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Modelling the isothermal inactivation curves of *Listeria innocua* CECT 910 in a vegetable beverage under low-temperature treatments and different pH levels

S. VEGA[1], D. SAUCEDO[1], D. RODRIGO[1*], C. PINA[1], C. ARMERO[2], A. MARTINEZ[1]

[1]Instituto de Agroquıímica y Tecnología de Alimentos, IATA-CSIC, Valencia, Spain

[2]Departament d'Estadística i I.O., Universitat de València,Valencia, Spain

[*]**Corresponding author:** Dolores Rodrigo, Instituto de Agroquímica y Tecnología de Alimentos, CSIC, Catedrático Agustin Escardino, 7, 46980. Paterna, Valencia, Spain. E-mail: lolesra@iata.csic.es

ABSTRACT

Thermal inactivation kinetics of *Listeria innocua* CECT 910 inoculated in a vegetable beverage at three pH conditions (4.25, 4.75, and 5.20), four levels of temperature (50, 55, 60, 65 °C), and different treatment times (0–75 min) were obtained. Survival curves did not follow a log-linear relationship, and consequently were fitted to various mathematical models: Weibull, Geeraerd, Cerf with shoulder, and the modified Gompertz equation. Results indicated that the best model for the treatment conditions was the modified Gompertz equation, which provides the best goodness of fit and the lowest Akaike information criterion value. Sensitivity analysis indicated that the most influential factors affecting the final microbial load were temperature and time in the case of the higher temperature level (65 °C), and time in the case of the lower temperature level (50 °C).

Keywords: Inactivation kinetic, *L. innocua*, thermal treatment, vegetable beverage, mathematical modeling.

INTRODUCTION

Today there is strong evidence that links the consumption of fruit and vegetables with prevention of some health disorders, such as cardiovascular disease and cancer associated with the absence or low concentration of some nutrients in foodstuffs. A daily intake of around 400 g of fruit and vegetables is recommended owing to the supply of micronutrients and other substances that produce health benefits (Steinmetz and Potter, 1996; WHO, 2003).

A very convenient way that can be used to supply these nutrients is by producing vegetable- and fruit-based beverages. These beverages can be drunk everywhere and not necessarily at home. However, to preserve the nutritional and sensory properties of these products, it is necessary to apply mild preservation processes. In the case of thermal processes, a tendency to use mild processes has been observed in recent years, and these processes are applied together with other control measures to produce the desired preservative effect as a whole (Hurdle Technology). Of course, foods preserved in such a way have a limited shelf life. One of the oldest and most widespread additional measures is acidification of foods. Acidic pH values reduce the heat resistance of microorganisms, directly affecting the primary kinetic parameter “D” (Ocio et al., 1994; Tejedor et al., 2001). However, it is necessary to apply enough heat to produce at least 5 log reductions in the pathogenic microbial load, considering the effect of all the control factors.

To calculate proper preservation processes, survival curves should be fitted to a mathematical model and some kinetic parameters should be deduced. Traditionally, the Bigelow model (Bigelow 1922) has been used to obtain the necessary death kinetic parameters for the development of heat preservation processes. But when minimum preservation processes are used (low processing temperature, high hydrostatic pressure,

pulsed electric fields, etc.) significant deviations from linearity in the survival curves have been observed. The deviations include shoulders or delay periods followed by exponential inactivation, tails, or subpopulations of bacteria that are more resistant to treatment (Fernandez et al., 1999). The use of non-linear modeling strategies to fit these non-log-linear curves is a recent development area. Various models have been developed and used: the survival function associated with a Weibull probability distribution based on the assumption that the stress resistance of a population (survival curve) is a cumulative form of a distribution of lethal events with time (Peleg and Cole, 1998; Fernández et al., 1999; Corradini and Peleg, 2003; Virto et al., 2005; Haimmer et al., 2006); the Cerf model, based on the hypothesis of the existence of two subpopulations with different levels of resistance to stress (Cerf et al., 1977); the Baranyi model (Baranyi and Roberts, 1994); the Geeraerd model (Geeraerd et al., 2000); and Gompertz (Bhaduri et al., 1991), among others. These models are able to adjust to sigmoidal functions.

This study is aimed at describing the behavior of *L. innocua* CECT 910, a nonpathogenic biological surrogate for heat inactivation of *L. monocytogenes* inoculated in a mixed vegetable drink, at three different pH levels (4.25, 4.75, 5.20), under very mild heat. Often biological indicators are used in studies because they are nonpathogenic and have similar physiology and metabolism characteristics to those of pathogenic species (Ponce et al., 1998; Murphy et al., 2002).

The suitability of the above-mentioned mathematical models was estimated from survival data. The discussion and subsequent selection of the most appropriate model for this type of process was based on traditional statistical indicators (Residual Mean Square (RSM), adjusted coefficient of multiple determination, the accuracy factor, and the bias factor), and the Akaike information criterion (AIC).

MATERIAL AND METHODS

Preparation of the mixed vegetable beverage

The composition of the vegetable beverage was: 31% tomato, 17% Italian pepper, 26% water, 9% celery, 5% cucumber, 4% carrot, 3% onion, 0.87% extra virgin olive oil (v/v), 0.2% solution of NaCl, 0.03% basil powder (w / v), and lemon juice. The three levels of pH in the beverage were obtained by varying the amount of lemon juice added; for pH 4.25 the percentage used was 2%, for intermediate pH (4.75) 0.5% was used, and in the absence of lemon juice (0%) a pH of 5.20 was achieved. The pH of the sterilized beverage was controlled by means of a pH meter (Crison Instruments®, GLP 21 pH meter). Beverages with different pH levels were frozen at –80 °C until used.

Microbial culture preparation

Lyophilized samples of *Listeria innocua* CECT 910 were obtained from the Spanish Type Culture Collection. For rehydration, they were transferred to 10 mL of tryptone soy broth (TSB) (Scharlab Chemie SA, Barcelona, Spain). After 30 min, 5 mL of the above culture was inoculated into 200 mL of TSB and incubated at 37 °C under continuous agitation (200 rpm). Forty mL of the resulting culture was transferred to 400 mL of culture medium and incubated for 12 h at 37 °C under continuous agitation (200 rpm). After this time, the culture was centrifuged twice at 4000 × g for 15 min at 4 °C and resuspended in 20 mL of TSB. *Listeria innocua* cells were deposited into sterile plastic cryovials, adding 2 mL of TSB with 20% glycerol in a 1:1 ratio. The cells were immediately stored at –80 °C until used for further studies. The approximate concentration was 5×10^9 CFU / mL

Thermal death studies

For inactivation studies, 1 mL of microorganism culture from cryovials was transferred to 9 mL of previously thawed vegetable beverage; this procedure was repeated for each formulation. Capillaries (BLAUBRAND®, Ref. 78744). previously sterilized at 240 °C for 24 h, were filled with 100 µl of the cell suspension and both ends were immediately sealed with the help of an oxygen-flame. Ten sets of six capillaries were filled for each pH level.

