



**OBJECTIVES**  
 Modelling inactivation kinetics of *Listeria innocua* 10528 in broth.  
 Study of the parameters temperature dependence.

**INTRODUCTION**

Food poisoning by pathogens directly affects human health and safety. Heat treatments are the most common and effective procedures for controlling the survival of microorganisms in food, and should be designed to provide an adequate safety margin against food-borne pathogens. The development of accurate and precise models, able to predict the behaviour of microorganisms populations, under specific environmental conditions, is of major importance to the food process industries for the development of new systems.

**METHODOLOGY**

➤ **Two-steps regression procedure**

The Gompertz model was fitted to inactivation data, obtained separately at different temperatures

Equations 1 to 4 were used to describe the dependence of the kinetic parameters ( $k_{max}$ , L) on temperature

➤ **One-step regression procedure**

Equations related with the temperature dependence of  $k_{max}$  and L were incorporated into the Gompertz model and a global regression analysis was performed using all the isothermal data.

**MATHEMATICAL MODELS**

**Square-root model**

$$\sqrt{k_{max}} = \sqrt{c} (T - d)$$

c, d constants

**Arrhenius type equation**

$$k_{max} = k_{ref} \exp\left(-\frac{E_a}{R} \left(\frac{1}{T} - \frac{1}{T_{ref}}\right)\right)$$

$k_{ref}$  rate constant at  $T_{ref}$   
 $E_a$  activation energy  
 $T_{ref} = 333 \text{ K}$

**Gompertz modified model**

$$\log N = \log N_0 - \log\left(\frac{N_0}{N_{res}}\right) \exp\left(-\exp\left(\frac{k_{max} e}{\log\left(\frac{N_0}{N_{res}}\right)} (L - t) + 1\right)\right)$$

**Williams-Landel-Ferry model**

$$L = 10^{\left(\frac{a(T - T_{min})}{b + (T - T_{min})}\right)}$$

$T_{min} = 310 \text{ K}$

Where:

- N population size (CFU/unit volume)
- $N_0$  initial population size =  $1 \times 10^7$  CFU/unit volume
- $N_{res}$  residual population size = 100 CFU/unit volume
- t processing time (s)
- T temperatura (K)
- L lag time (s)
- $k_{max}$  reaction rate ( $s^{-1}$ )

a, b constants

**Arrhenius type equation**

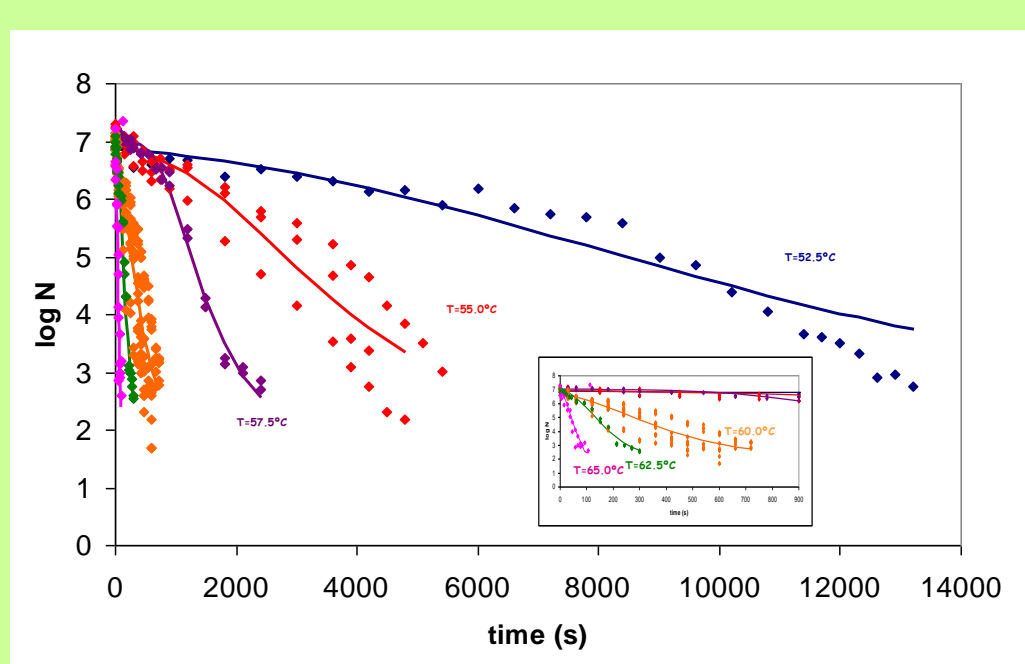
$$L = a \exp\left(b \left(\frac{1}{T} - \frac{1}{T_{ref}}\right)\right)$$

