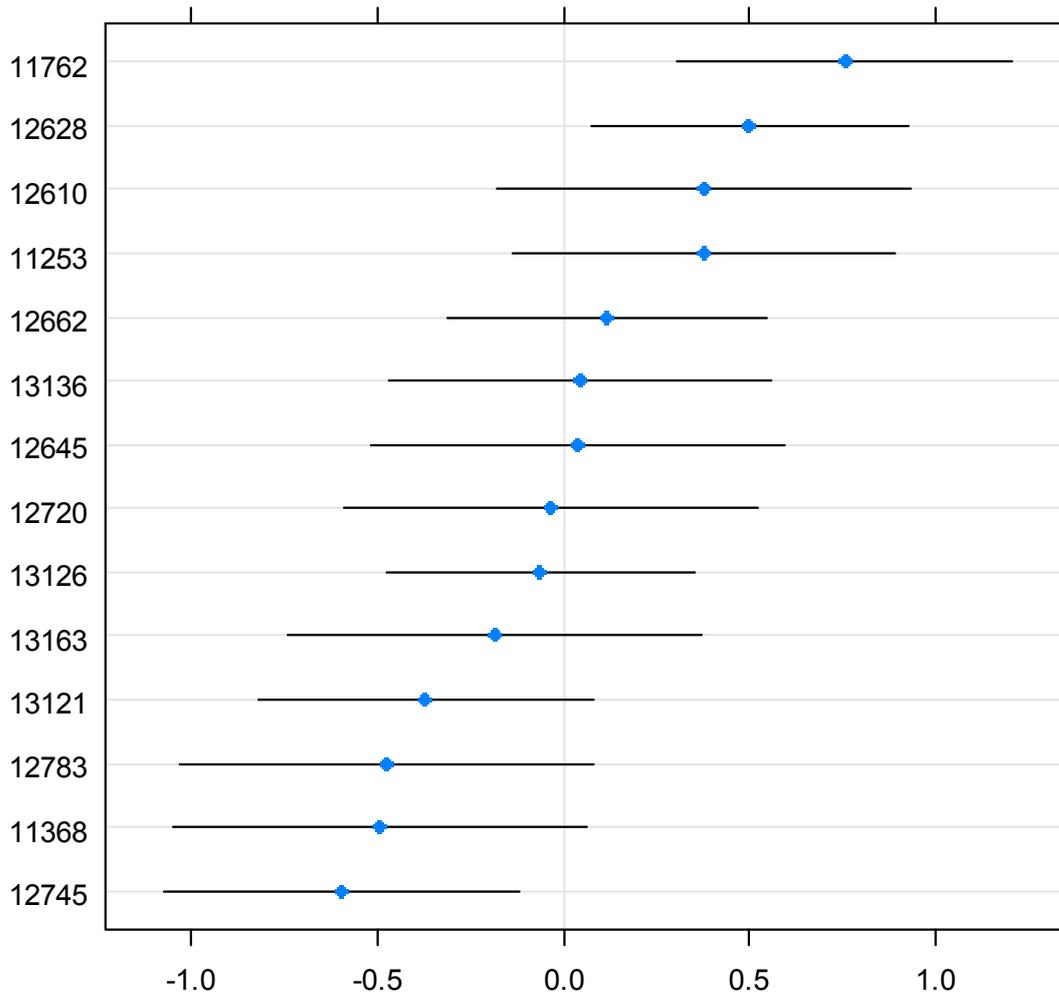
Figure 395. Caterpillar-plot illustrating the rank-order of *Campylobacter* strains as determined by their resistance to higher incubation temperatures. The values shown by the horizontal axis refer to the Best Linear Unbiased Predictors (BLUP) presented in Table 385.

3.3.2 *Experimental Media Simulations and Sous Vide Cooking:*

Predictive models were fit to data using four classes of non-linear model. An assessment of model adequacy is provided by two diagnostic measures of model fit, namely Root Mean Sum of Squared Errors (RMSE) and the adjusted-R^2 (Table 370). The D-value was determined from the model predictions and describes the time to 1 log-reduction in the numbers of cells. The fit of models to data was good. Models demonstrating the highest degree of accuracy were found for strains 12662 (ST-257, CC-257) at 52^0C (RMSE = 0.164, adjusted-R2 = 0.946) and 12628 (ST-1773, CC-828) (RMSE = 0.338, adjusted-R2 = 0.961) at 56^0C. In contrast, accuracy of model fit for strain 13136 (ST-45, CC-45) was lower (RMSE = 0.373, adjusted-R^2 = 0.637). At both 52^0C and 56^0C models predicted higher D-values for strain 12662 (ST-257, CC-257) (12.720 and 2.822 minutes respectively) than compared to other strains and time-temperature combinations (Table 370).

The log-linear model was used to determine the survival for strains 12628 (ST-1773, CC-828) and 13136 (ST-45, CC-45) at 52^0C (Tables 371 and 373), the Weibull model was to describe the response of strain 12662 (ST-257, CC-257) also following heating at 52^0C (Table 372). In contrast, the mixed Weibull distribution model was used to describe the survival of strains 12628 (ST-1773, CC-828) and 12662 (ST-257, CC-257) (Tables 374 and 375) following heating at 56^oC, and a modified Weibull model incorporating an asymptotic function was fit to strain 13136 (ST-45, CC-45) also following heating at 56^0C (Table 376). Parameter estimates for individual models are also provided (Tables 371 – 375). The standard error (SE) relating to the $\delta 2$ parameter estimated by the mixed Weibull distribution model for strain 12662 (ST-257, CC-257) is the same order of magnitude as the parameter estimate (Table 375), an indicator of imprecision in model predictions. This assumption can be validated by examining the corresponding predicted response curve, where variability in observations recorded at 8 and 10 minutes may contribute to relatively high standard error (Figure 385). Predicted response curves are provided for strains 12628 (ST-1773, CC-828) (Figure 3), 12662 (ST-257, CC-257) (Figure 4) and 13136 (ST-45, CC-45) (Figure 383) following heating at 52^0C. In addition, for 12628 (ST-1773, CC-828) (Figure 384), 12662 (ST-257, CC-257) (Figure 385) and 13136 (ST-45, CC-45) (Figure 386) following heating at 56^0C.

3.3.3   Time-temperature Simulations: 52^oC and 56^oC:

Campylobacter Survival in CAB-FBP Media

Table 386. Assessment of individual model fit for strains following gradual heating at 52^0C and 56^0C.

Strain	Challenge Type	RMSE	$R^2_{adjusted}$	D-value
12628 (ST-1773, CC-828)	52^0C	0.360	0.877	6.080
12662 (ST-257, CC-257)	52^0C	0.164	0.946	12.790
13136 (ST-45, CC-45)	52^0C	0.373	0.637	9.240
12628 (ST-1773, CC-828)	56^0C	0.338	0.961	2.426
12662 (ST-257, CC-257)	56^0C	0.599	0.829	2.822
13136 (ST-45, CC-45)	56^0C	0.588	0.899	2.056

Table 387. Log-linear model for survival of strain 12628 (ST-1773, CC-828) following heating at 52^0C.

Parameters	Estimates	Standard Error
Kmax	0.379	0.038
N0	7.007	0.161

Table 388. Weibull model for survival of strain 12662 (ST-257, CC-257) following heating at 52^0C.

Parameters	Estimates	Standard Error
Δ	12.792	0.588
p	2.571	0.413
N0	6.760	0.074

Table 389. Log-linear model for survival of strain 13136 (ST-45, CC-45) following heating at 52^oC.

Parameters	Estimates	Standard Error
Kmax	0.250	0.373
N0	6.810	0.180

Table 390. Mixed Weibull distribution model for survival of strain 12628 (ST-1773, CC-828) following heating at 56^oC.

Parameters	Estimates	Standard Error
α	3.738	0.302
δ1	2.426	0.284
p	2.273	0.470
N0	6.930	0.149
δ2	13.626	5.117

Table 391. Weibull model for survival of strain 12662 (ST-257, CC-257) following heating at 56°C.