Capillary tubes with microorganism were immersed in a water bath (Haake CF3) and heated at 50, 55, 60, and 65 °C. The heat treatment was isothermal, with a "Come Up Time" (CUT) of 3 seconds, considered as the time needed to reach the same temperature throughout the capillary measured by a type T thermocouple (Control Company, Texas, USA). Capillary tubes were removed from the bath at regular time intervals (0–75 min), immersed in an ice-water bath, and plated to estimate the survivors for each treatment. All treatments were given in quadruplicate. A series of untreated capillary tubes was used as control.

Count of survivors

For the suspension of treated cells and controls a series of decimal dilutions was carried out with sterile peptone water at 1% (Scharlab Chemie SA). The medium used for viable cell enumeration was tryptone soya agar (TSA) (Scharlab Chemie SA). Selected dilutions were incubated at 37 °C for 48 h. The decimal reductions in the number of survivors were calculated from the count of viables.

Mathematical models

Survival curves were fitted to the Weibull, Geeraerd, and Cerf with shoulder models using the GInaFIT tool (Geeraerd et al., 2005) The fit to the modified Gompertz equation was carried out with a nonlinear regression model, using Statgraphics Centurion XV

(StatPoint, Inc.). The models were estimated by using nonlinear least squares assuming that residuals are randomly distributed, following a normal distribution with an average equal to zero (Cunha et al., 1988).

WEIBULL DISTRIBUTION

The survival function of a Weibull distribution has been used to describe the inactivation of microorganisms, determining different single cell resistances to heat treatments. It is identified as follows (Van Boekel 2002; Geeraerd et al.,.,, 2005):

$$\left(\frac{N}{N(0)}\right) = 10^{-\left(\frac{t}{\delta}\right)^{p}} \qquad (1)$$

where N (CFU / mL) represents the final concentration of cells, $N\ (0)$ (CFU / mL) is the initial concentration of cells; t is the time (min); δ is the scale parameter; p is the shape parameter, which corresponds to a concave upward curve if $p < 1$, a downward convex curve if $p > 1$, and if $p = 1$ it describes a linear behavior (Peleg and Cole, 1998).

The reparameterization of the density function included in GInaFIT (Geeraerd et al.,.,, 2005), is the one proposed by Mafart Weibull et al.,., (2002), which makes it possible for parameter δ to represent the time to the first decimal reduction:

$$Log_{10}\left(N\right) = Log_{10}\left(N(0)\right) - \left(\frac{t}{\delta}\right)^{p} \qquad (2)$$

GEERAERD MODEL

This model is based on several arguments that describe the behavior of microorganism inactivation curves when shoulder, log-linear phase, and tail appear (Cerf 1977; Casolari 1988; Mossel et al., 1995; Geeraerd et al., 2000; Geeraerd et al., 2005). Incorporation of

each of the phenomena (shoulder, log-linear phase, and tail) is described by the following formula:

$$N(t) = (N(0) - N_{res}) \cdot e^{-k_{\max} t} \cdot \left(\frac{e^{k_{\max} S_1}}{1 + (e^{k_{\max} S_1} - 1) \cdot e^{-k_{\max} t}} \right) + N_{res} \qquad (3)$$

where N (CFU / mL) represents the final concentration of cells; N (0) (CFU/mL) is the initial concentration of cells; N_{res} is the concentration of residual cells (CFU/mL); k_{max} is the specific inactivation rate (1/unit time); S_1 is the parameter representing the shoulder (time units); t is the time (min).

CERF MODEL WITH SHOULDER

Cerf (1977) proposed a model of two fractions which is formulated as follows:

$$Log_{10}(N) = Log_{10}(N(o)) + Log_{10}(f \cdot e^{-k_{\max 1} t} + (1 - f) \cdot e^{k_{\max 2} t}) \qquad (4)$$

where N (CFU/mL) represents the final concentration of cells; $N(0)$ (CFU/mL) is the initial concentration of cells; f is the fraction of the initial population considered as the bigger subpopulation, (1–f) is the fraction of the initial population considered as the smaller subpopulation (which is more heat resistant than the previous subset); k_{max1} and k_{max2} (1/time unit) are the rates of inactivation for the two subpopulations, respectively; t is the time (min).

GOMPERTZ MODIFIED EQUATION

The initial application of the modified Gompertz equation was to describe sigmoidal growth curves (Gibson et al., 1987). The utility of the formula in describing survival curves was demonstrated by Bhaduri et al., (1991) for the inactivation of *L. monocytogenes* after heat treatments. The empirical formula used has the following form:

$$Log_{10}(N) = A - Ce^{-e^{-B(t-M)}} \quad (5)$$

where N (CFU/mL) represents the final concentration of cells; A is the highest value of the asymptote (CFU/mL); B is the rate of death at M (1/time unit); C is the difference between the highest and lowest asymptote (CFU/mL) values; M is the time (min) at which the absolute death rate is maximal; t is the time (min). A minus sign before the C parameter means inactivation of microorganisms.

From the parameters of the modified Gompertz equation it is possible to derive kinetic parameters related to the growth or death of microorganisms such as maximum growth rate, generation time, and lag phase (McMeekin et al., 1993).

Xiong et al., (1999) made it possible to characterize kinetic parameters related to inactivation of microorganisms through the equations proposed by McMeekin et al., (1993). The inactivation kinetic parameter μ_{max} (maximum death rate) can be determined by the relationship between parameters B and C:

$$\mu_{\max} = \frac{BC}{e} \quad (6)$$

Model comparison

The criteria used to compare the goodness of fit of the models were:

Adjusted coefficient of multiple determination:

$$CorrectedR^2 = 1 - \frac{(m-1)\left(1 - \frac{SSE}{SST}\right)}{(m-j)} \quad (7)$$

Estimated standard deviation of the regression error term:

$$RMS = SD = \sqrt{\frac{SSE}{m - j}} \tag{8}$$

where *m* is the number of observations, *j* is the number of model parameters, *SSE* and *SST* are the residual and total sum of squares, respectively, and *SD* or *RMS* is the standard deviation of the residuals (Reyns et al., 2000; Ly-Nguyen et al.,.,, 2003).