$T_{ref} = 333 \text{ K}$

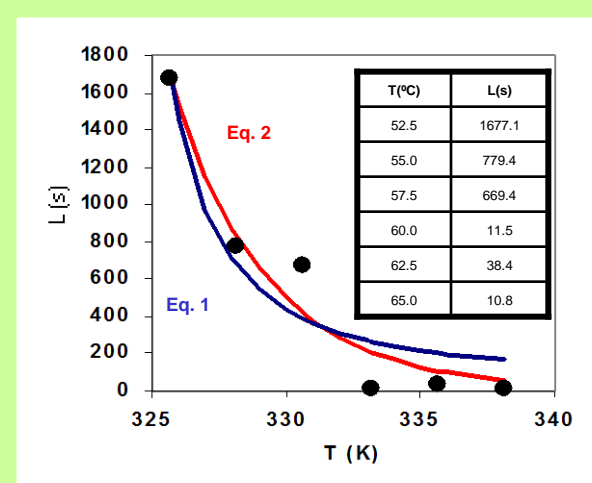
**CASE STUDY**

Inactivation data of *L. innocua* obtained in liquid medium at 52.5, 55, 57.5, 60, 62.5 and 65°C (Miller et al. 2003)

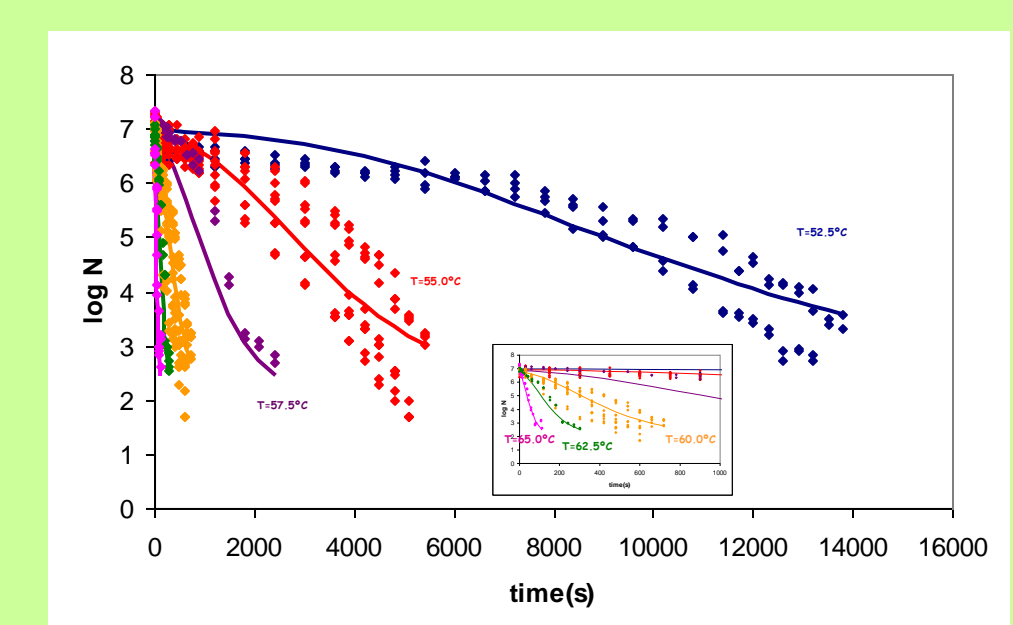
**Gompertz**



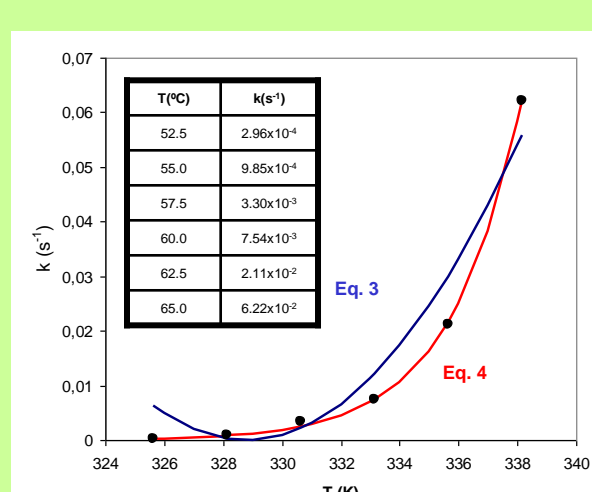
Two-step



$L = f(T)$



One-step



$k_{max} = f(T)$

Equations 2 and 4 selected

**Parameters estimation and relevant statistical data**

**Two-step regression procedure**

Model	Parameters estimates				Regression analysis		
	$k_{ref}$ or c ( $s^{-1}$ ) or ( $s^{-1}K^{-2}$ )	$E_a$ or d ( $J/mol$ ) or (K)	SHW <sub>95%</sub> of $k_{ref}$ or c	SHW <sub>95%</sub> of $E_a$ or d	$R^2_{adj}$	SSR/(n-p)	Randomness of residuals
Arrhenius	$7.342 \times 10^{-3}$	$3.998 \times 10^5$	20.21	9.940	0.9996	$2.400 \times 10^{-7}$	✓
Square-Root	$6.388 \times 10^{-4}$	$3.288 \times 10^2$	138.6	1.654	0.9222	$4.455 \times 10^{-6}$	✓

Model	Parameters estimates				Regression analysis		
	a (s)	b (K)	SHW <sub>95%</sub> of a	SHW <sub>95%</sub> of b	$R^2_{adj}$	SSR/(n-p)	Randomness of residuals
Arrhenius	$2.192 \times 10^2$	$3.007 \times 10^4$	195.2	104.6	0.9350	$2.830 \times 10^4$	✓
WLF	1.593	-7.941	73.2	74.0	0.8815	$5.163 \times 10^4$	✓

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**One-step regression procedure**

Model	Parameters estimates								Regression analysis		
	$k_{max}$ ( $s^{-1}$ )				L (s)				$R^2_{adj}$	SSR/(n-p)	Randomness
	$k_{ref}$	$E_a$	SHW <sub>95%</sub> of $k_{ref}$	SHW <sub>95%</sub> of $E_a$	a	b	SHW <sub>95%</sub> of a	SHW <sub>95%</sub> of b			
Arrhenius	$7.616 \times 10^{-3}$	$3.727 \times 10^5$	$1.182 \times 10^{-1}$	$5.999 \times 10^2$	$4.610 \times 10^1$	$6.134 \times 10^4$	$8.113 \times 10^1$	$2.139 \times 10^1$	0.8616	0.3077	✓