Parameters	Estimates	Standard Error
α	1.726	0.588
$\delta 1$	2.882	0.808
p	2.278	1.175
N0	6.832	0.278
$\delta 2$	7.149	2.119

Table 392. Weibull model incorporating an asymptotic function for survival of strain 13136 (ST-45, CC-45) following heating at 56^{0}C.

Parameters	Estimates	Standard Error
Nres	2.369	0.342
δ	2.056	0.610
p	1.362	0.392
N0	6.763	0.308

3.3.4 Time-temperature Simulations 52°C and 56°C:

Campylobacter Survival in CAB-FBP Media
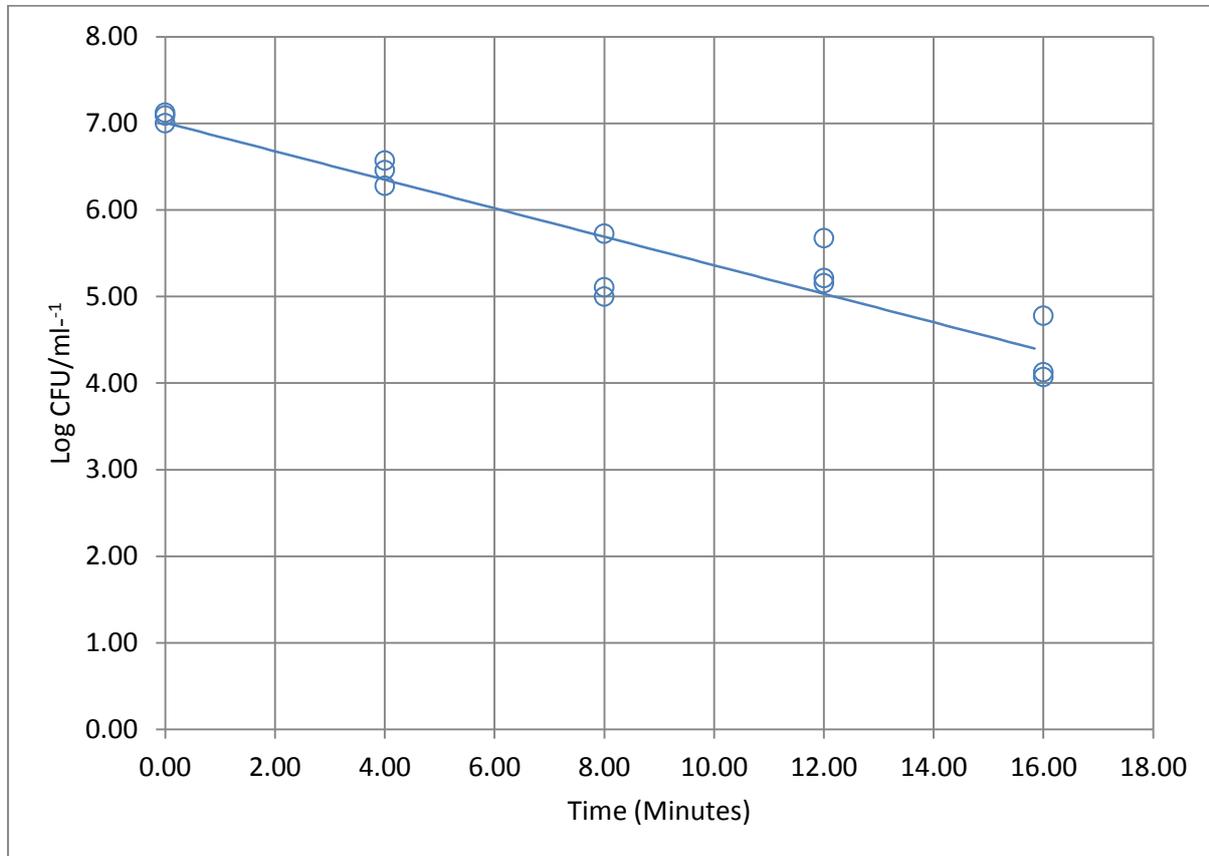
Figure 396. Predicted Response Curve using a Log-linear model for survival of strain 12628 (ST-1773, CC-828) from CEB-FBP media following heating at 52^{0}C in commercial sous vide machine.
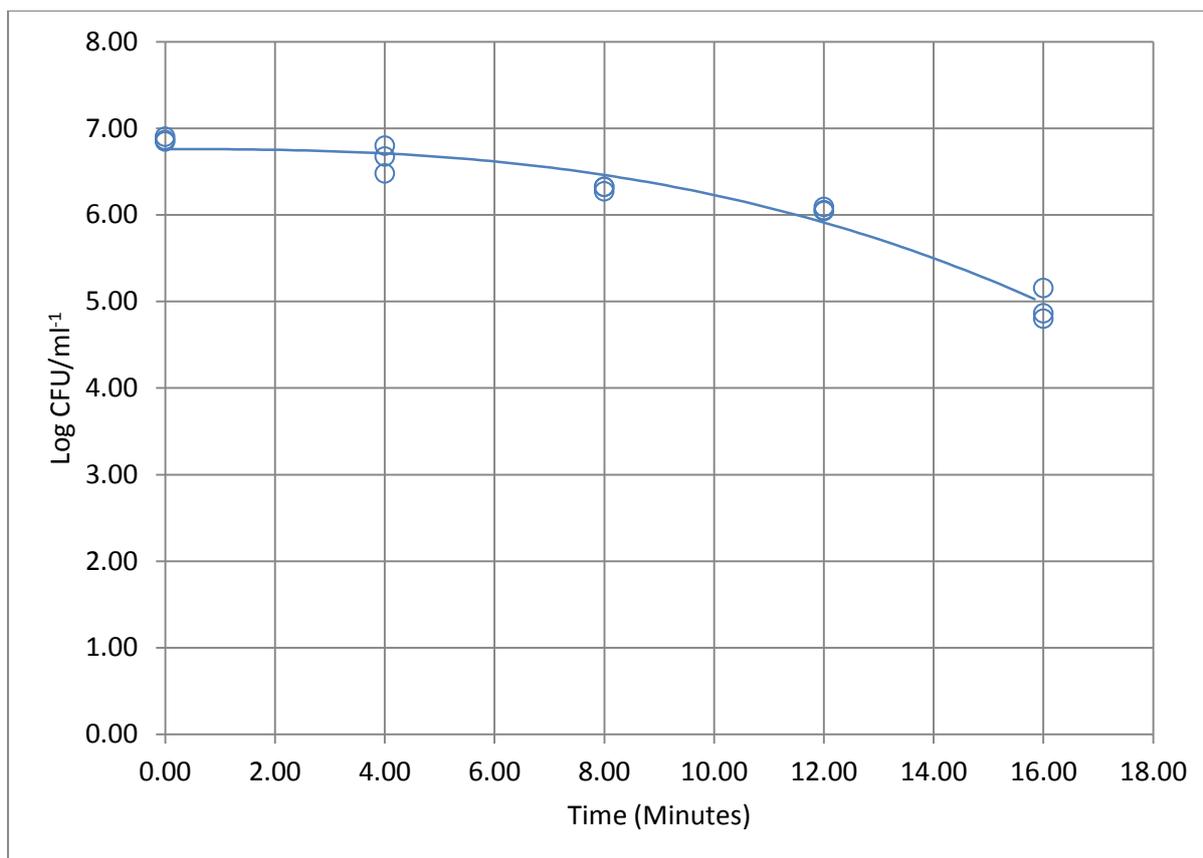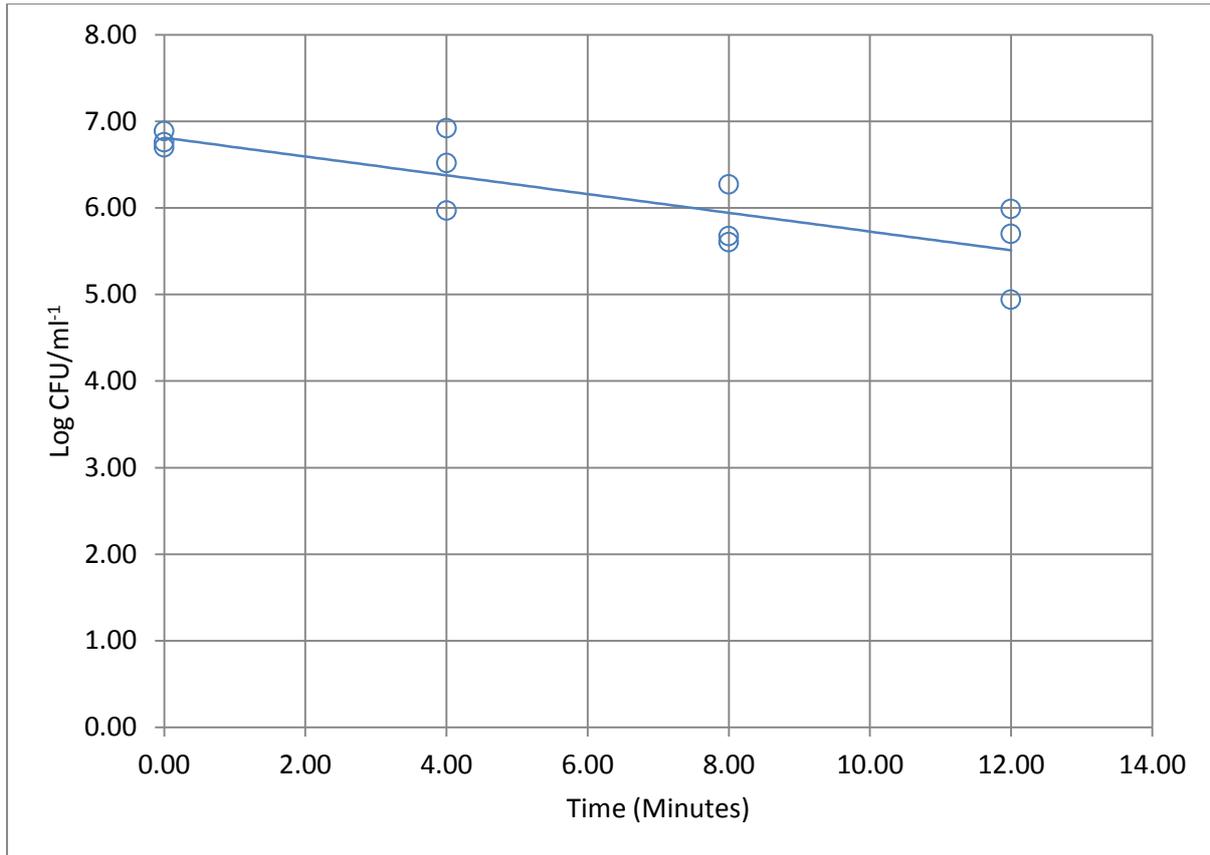
Figure 397. Predicted Response Curve using a Weibull model for survival of strain 12662 (ST-257, CC-257) from CEB-FBP media following heating at 52^0C in commercial sous vide machine.