Accuracy factor (Af) and Bias factor (Bf):

These statistics have been generally used in model validation and were proposed by Ross (1996) with a set of *m* randomly selected experimental data reserved for this purpose:

$$A_f = 10^{\sum \left| \log\left(Y_p / Y_o\right) \right| / m} \tag{9}$$

$$B_f = 10^{\sum \left(\log\left(Y_p / Y_o\right) \right) / m} \tag{10}$$

where *m* is the total number of observations; *Yo* represent the observations of the response variable of the new set of data, and *Yp* the subsequent predicted values set with the initial regression model.

Information theory criteria for model selection

It is perfectly reasonable that several models would serve nearly equally well in approximating a set of data. Inference must admit that there are sometimes competing models and the data do not support selecting only one. Using the Principle of Parsimony, if several models fit the data equally well, the one with the fewest number of parameters might be preferred; however, some consideration should be given to the other (few) competing models that are essentially tied as the best approximating model.

For this type of analysis the Akaike information criterion (*AIC*) was used (Burnham and Anderson 2002; Stoica and Selen 2004)

$$AIC = m \ln SSE - m \ln m + 2j \tag{11}$$

where m is the number of observations, j is the number of model parameters, and *SSE* is the residual sum of squares.

The first term of the Akaike statistic decreases with the number of parameters and increases with the number of data, the second increases with the number of data, and the third increases with the number of parameters. The smaller the *AIC* computed value, the better the subsequent model can describe a particular data set (Burnham and Anderson, 2002; Stoica and Selen, 2004).

Burnham and Anderson (2002) strongly recommend using the corrected Akaike information criterion, *AICc*, when m is small or j is large. Since *AICc* converges to *AIC* as m becomes larger, *AICc* should generally be employed regardless. Thus, *AICc* is *AIC* with a greater penalty for extra parameters. *AICc* can be calculated by the following equation:

$$AICc = AIC + \frac{2j(j+1)}{m-j-1} \tag{12}$$

Note that when all the candidate models have the same dimension j, *AICc* and *AIC* will give identical (relative) results. In this situation, *AIC* can always be used. Because *AIC* or *AICc* is on a relative scale, computing the *AIC* differences for each M_i model is recommended rather than the actual *AIC* or *AICc* values

$$\Delta i = AICi - \min AIC \tag{13}$$

where *min AIC* corresponds to the best candidate model.

Models for which $\Delta i \leq 2$ have substantial support and should receive consideration when making inferences. Models having Δi between 4 and 7 have considerably less support, while models with $\Delta i \geq 10$ have essentially no support, and might be omitted from further consideration because they fail to explain some substantial variability of the data.

It is useful to normalize the model likelihoods so that they sum to 1 and treat them as probabilities. The *AIC* value for each model *Mi* can be transformed to construct the so-called Akaike weights, *Wi,* which for each model can be interpreted as the probability that *Mi* is the best model, given the data and the set of candidate models (e.g., Burnham and Anderson, 2001).

$$Wi = \frac{\exp\left(-\frac{\Delta i}{2}\right)}{\sum_{i=1}^{I} \exp\left(-\frac{\Delta i}{2}\right)} \tag{14}$$

Sensitivity analysis

Sensitivity Analysis allows determination of the effects of variable inputs on variable outputs. In this study the input variables are the initial number of microorganisms, the temperature, the pH value, and treatment time, and the output variable is the number of survivors for each combination of temperature, initial number of microorganisms, pH, and treatment time. To perform this analysis, @Risk from Palisade was used.

Statistical analysis

To evaluate the effect of time and temperature intensity on microbial inactivation of *L. innocua,* multivariate analysis of variance (ANOVA) models were used. To determine the significant levels associated with the difference between any pair of means, a multiple range test (MRT) was applied, using the Fisher distribution (LSD) for the equality of

variances. All statistical computations were done with Statgraphics Centurion XV (StatPoint, Inc.).

RESULTS AND DISCUSSION

Survival curves

Inactivation curves for *Listeria innocua* cells inoculated in a vegetable beverage were obtained at three pH levels (4.25, 4.75, 5.20), 4 temperature levels (50, 55, 60, and 65), and various exposure times (0–75 min) (Figure 1).

As can be seen in the figure, the survival curves have non-linear behavior and there is a noticeable presence of shoulders and tails for the various temperature and pH levels. This type of behavior is very common in minimum processes and requires the use of mathematical models other than the log-linear Bigelow model (Bigelow 1922). The curves clearly show that increasing the temperature and exposure time increases the inactivation achieved.

Significant differences ($p<0.05$) in the logarithm of the final cell count for each temperature and time combination were observed. Likewise, a marked effect of pH was observed at each temperature studied.

As the pH of the beverage decreases, the number of microorganisms surviving each treatment decreases too, the effect being greater as the temperature increases. At the highest temperature (65 °C) (figure 1d), more than five log unit reductions were achieved for times lower than 1 min in the cases of pH 4.25 and 4.75 and lower than 2 min. at pH 5.20 . This difference could be due to the combined effect of temperature and pH on the variation of exposure time to be applied to achieve significant reductions of the initial population of microorganisms.

Fitting of mathematical models

Given the shape of the inactivation curves, four models frequently used in kinetic studies were selected: the Weibull frequency distribution model (Equation 2); the Geeraerd model (Equation 3); the Cerf with shoulder model (Equation 4), and the Gompertz modified equation (Equation 5) (Bhaduri et al., 1991; Linton 1995; Xiong et al.,1999; Chen and Hoover, 2004; Geeraerd et al., 2006; Buzrul et al., 2008).