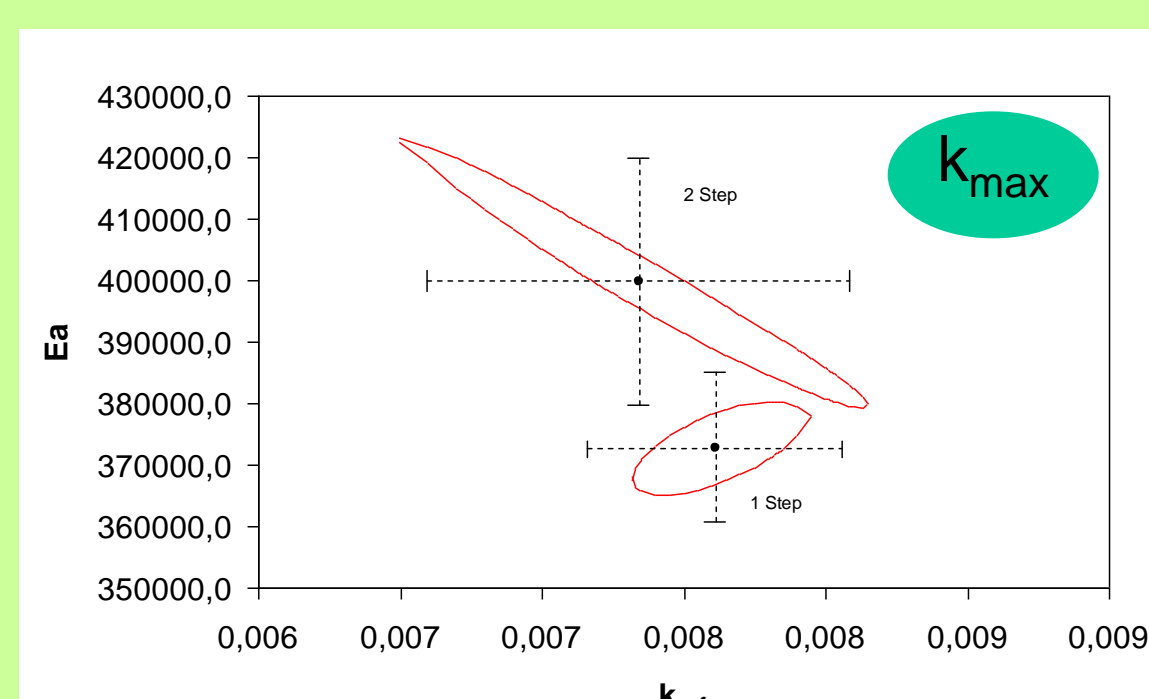
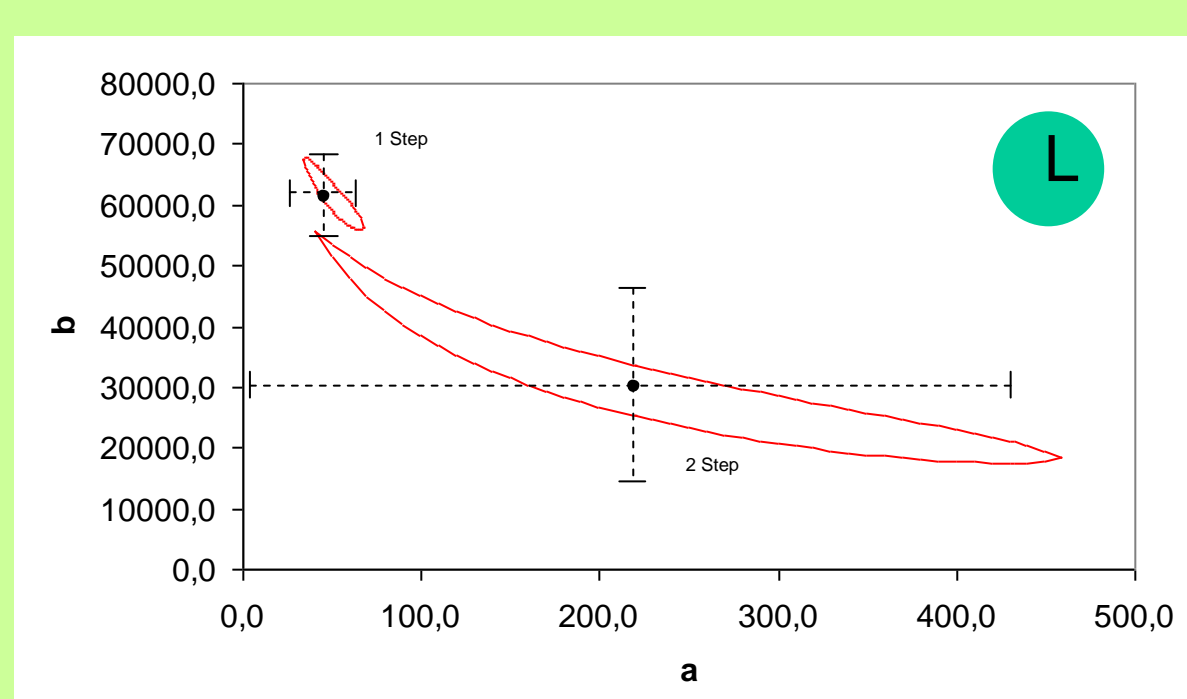
SHW<sub>95%</sub> standardised half width at 95%  
 SSR sum of squares of residuals  
 n # experimental points  
 p # model parameters

The criteria used, to conclude about the best models, were the quality of the residuals, the value of  $R^2_{adj}$  and the precision of the estimates (evaluated by SHW at 95%)

➤ The **Arrhenius equation** describes better the dependence of  $k_{max}$  on temperature, as well as the dependence of L on temperature

➤ These models were used in 1-step regression procedure

**Joint confidence regions of the estimates at 90%**



The dashed lines are the confidence intervals at 95%

**CONCLUSIONS**

- The global fit improved parameters estimation, as expected ➔ narrow confidence intervals
- Nevertheless, the two different regression procedures lead to different parameters estimates, as observed by the contours of the joint confidence regions

**REFERENCES**

Miller, F. A., Brandão, T. R. S., Teixeira, P. and Silva, C. L. M. (2003). Heat resistance of *Listeria innocua* in liquid medium as affected by the culture growth phase. Poster presented at: Biotec' 2003 (X Congresso Nacional de Biotecnologia), Lisboa - Algés, Portugal, 6-8 December

T(°C)	L(μ)
52.5	1677.1
55.0	779.4
57.5	669.4
60.0	11.5
62.5	38.4
65.0	10.8