Figure 398. Predicted Response Curve using a Log-linear model for survival of strain 13136 (ST-45, CC-45) from CEB-FBP media following heating at 52^0C in commercial sous vide machine.
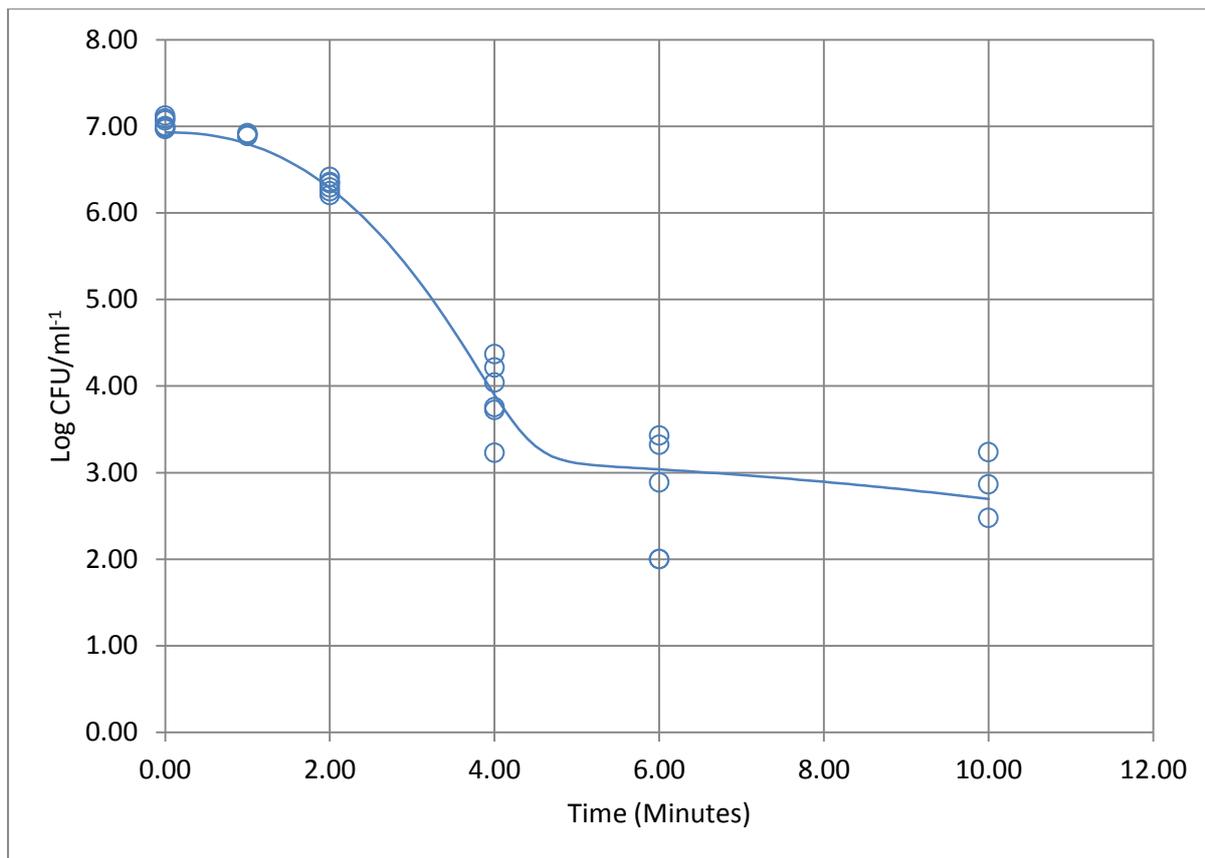
Figure 399. Predicted Response Curve using a mixed Weibull Distribution model for survival of strain 12628 (ST-1773, CC-828) from CEB-FBP media following heating at 52^0C in commercial sous vide machine.
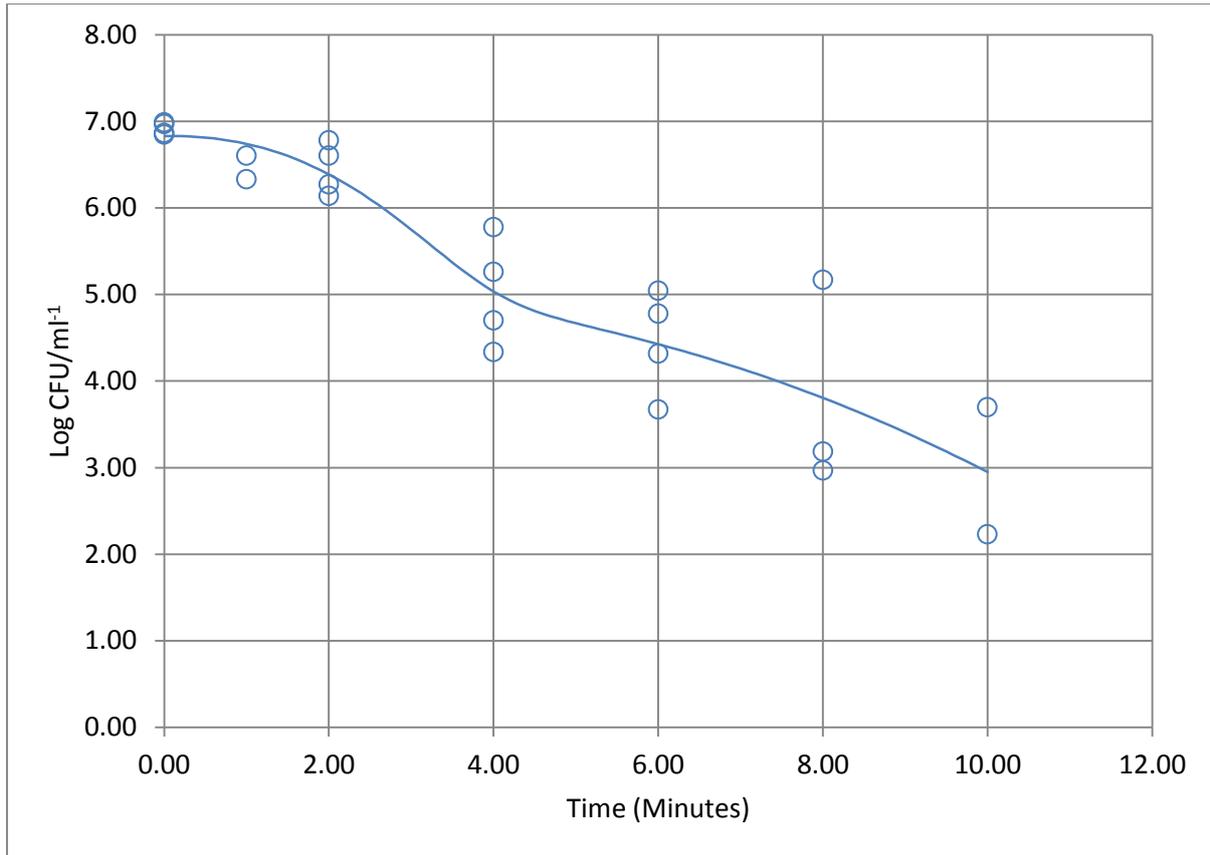
Figure 400. Predicted Response Curve using a mixed Weibull Distribution model for survival of strain 12662 (ST-257, CC-257) from CEB-FBP media following heating at 52^{0}C in commercial sous vide machine.
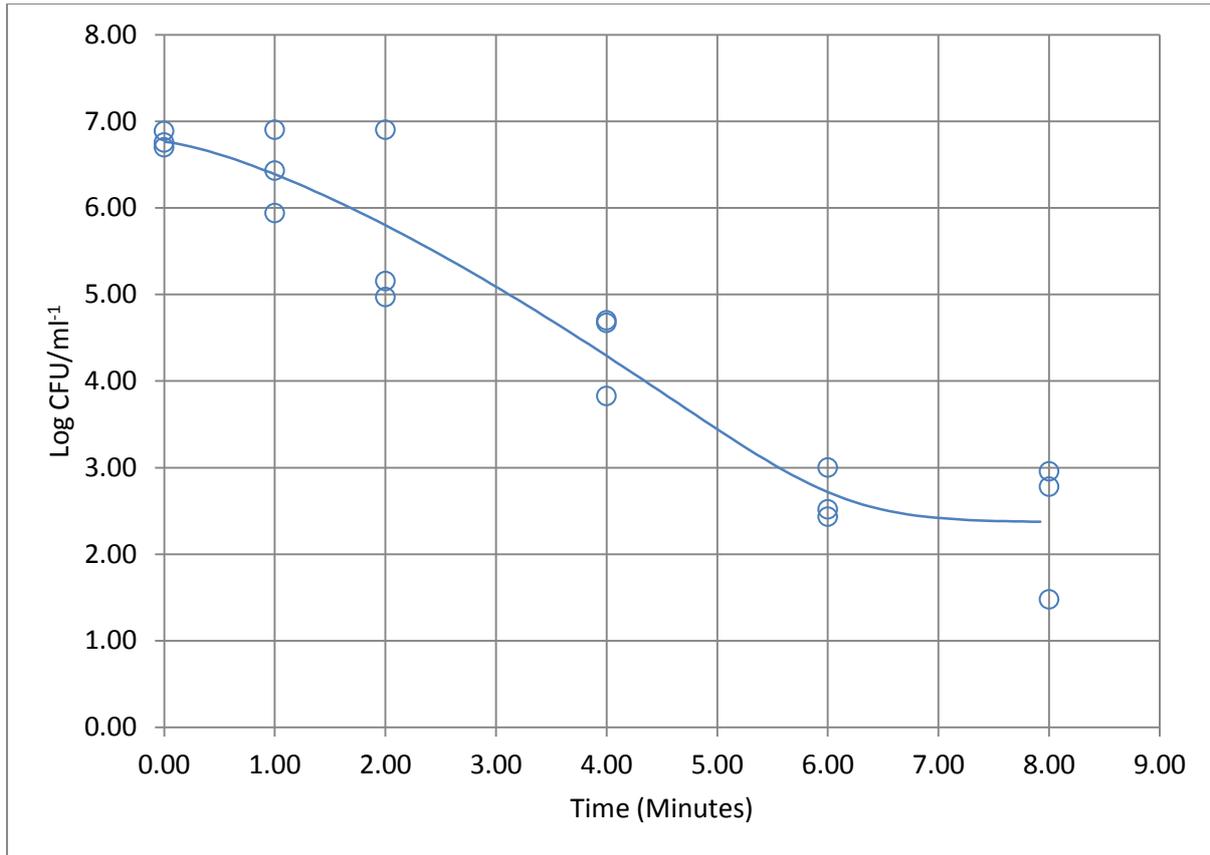
Figure 401. Predicted Response Curve using a mixed Weibull Distribution model for survival of strain 13136 (ST-45, CC-45) from CEB-FBP media following heating at 52^0C in commercial sous vide machine.