The various non-linear regression models considered in this study produced different estimations of the respective parameters for each temperature and pH combination. Table 1 shows the values for the various kinetic parameters describing the inactivation of microorganisms, as well as the significant differences ($p<0.05$) for each temperature and pH combination. As can be seen, only the μ_{max} kinetic parameter of the Gompertz equation varied as the treatment temperature varied: for all pH levels, the parameter increased as the temperature increased. Nevertheless it is necessary to note that in the case of the Weibull model, the scale parameter (δ) considered as a reaction rate constant (Cunha et al 1998), could not be a function of temperature if the shape parameter (p) value is not constant for all "δ" values corresponding to one pH value (Mafart et al., 2002).

The suitability of each model for fitting the experimental data was evaluated by R^2_c (Equation 7) and *RMS* (Equation 8), (Table 2). The regression coefficients obtained were significant at 90% in all cases except 50 °C and pH 4.75 and 65 °C and pH 5.20, where they were significant at 80%. According to these results, for each pH level it was the Gompertz modified equation that presented the best goodness of fit, followed by the Geeraerd and Cerf models, the Weibull distribution function being the last one. Figure 2 shows an example of model fitting.

Model validation

To assess the ability of a model to predict the response of microorganisms under certain environmental conditions, the Accuracy Factor (A_f) and the Bias Factor (B_f) have been proposed (McMeekin et al., 1993; Ross 1996). In this paper, the validation of the mathematical models was carried out by these indices, (A_f) (Equation 9) and (B_f) (Equation 10), in a set of experimental data not used previously in the estimated regression models. The values of these two factors for each temperature and pH combination are shown in Table 3.

The Accuracy Factor (A_f) indicates the difference between the observed and predicted values. Unlike other models, the modified Gompertz equation presents minimal prediction error. The Bias Factor (B_f) assesses the model reliability in predicting the response of bacterial inactivation. A model that is considered reliable must have a B_f value ≤ 1. The Gompertz modified equation was the most reliable at the three pH levels, followed by the Geeraerd model.

Information theory criteria for model selection results

Table 4 shows the values for the Akaike increments (Δ_i). According to the definition given in the Material and Methods section, models with $\Delta_i \leq 2$ have substantial support and should receive consideration when making inferences. Models having Δ_i of about 4 to 7 have considerably less support, while models with $\Delta_i \geq 10$ have essentially no support, and might be omitted from further consideration. In our study, the Weibull model should receive consideration when making inferences in 33.33% of the curves tested (12), Geeraerd in 8.33%, Cerf with shoulder in 16.67%, and finally the Gompertz model in 58.33%. According to these calculations, the Gompertz model should be the first choice for interpreting the non-linear survival curves obtained at low treatment temperatures, followed by the Weibull model.

If we compare the estimated models by means of the various tools used in this study, we detect discrepancies in the case of the Cerf with shoulder model, the Weibull distribution function, and the Geeraerd model. This is probably due to the fact that the Weibull distribution function has fewer parameters than the other two models. It is well known that information theory criteria penalize models with a higher number of parameters.

Secondary kinetic parameter estimation of $Z_{(T)}$

According to the analysis carried out in the previous sections, the Gompertz modified equation was the most suitable one to describe the inactivation curves of *L. innocua*. As mentioned above, the estimates of the kinetic parameters (μmax) increased with treatment intensity level for each pH (Table 1).

With the linear relationship between the logarithm of μ_{max} versus temperature it was possible to estimate $Z_{(T)}$ values for each pH level. Regression coefficients for each linear relationship obtained are significant at 95%. Values of $Z_{(T)}$ for each pH were 7.11, 7.95, and 7.56 °C for pH values of 4.25, 4.75, and 5.20, respectively.

Sensitivity analysis

Advanced Sensitivity Analysis allows determination of the effects of inputs on @RISK outputs. The results show how simulation outcomes changed as the input values changed. The initial number of microorganisms, temperature, pH, and heating time were used as input variables and the final number of microorganisms as the output variable.

Figure 3 shows a tornado graph for the final number of microorganisms with regard to the inputs defined in the analysis: bars indicate the relative importance of each input and are arranged in decreasing order. According to the tornado graph, the most influential input variables depend on the severity of the process. For example, as can be seen in Figure 3a, time was the most influential parameter when the treatment temperature was

low (50 °C), while for the higher temperature level (65 °C) the most influential parameter was temperature (Figure 3b). In this kind of very mild heating process, good control of the process temperature is essential in order to achieve the goal of 5 log reductions in the initial load of *Listeria innocua*. Figure 4 show the impact of modifying the base input value for each parameter on the estimated final microbial load. As can be seen in figure 4a, 10% change on initial microbial load and pH have an important impact in the final load for very low thermal treatments while at 65ºC of treatment (Figure 4b) changes on those factors had a meaningless impact in the final microbial load.

CONCLUSIONS

The survival curves obtained at low processing temperatures (50–65 °C) differed from log-linear behavior and showed shoulders or tails, and in some cases they were sigmoidal. According to the various tools used to discriminate among the models studied, the Gompertz modified equation was the one that best described the experimental data, with the lowest Akaike increment in 58.33% of the inactivation curves obtained. Consequently, the Gompertz modified equation should be the first choice for describing the thermal inactivation of *Listeria innocua* at low treatment temperatures in the vegetable beverage tested in this work. Although, with the traditional indices for comparing models, the Weibull distribution function was the worst one, when the Akaike increment was applied as a tool to discriminate between models this distribution function was the second choice for describing the experimental data.

For these very low heat treatments in combination with low pH, the sensitivity analysis concluded that very good control of the temperature is necessary because of the important

impact that small changes can make on the final load of the pathogenic or spoilage microorganism.

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Figure 1. *L. innocua* survival curves at different temperatures (50, 55, 60, and 65 °C) and pHs (4.25, 4.75, and 5.20)

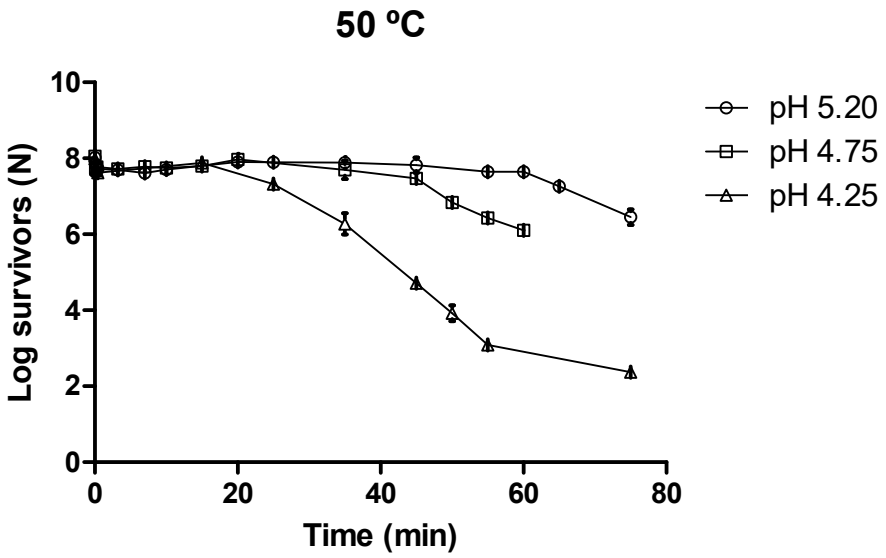
Figure 2: Example of fitting to the different model tested in this study at the temperature of 60ºC pH 5.2

Figure 3: Tornado graph for the final number of microorganisms as affected by input parameters at a temperature of 50 °C (a) and 65 °C (b)

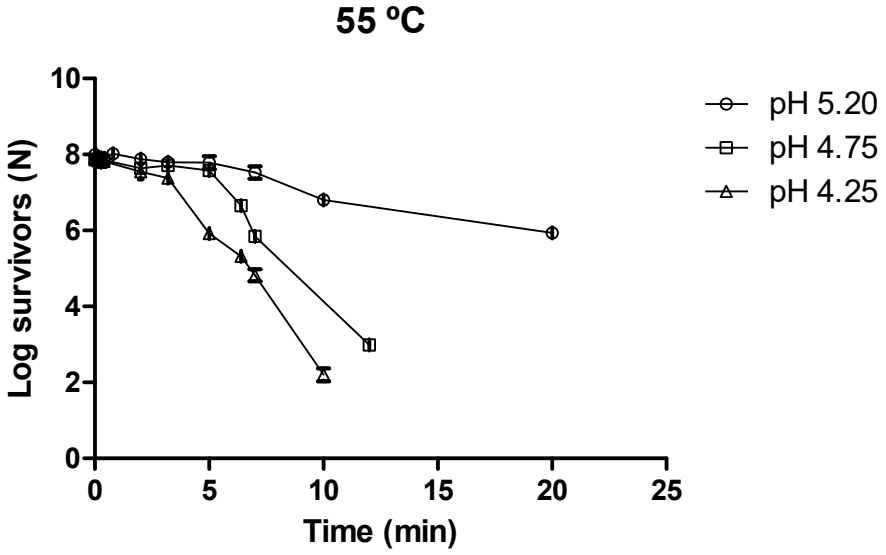
Figure 4 Effect of percentage change of input parameters from a base value on the estimated final load for a thermal treatment of 50 °C (a) and 65 °C (b).

FIGURE 1:

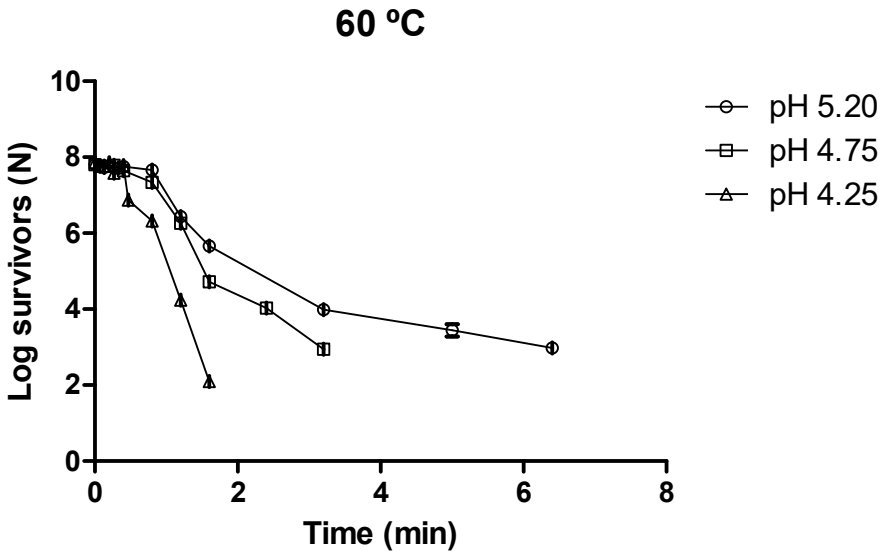
a)


50 ºC
Log survivors (N)
Time (min)
pH 5.20
pH 4.75
pH 4.25

b)


55 ºC
Log survivors (N)
Time (min)
pH 5.20
pH 4.75
pH 4.25

c)


60 ºC
Log survivors (N)
Time (min)
pH 5.20
pH 4.75
pH 4.25

d)