3.3.5 Time-Temperature Simulations: 50^0C, 52^0C, 54^0C and 56^0C

Food-matrices and Sous Vide Cooking

Predictive models were fit to data for all experimental simulations with the exception of food matrix simulations for strain 12628 (ST-1773, CC-828) and 12662 (ST-257, CC-257) following heating at 56^0C, where insufficient observations were obtained during sampling due to experimental heterogeneity. Predictive models were fit to data using four classes of non-linear model. An assessment of model adequacy is provided by two diagnostic measures of model fit, namely Root Mean Sum of Squared Errors (RMSE) and the adjusted-R^2 (Table 377). The D-value was determined from the model predictions and describes the time to first decimal reduction in the numbers of cells. Models demonstrating the highest degree of accuracy were found for strains 12628 (ST-1773, CC-828) at 52^0C (RMSE = 0.493, adjusted-R2 = 0.920) and 12662 (ST-257, CC-257) (RMSE = 0.460, adjusted-R2 = 0.906) at 54^0C. The accuracy and overall model fit for strain 12662 (ST-257, CC-257) following heating at 52^0C was lower (RMSE = 0.438, adjusted-R^2 = 0.558). A review of D-values for all strain and temperature combinations revealed that strain 12662 (ST-257, CC-257) to be the most resistant with regards to the time taken to achieve a 1 log-reduction in the numbers of cells (Table 377).

Parameter estimates for all models are presented in Tables 378 – 382. Parameter estimates and corresponding standard errors suggest a high degree of precision in model predictions. The Weibull model was used to determine the survival for strain 12662 (ST-257, CC-257) at 50^0C and 52^0C (Tables 378 and 380). The standard error for parameter estimates of all models indicates high degree of precision in model predictions. The Log-linear model incorporating a shoulder effect, was used to describe the underlying response of strains 12628 (ST-1773, CC-828) at 52^0C (Table 379) and strain 12662 (ST-257, CC-257) at 54^0C (Table 382). In comparison, The Weibull model incorporating an asymptotic function was used to describe the underlying response of strain 13136 (ST-45, CC-45) at 52^0C (Table 380).

Predicted response curves are provided for strains 12662 (ST-257, CC-257) at 50^0C (Figure 387), 12628 (ST-1773, CC-828) (Figure 388), 12662 (ST-257, CC-257) (Figure 389) and 13136 (ST-45, CC-45) (Figure 390) following heating at 52^0C. In addition, predicted response curve for 12662 (ST-257, CC-257) at 54^0C is shown in Figure 391. Predictive models could not be generated for strains during simulations undertaken at 56^oC due to difficulties in recovery of cells.

Table 393. Assessment of individual model fit for strains following direct heating at 50^{0}C.

Strain	Challenge Type	RMSE	$R^2_{adjusted}$	D-value
12662 (ST-257, CC-257)	50^{0}C	0.353	0.888	114.984
12628 (ST-1773, CC-828)	52^{0}C	0.493	0.920	28.005
12662 (ST-257, CC-257)	52^{0}C	0.438	0.558	54.240
13136 (ST-45, CC-45)	52^{0}C	0.535	0.830	27.303
12662 (ST-257, CC-257)	54^{0}C	0.460	0.900	38.110
12628 (ST-1773, CC-828)	56^{0}C			
12662 (ST-257, CC-257)	56^{0}C			

Table 394. Weibull incorporating a shoulder effect for survival of strain 12662 (ST-257, CC-257) following heating at 50^{0}C.

Parameters	Estimates	Standard Error
Δ	114.984	20.919
P	2.147	0.688
$N0$	6.248	0.284

Table 395. Log-linear model incorporating a shoulder effect for survival of strain 12628 (ST-1773, CC-828) following heating at 52^{0}C.

Parameters	Estimates	Standard Error
SI	17.895	4.731
Kmax	0.218	0.002
$N0$	6.237	0.237

Table 396. Weibull model for survival of strain 12662 (ST-257, CC-257) following heating at 52^{0}C.

Parameters	Estimates	Standard Error
δ	52.240	4.640
p	2.301	0.863
$N0$	5.765	0.145

Table 397. Weibull model incorporating an asymptotic function for survival of strain 13136 (ST-45, CC-45) following heating at 52^{0}C.

Parameters	Estimates	Standard Error
Nres	2.966	0.227
δ	27.303	4.909
p	1.952	0.745
$N0$	5.917	0.218

Table 398. Log-linear model incorporating a shoulder effect for survival of strain 12662 (ST-257, CC-257) following heating at 54^0C.

Parameters	Estimates	Standard Error
SI	30.128	2.276
Kmax	0.275	0.025
N0	5.718	0.120

3.3.6 *Sous-Vide Food Matrix Time-Temperature Simulations at 50^{0}C, 52^{0}C and 54^{0}C*