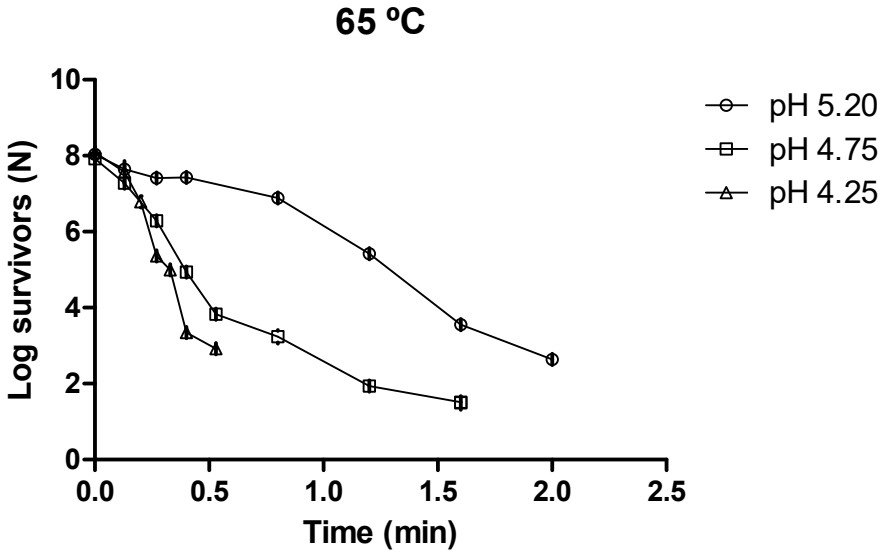

65 ºC
Log survivors (N)
Time (min)
pH 5.20
pH 4.75
pH 4.25

Figure 2

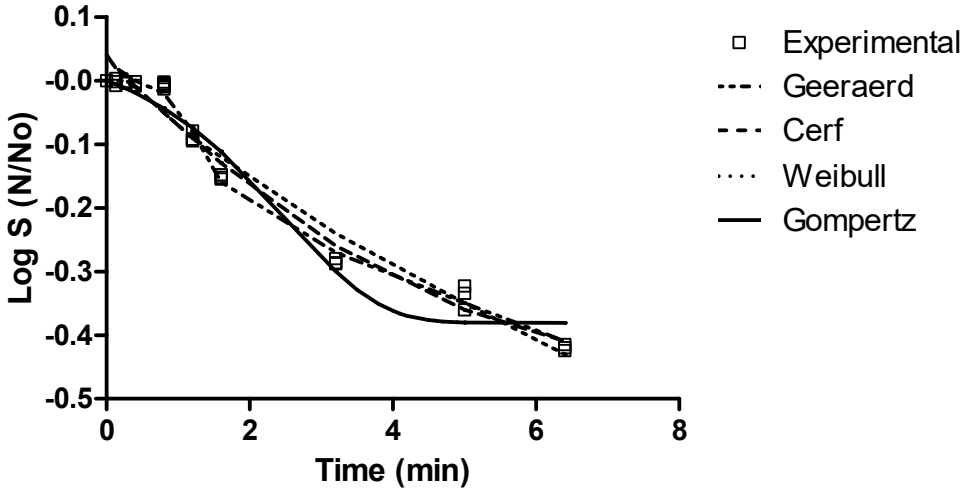
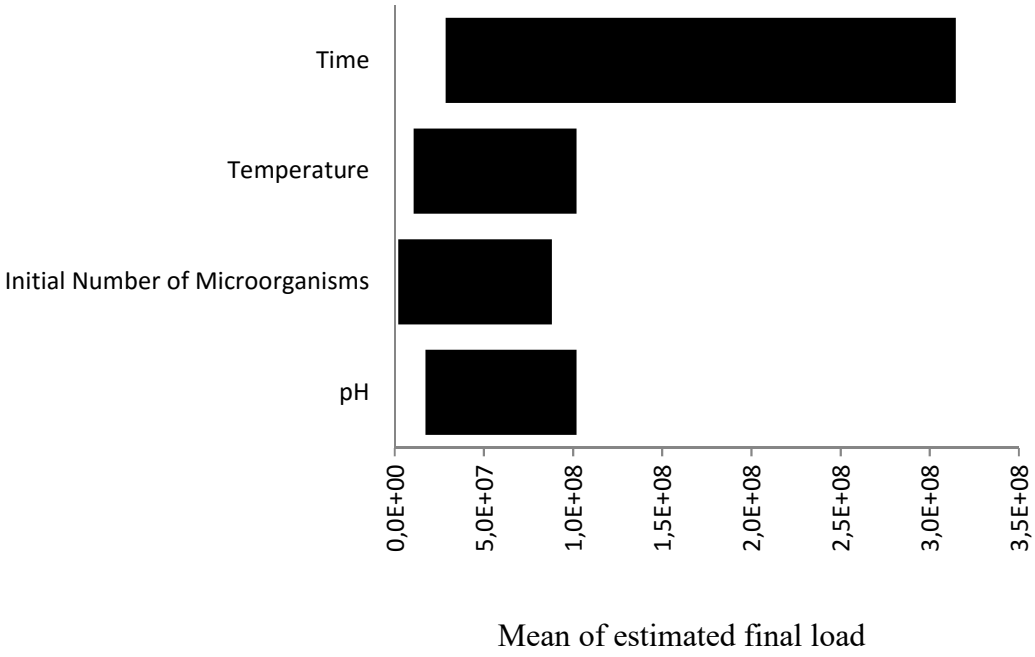

0.1
-0.0
-0.1
-0.2
-0.3
-0.4
-0.5
Log S (N/No)
0
2
4
6
8
Time (min)
Experimental
Geeraerd
Cerf
Weibull
Gompertz

FIGURE 3:

a)


Time
Temperature
Initial Number of Microorganisms
pH
0,0E+00
5,0E+07
1,0E+08
1,5E+08
2,0E+08
2,5E+08
3,0E+08
3,5E+08
Mean of estimated final load

b)

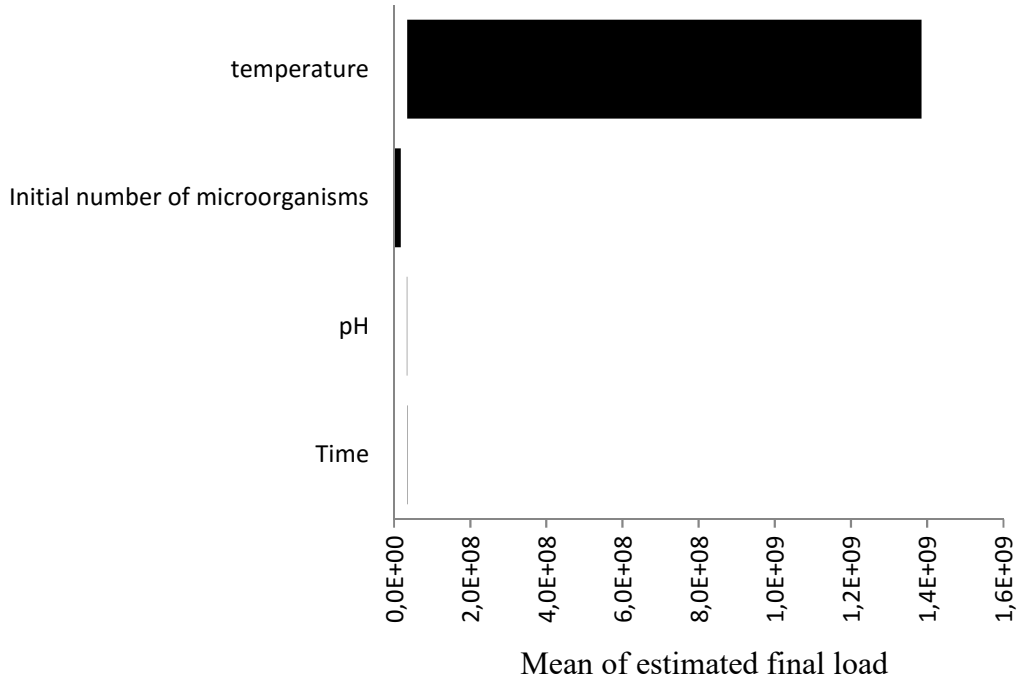
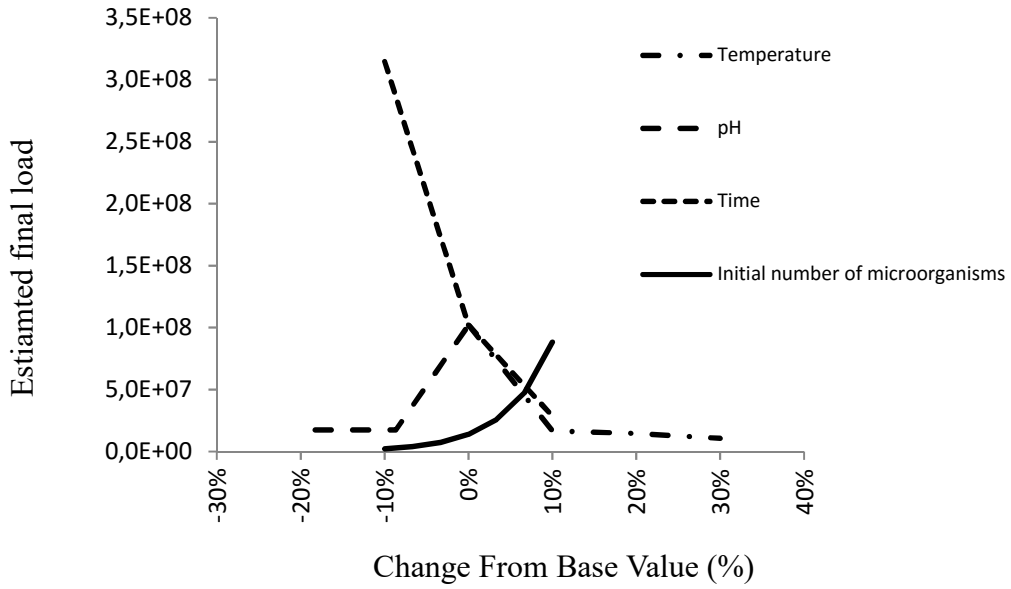

temperature
Initial number of microorganisms
pH
Time
0,0E+00
2,0E+08
4,0E+08
6,0E+08
8,0E+08
1,0E+09
1,2E+09
1,4E+09
1,6E+09
Mean of estimated final load

FIGURE 4:

a)


3,5E+08
3,0E+08
2,5E+08
2,0E+08
1,5E+08
1,0E+08
5,0E+07
0,0E+00
Estiamted final load
-30%
-20%
-10%
0%
10%
20%
30%
40%
Change From Base Value (%)
Temperature
pH
Time
Initial number of microorganisms

b)

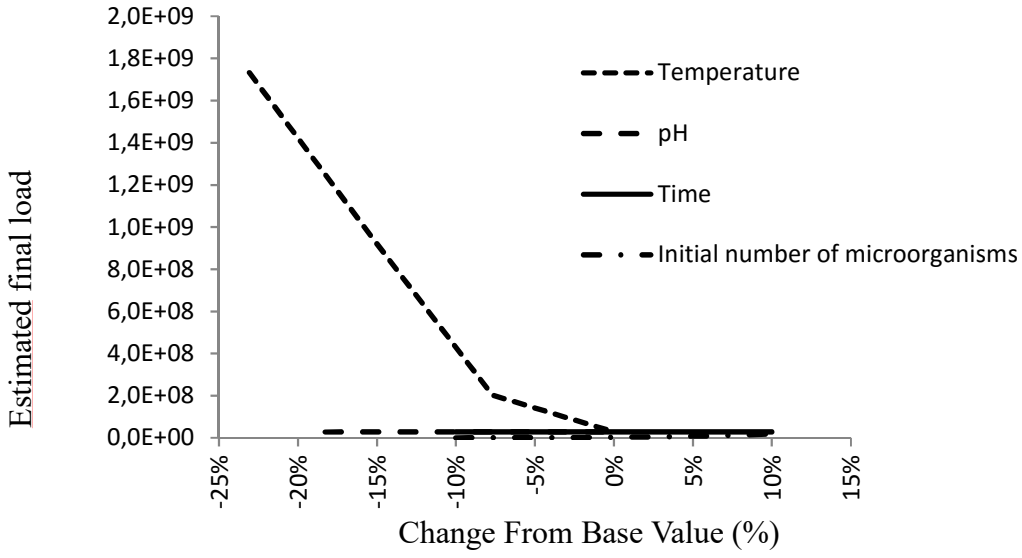

2,0E+09
1,8E+09
1,6E+09
1,4E+09
1,2E+09
1,0E+09
8,0E+08
6,0E+08
4,0E+08
2,0E+08
0,0E+00
Estimated final load
-25%
-20%
-15%
-10%
-5%
0%
5%
10%
15%
Change From Base Value (%)
Temperature
pH
Time
Initial number of microorganisms

Table 1. Parameter values for the various models used to describe experimental data