Predicted Response Curves:
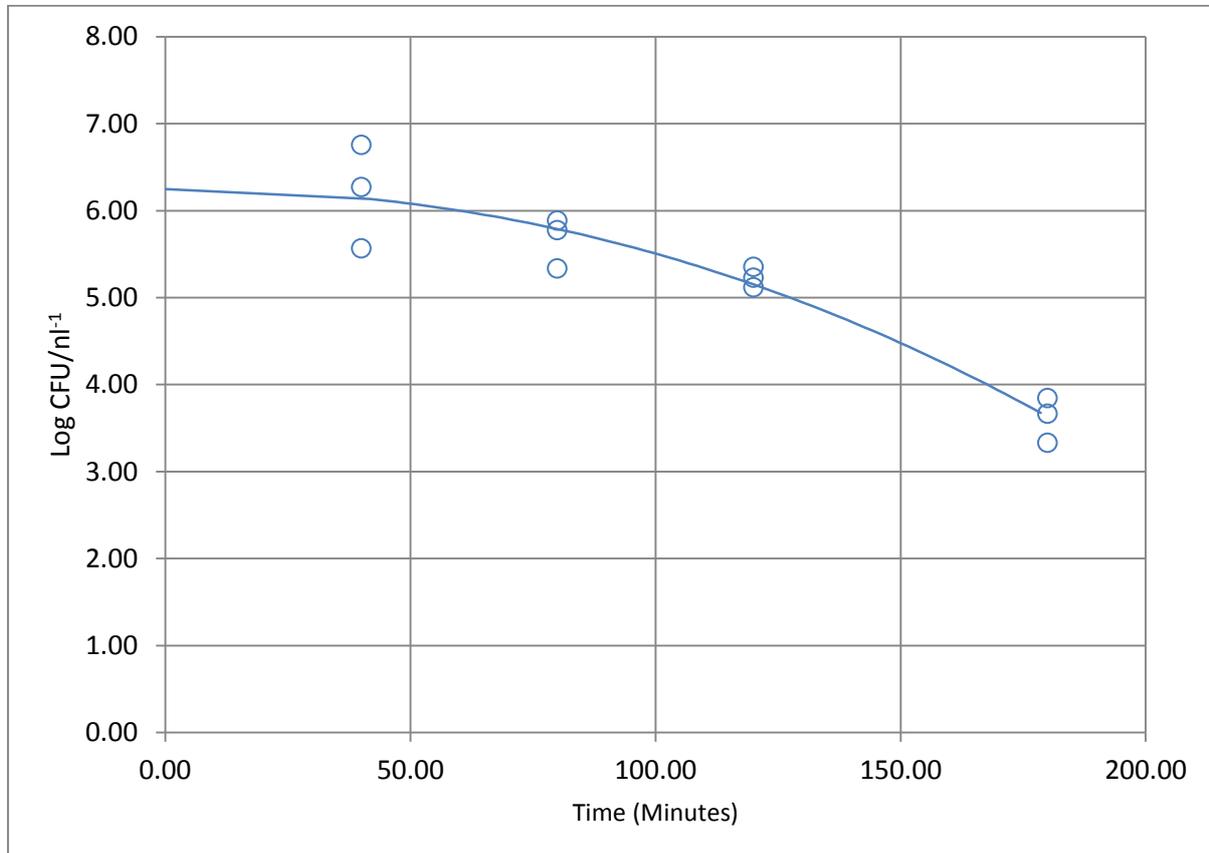
Figure 402. Predicted response curve using Weibull model for strain 12662 (ST-257, CC-257) following heating of food matrices using commercial sous vide method at 50^{0}C.
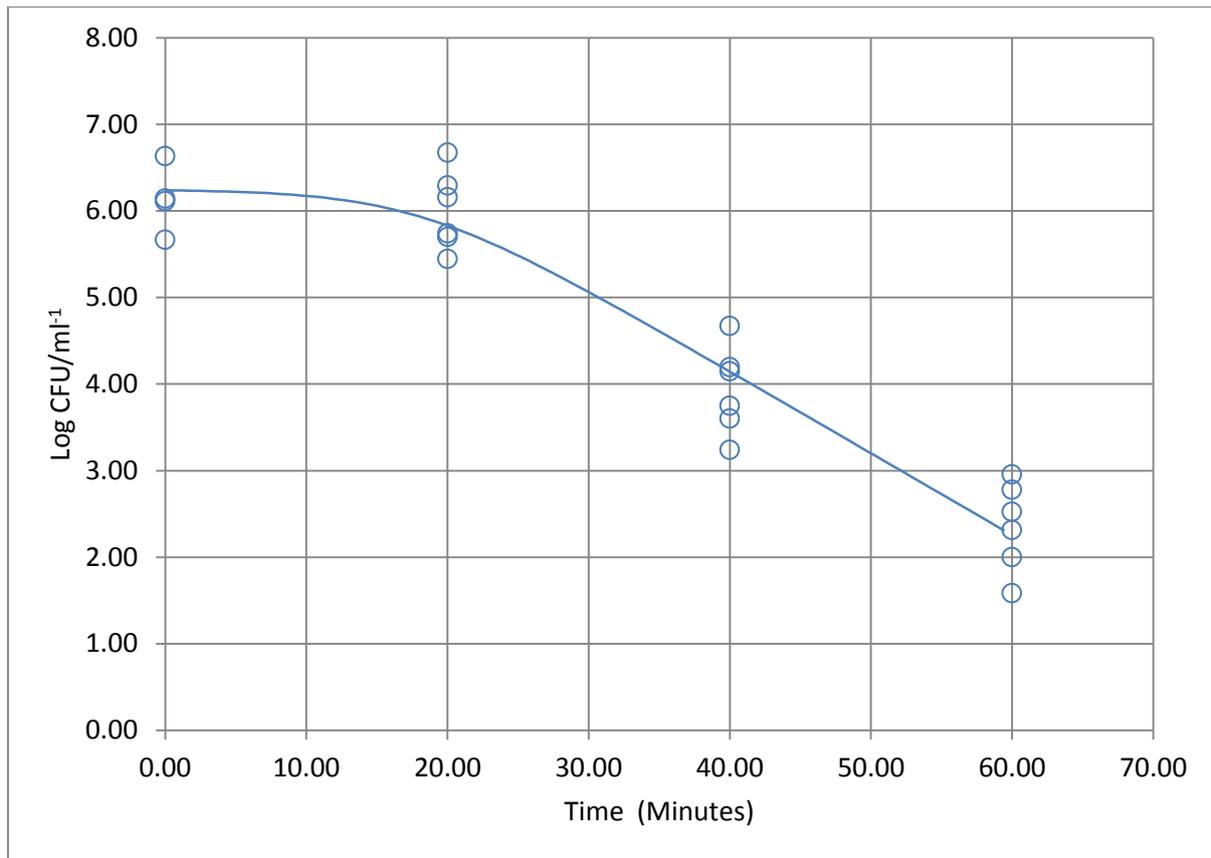
Figure 403. Predicted response curve using a Log-linear model incorporating a shoulder-effect for survival of strain 12628 (ST-1773, CC-828) following heating of food matrices using commercial sous vide method at 52^0C.
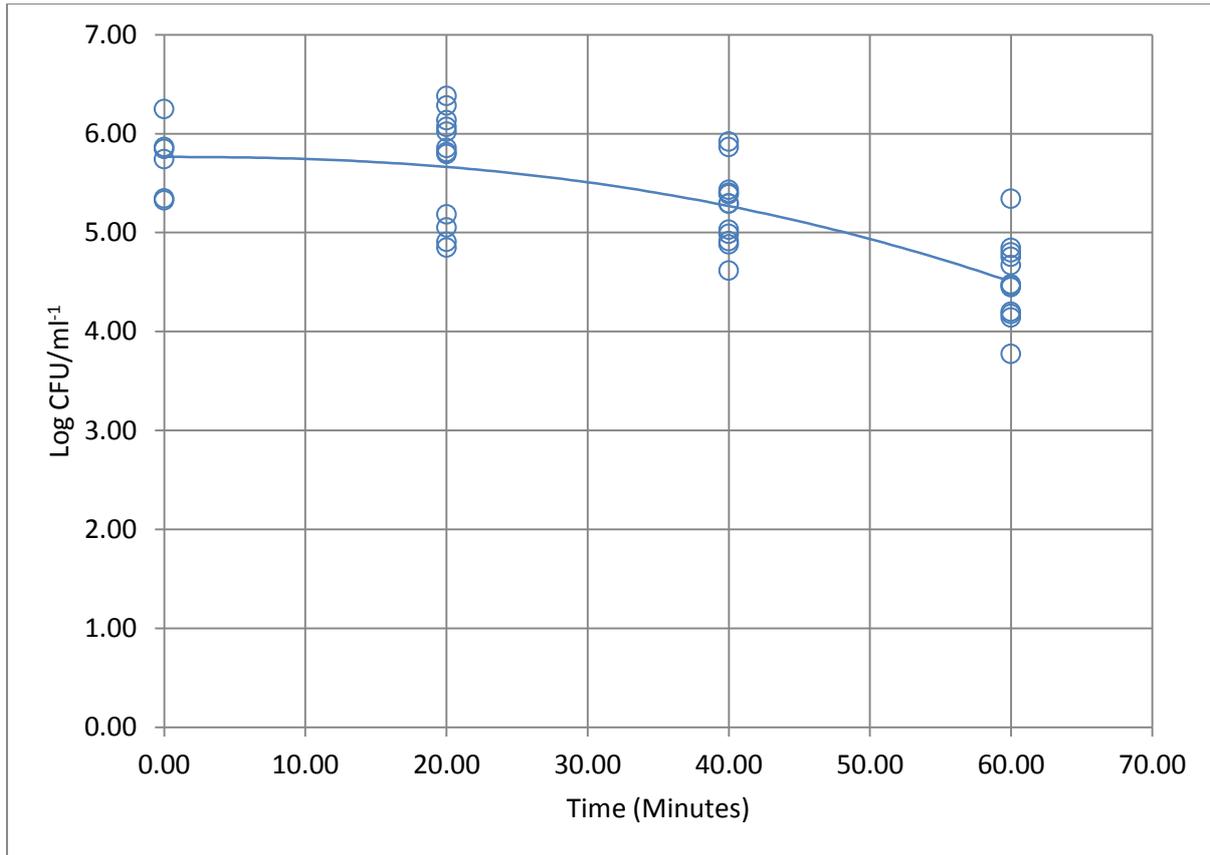
Figure 404. Predicted response curve using a Weibull for survival of strain 12662 (ST-257, CC-257) following heating of food matrices using commercial sous vide method at 52^{0}C.
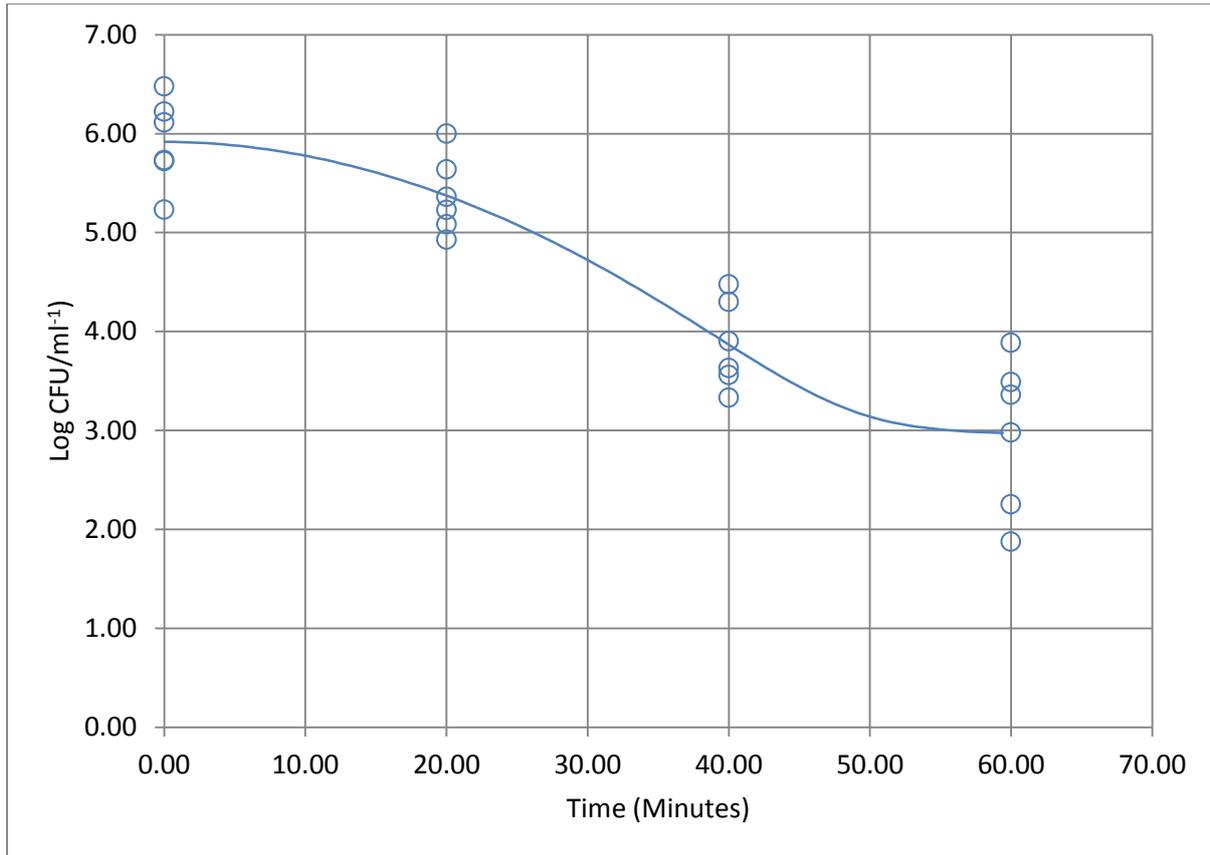
Figure 405. Predicted response curve using a Weibull for survival of strain 13136 (ST-45, CC-45) following heating of food matrices using commercial sous vide method at 52^0C.
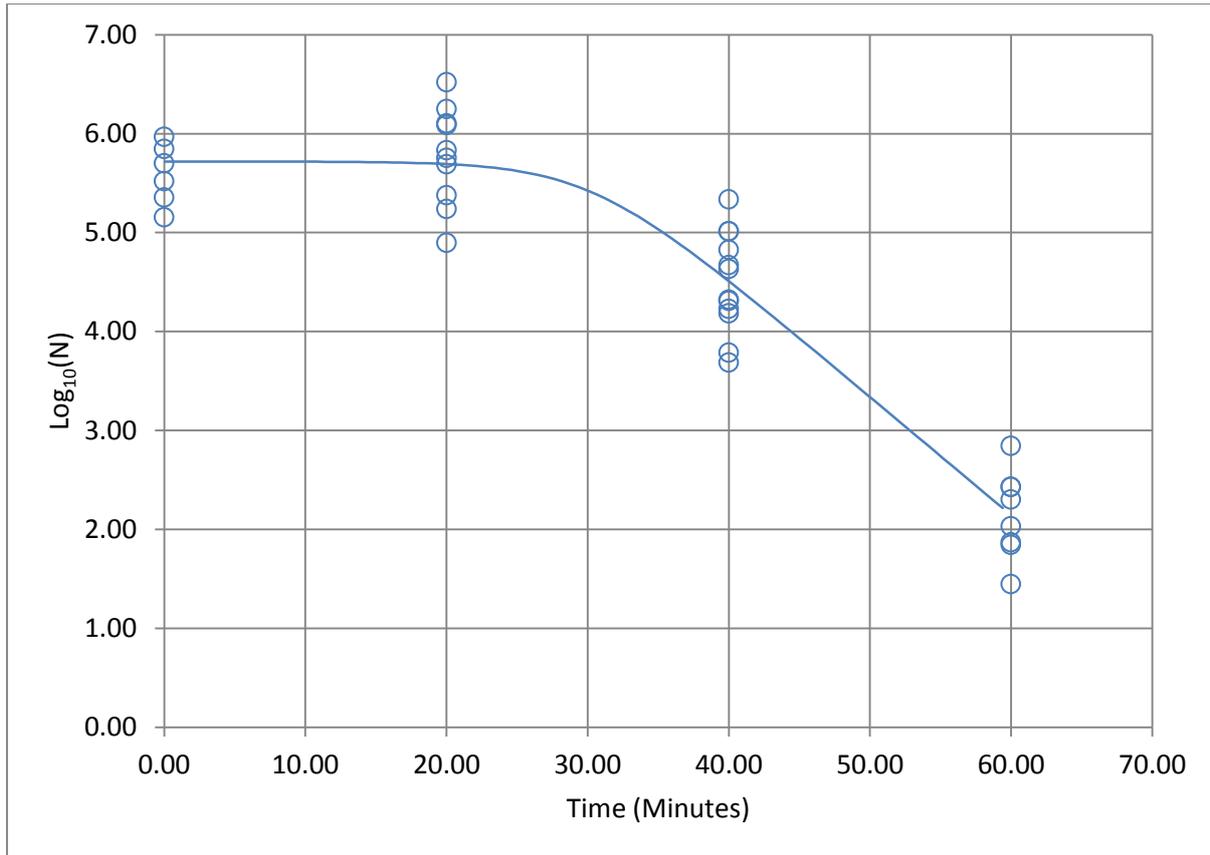
Figure 406. Predicted response curve using a Log-linear model incorporating a shoulder-effect for survival of strain 12662 (ST-257, CC-257) following heating of food matrices using commercial sous vide method at 54^0C.

3.3.7 Time-temperature Simulations: 52^0C

Food-matrices Interior Survival

Predictive models were fit to data describing the survival of *Campylobacter* in the interior of food matrices at 52^0C. Two classes of non-linear model were used. A Weibull model with asymptotic function was fit to data for strain 12628 (ST-1773, CC-828) and a mixed Weibull distribution model was used to describe the underlying response of strain 12662 (ST-257, CC-257). An assessment of model adequacy was provided by Root Mean Sum of Squared Errors (RMSE) and the adjusted-R^2 (Table 383). A high RMSE value (0.637) for model relating to strain 12628 (ST-1773, CC-828) indicates a potentially high level of degree of heterogeneity. This is supported by the corresponding predicted response curve that indicates the presence of heterogeneity at 10 minute sampling point (Figure 14). However, the adjusted-R2 = 0.869 indicates an adequate level of fit to the data. Model fit indices indicate that the mixed Weibull distribution model used to describe the response of strain 12662 (ST-257, CC-257) was also adequate (RMSE = 0.462, $R^2adj.$ = 0.847). The time to 1 log-reduction in numbers of cells was comparable between strains 12628 (ST-1773, CC-828) and 12662 (ST-257, CC-257) (Table 383). Parameter estimates for models for each strain are provided in Tables 384 and 385. The standard error of the estimate for the shape parameter of the mixed Weibull distribution model (Table 385) indicates moderate degree of imprecision. This may in part be due to reduced numbers of observations recorded at later sampling points (20 minutes) and/or heterogeneity in recorded numbers (Figure 381).

Table 399. Assessment of individual model fit for strains following heating using sous vide method and interior survival in food-matrices at 52^0C.

Strain	Challenge Type	RMSE	$R^2_{adjusted}$	D-value
12628 (ST-1773, CC-828)	52^0C	0.637	0.869	5.288
12662 (ST-257, CC-257)	52^0C	0.462	0.847	5.587

Table 400. Weibull model incorporating an asymptotic function for survival of strain 12628 (ST-1773, CC-828) following heating using sous vide method and interior survival in food-matrices at 52^0C.

Parameters	Estimates	Standard Error
Nres	2.121	0.368
δ	5.288	1.537
p	1.847	0.753
$N0$	6.048	0.368

Table 401. Mixed Weibull distribution model for survival of strain 12662 (ST-257, CC-257) following heating using sous vide method and interior survival in food-matrices at 52^0C.

Parameters	Estimates	Standard Error
α	1.579	0.468
$\delta1$	5.587	0.861
p	6.000	4.078
$N0$	6.194	0.283
$\delta2$	17.453	1.946