		WEIBULL	**GEERAERD**	**CERF WITH SHOULDER**		**GOMPERTZ MODIFIED EQUATION**
pH	T [°C]	$\delta \pm \sigma^{(a)}$ [min*mL/UFC]	$k_{max} \pm \sigma^{(a)}$ [UFC/mL min]	$k_{max1} \pm \sigma^{(a)}$ [UFC/mL min]	$k_{max2} \pm \sigma^{(a)}$ [UFC/mL min]	$\mu_{max} \pm \sigma^{(a)}$ [UFC/mL min]
4.25	50	25.538 ± 0.318	0.250 ± 0.006	0.250 ± 0.006	0.250 ± 0.006	0.134 ± 0.007
	55	3.635 ± 0.073	1.802 ± 0.032	1.802 ± 0.032	1.802 ± 0.032	0.873 ± 0.043
	60	0.536 ± 0.030	10.722 ± 0.481	41.988 ± 4.340	9.593 ± 0.915	5.334 ± 0.218
	65	0.119 ± 0.001	30.429 ± 1.100	34.867 ± 1.754	8.342 ± 1.018	16.250 ± 1.374
4.75	50	24.619 ± 0.774	0.294 ± 0.019	0.389 ± 0.167	0.206 ± 0.153	0.178 ± 0.011
	55	5.347 ± 0.258	1.246 ± 0.045	1.246 ± 0.045	1.246 ± 0.045	1.433 ± 0.092
	60	0.340 ± 0.028	9.294 ± 0.552	9.069 ± 0.710	0 ± 0.0	4.202 ± 0.450
	65	0.467 ± 0.044	12.237 ± 0.382	17.541 ± 0.600	4.644 ± 0.481	15.581 ± 4.909
5.20	50	51.379 ± 0.858	0.201 ± 0.029	0.201 ± 0.029	0.201 ± 0.029	0.100 ± 0.010
	55	11.249 ± 0.117	0.457 ± 0.214	0.326 ± 0.014	0.326 ± 0.014	0.283 ± 0.049
	60	0.086 ± 0.002	7.123 ± 0.337	7.135 ± 0.318	0.363 ± 0.059	3.226 ± 0.189
	65	0.564 ± 0.158	2.013 ± 0.966	11.560 ± 3.692	1.537 ± 0.384	7.094 ± 1.379

(a) σ, standard deviation.

Table 2. Goodness of fit for the various models used to describe the experimental data

		WEIBULL		GEERAERD		CERF WITH SHOULDER		GOMPERTZ MODIFIED EQUATION	
pH	T (°C)	*RMS*[(a)]	R^2_c [(b)]	*RMS*[(a)]	R^2_c [(b)]	*RMS*[(a)]	R^2_c [(b)]	*RMS* [(a)]	R^2_c [(b)]
4.25	50	0.354	0.966	0.229	0.986	0.235	0.985	0.195	0.990
	55	0.170	0.991	0.183	0.990	0.187	0.990	0.161	0.992
	60	0.226	0.985	0.203	0.988	0.492	0.893	0.175	0.991
	65	0.422	0.933	0.379	0.946	0.309	0.964	0.298	0.967
	Average	**0.293**	**0.969**	**0.248**	**0.978**	**0.306**	**0.958**	**0.207**	**0.985**
4.75	50	0.169	0.642*	0.170	0.639*	0.166	0.650*	0.140	0.752*
	55	0.309	0.964	0.256	0.975	0.261	0.974	0.108	0.996
	60	0.291	0.954	0.156	0.983	0.174	0.979	0.177	0.979
	65	0.908	0.770*	0.272	0.979	0.177	0.991	0.183	0.990
	Average	**0.419**	**0.832**	**0.213**	**0.894**	**0.195**	**0.899**	**0.152**	**0.929**
5.20	50	0.133	0.910	0.139	0.903	0.142	0.899	0.121	0.917
	55	0.178	0.918	0.155	0.935	0.177	0.919	0.106	0.971
	60	0.657	0.864	0.224	0.984	0.208	0.986	0.248	0.980
	65	0.221	0.745*	0.223	0.741*	0.225	0.736*	0.310	0.975
	Average	**0.297**	**0.859**	**0.185**	**0.891**	**0.188**	**0.885**	**0.196**	**0.961**

[(a)] Estimated standard deviation of the non-linear regression model.

Table 3. Validation of models used to describe the experimental data by A_f and B_f (Ross, 1996).

		WEIBULL		**GEERAERD**		**CERF WITH SHOULDER**		**GOMPERTZ MODIFIED EQUATION**	
pH	T (°C)	A_f[(a)]	B_f[(b)]	A_f[(a)]	B_f[(b)]	A_f[(a)]	B_f[(b)]	A_f[(a)]	B_f[(b)]
4.25	45	1.020	0.990	1.031	0.993	1.022	0.990	1.025	0.994
	50	1.023	0.998	1.014	0.999	1.014	0.999	1.016	0.997
	55	1.034	1.016	1.036	1.000	1.065	1.047	1.018	1.002
	60	1.114	1.016	1.138	1.008	1.094	1.030	1.072	1.009
	Promedio	**1.048**	**1.005**	**1.055**	**1.000**	**1.049**	**1.017**	**1.033**	**1.000**
4.75	45	1.025	0.986	1.022	0.984	1.022	0.984	1.020	0.992
	50	1.022	1.000	1.022	1.007	1.022	1.007	1.020	1.000
	55	1.031	1.006	1.028	0.999	1.028	0.999	1.032	0.997
	60	1.025	1.005	1.013	1.002	1.014	1.002	1.084	0.934
	Promedio	**1.026**	**0.999**	**1.021**	**0.998**	**1.022**	**0.998**	**1.039**	**0.981**
5.20	45	1.016	0.997	1.016	0.997	1.016	0.997	1.017	0.996
	50	1.029	1.003	1.022	1.004	1.023	1.004	1.021	1.005
	55	1.020	0.988	1.019	0.988	1.019	0.988	1.018	0.988
	60	1.060	1.018	1.056	1.011	1.055	1.010	1.044	1.011
	Promedio	**1.031**	**1.002**	**1.028**	**1.000**	**1.028**	**1.000**	**1.025**	**1.000**

(a) A_f, Accuracy Factor.
(b) B_f, Bias Factor.

Table 4: Akaike increments (Δ_i) for the various models used to interpret the experimental data

pH	Model	Temperature °C			
		50	**55**	**60**	**65**
4.2	Weibull	87.19	14.24	61.30	34.37
	Geeraerd	24.91	22.26	35.09	21.64
	Cerf with shoulder	28.43	25.50	226.31	3.26
	Gompertz	0.00	0.00	0.00	0.00
4.7	Weibull	0.00	119.14	352.40	141.36
	Geeraerd	61.76	132.51	0.00	38.67
	Cerf with shoulder	58.52	135.41	23.59	0.00
	Gompertz	32.30	0.00	24.25	3.00
5.2	Weibull	0.00	0.00	290.96	0.00
	Geeraerd	115.93	152.25	290.96	160.76
	Cerf with shoulder	118.83	172.16	40.34	161.56
	Gompertz	158.91	154.90	0.00	186.85

Models with $\Delta_i \leq 2$ have substantial support and should receive consideration when making inferences. Models having Δ_i of about 4 to 7 have considerably less support, while models with $\Delta_i \geq 10$ have essentially no support and might be omitted from further consideration.