3.3.8 Time-temperature Simulations: 52^{0}C

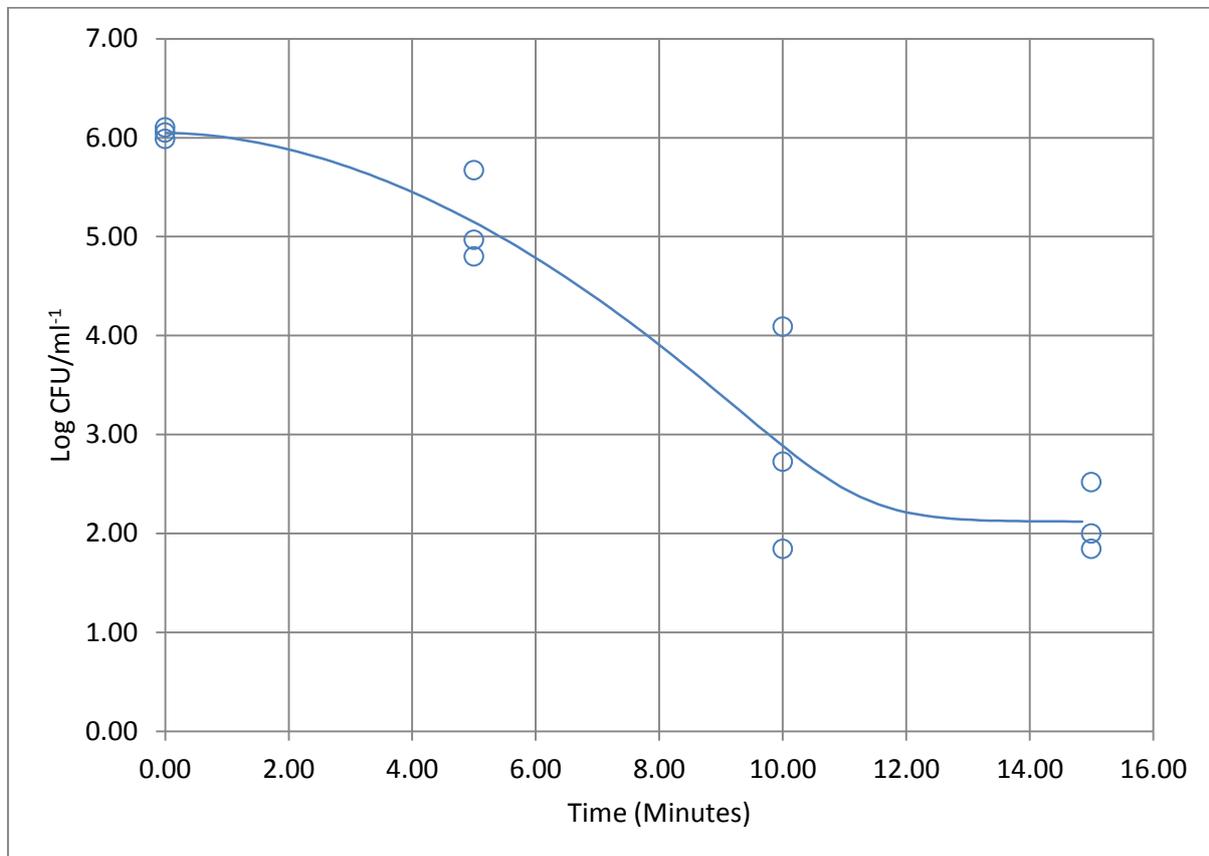*Food Matrices Interior Survival Predicted Response Curves*

Figure 407. Predicted response curve using a Weibull model incorporating an asymptotic function for strain 12628 (ST-1773, CC-828) following heating using commercial sous vide method and interior sampling of food matrices at 52^{0}C.
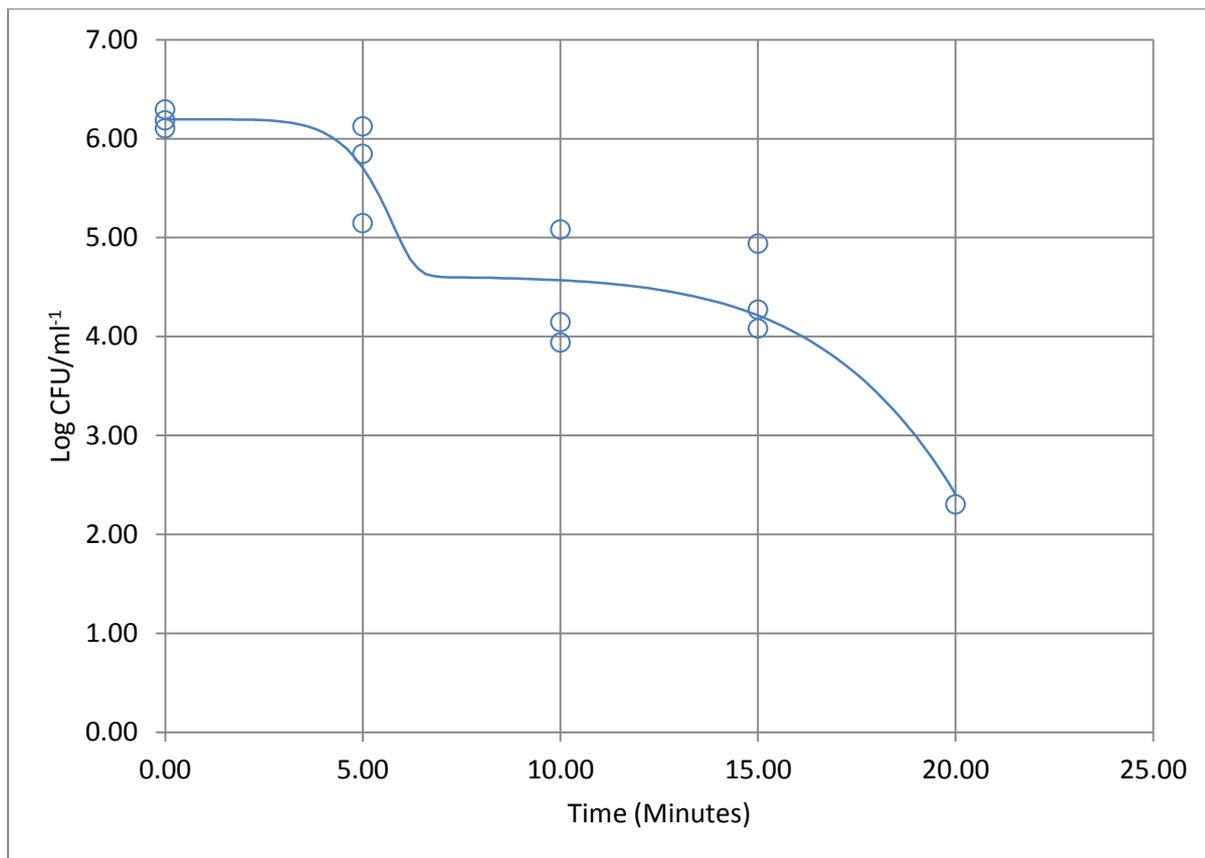
Figure 408. Predicted response curve using a mixed Weibull distribution model for strain 12662 (ST-257, CC-257) following heating using commercial sous vide method and interior sampling of food matrices at 52^{0}C.

3.4   DISCUSSION

Assuming generality in responses between strains for *Campylobacter* growth limits requires caution. Overall, low numbers of observations for each strain has contributed to a moderate degree of variability surrounding BLUP (Figure 395). While the experimental protocol was designed to achieve sufficient replication for each strain, difficulties in obtaining an accurate assessment of the growth-limit during each temperature simulation restricted the maximum number of observations per strain to n = 6. Low sample size may have potentially contributed to the high degree of variability in numbers of cells recorded during higher temperatures (Figure 394). As such, we are unable to exclude the possibility that the rank order assessment identified by the model predictions may also, in part, be influenced by experimental heterogeneity as much as inherent inter-strain variability. Therefore, we suggest caution be exercised when making conclusions governing the resistance of particular strains to heat challenge. Nevertheless, the overall fit of the model indicates that the model performed adequately in explaining the underlying variation in the detection of growth-limits for *Campylobacter* (R^2 = 0.818).

GlnaFiT (Geeraerd *et al.*, 2005) was used to develop predictive models to describe the underlying response of *Campylobacter* strains following exposure to a range of time-temperature challenges. In general, non-linear models selected to analyse the survival of individual strains performed well. We used two model fit indices to assess the predictive accuracy of models, namely RMSE and R^2_{adj}. Although RMSE is considered a more reliable indicator of the overall goodness of fit of a model to data, we retain R^2_{adj} as the classical and most widely used metric for adjudging model accuracy. However, the application and interpretation of RMSE differs to the classical interpretation of coefficient of determination (R^2_{adj}). Values of the R^2_{adj} close to 1.0 indicate a high degree of model fit, whereas the interpretation of RMSE is based upon the minimization of the corresponding test statistic. As such, RMSE not only acts as a measure of model accuracy, but may also signify experimental imprecision. The interpretation and potential cause of higher values of RMSE may be assisted by visual examination of the predicted response curves. High levels of dispersion between observations surrounding individual sampling points may indicate experimental heterogeneity, providing an opportunity to review or refine future experimental design and implementation.

3.5   REFERENCES:

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