Predictions of microbial thermal inactivation in solid foods: isothermal and non-isothermal conditions

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Objectives

The main objective of this work was to assess the use of a ***Gompertz-inspired model*** expressed in terms of relevant factors (temperature, pH and water activity), developed on the basis of experiments carried out in broth, in predictions of ***Listeria innocua*** inactivation on parsley surface. Both ***isothermal and time-varying temperature*** conditions were considered.

Relevance

When the microorganism is on a solid food surface, its kinetic response may be considerably different

For inactivation, it is widely known that bacterial pathogens tend to be more resistant to heat in real food products than in broth-based media

Kinetic models that predict accurately microbial behaviour in real foods are an excellent tool to design adequate processing conditions

Assumption that broth-data-based models are conservative, because of the ideal conditions, might not be valid

Relevance

SO WHAT?

- Mathematical models developed from data obtained in broth should be validated in “real” food systems, where there is wider influencing factors rather than the stressing conditions such as temperature, pH or water activity.

- The binomial microorganism/food is determinative of the kinetic behaviour and should always be validated.

Model description

***Gompertz-inspired model* - ** 3 parameter model (shoulder, maximum inactivation rate and tail)

Model parameters

Model parameters

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

N	population size (CFU/unit volume)
N_0	initial population size (CFU/unit volume)
N_{res}	residual population size (CFU/unit volume)
t	processing time (s)
L	shoulder (s)
k_{max}	maximum inactivation rate (min^{-1}; s^{-1})

Model description

T, pH and a_w effects
Isothermal conditions

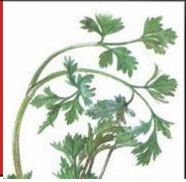
Gompertz-inspired model expressed in terms of relevant factors

- Temperature is the key factor controlling the survival/inactivation of bacteria.

- In thermal processes, factors such as pH and water activity influence microbial thermal resistance.

- The interactive effects of stressing environmental conditions may influence microbial inactivation behaviour.

- The study of main and combined effects of temperature, water activity, and pH on kinetic parameters is important for a complete process assessment and control.



Model description

Gompertz-inspired model expressed in terms of relevant factors

*The temperature, pH and water activity **main** and **combined effects** on maximum inactivation rate, shoulder parameter and tail were considered:*

pH and a_w dependent

temperature, pH and a_w dependent

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

Model description

T, pH and a_w effects
Isothermal conditions

The log variations of k_{max} and L on temperature, pH and water activity (a_w) were described by polynomials:

$$\log(k_{\max}) = \sum_{i=0}^{n}\sum_{j=0}^{n}\sum_{k=0}^{n} G_{k_{\max}\,ijk}\; a_w^{\,k}\; pH^{\,j}\; T^{\,i}$$

$$\log(L) = \sum_{i=0}^{n}\sum_{j=0}^{n}\sum_{k=0}^{n} G_{L\,ijk}\; a_w^{\,k}\; pH^{\,j}\; T^{\,i}$$

Regarding the tail parameter ($\log(N_{res}/N_0)$), the dependence on pH and water activity (a_w) were also described by polynomials:

$$\log\left(\frac{N_{res}}{N_0}\right) = \sum_{i=0}^{n}\sum_{j=0}^{n} G_{Tail\,ij}\; a_w^{\,i}\; pH^{\,j}$$

where n is the order of the polynomial to be assumed and G are polynomials coefficients (i=1,…, n; j=1,…, n; k=1,…, n).

Model description

T, pH and a_w effects
Isothermal conditions

Mathematical modelling

k_{max}

$$\log(k_{max}) = G_{k\,max_{T3}}\,T^3 + G_{k\,max_{T2}}\,T^2 + G_{k\,max_{T1}}\,T + G_{k\,max_{T0}} \leftarrow T$$

$$G_{k\,max_{Ti}} = G_{k\,max_{TipH2}}\,pH^2 + G_{k\,max_{TipH1}}\,pH + G_{k\,max_{TipH0}} \leftarrow pH$$

$$G_{k\,max_{TipHj}} = G_{k\,max_{TipHjaw1}}\,a_w + G_{k\,max_{TipHjaw0}} \leftarrow a_w$$

L

$$\log(L) = G_{L_{T3}}\,T^3 + G_{L_{T2}}\,T^2 + G_{L_{T1}}\,T + G_{L_{T0}} \leftarrow T$$

$$G_{L_{Ti}} = G_{L_{TipH2}}\,pH^2 + G_{L_{TipH1}}\,pH + G_{L_{TipH0}} \leftarrow pH$$

$$G_{L_{TipHj}} = G_{L_{TipHjaw1}}\,a_w + G_{L_{TipHjaw0}} \leftarrow a_w$$

$\log(N_{res}/N_0)$

$$\log\left(\frac{N_{res}}{N_0}\right) = G_{Tail_{pH2}}\,pH^2 + G_{Tail_{pH1}}\,pH + G_{Tail_{pH0}} \leftarrow pH$$

$$G_{Tail_{pHj}} = G_{Tail_{pHjaw1}}\,a_w + G_{Tail_{pHjaw0}} \leftarrow a_w$$

The values of the parameters (coefficients of the polynomial equation) used in the simulation were the ones previously estimated considering inactivation experimental data of *L. innocua* obtained at different conditions in broth, by Miller *et al.* 2011.

Miller, FA, Ramos, BF, Gil, MM, Brandão, TRS, Teixeira, P, Silva, CLM. Heat inactivation of *Listeria innocua* in broth and food products under non-isothermal conditions. *Food Control* 2011: **22**: 20-26.

Model description

T, pH and a_w effects
Isothermal conditions

To predict *Listeria innocua* inactivation on **parsley surface**, **polynomials** were merged into the **Gompertz-inspired model**, using a **black box polynomial model**:

$$\log\left(\frac{N_{res}}{N_0}\right) = \sum_{i=0}^{n}\sum_{j=0}^{n} G_{Tail_{ij}}\ a_w^{\,i}\, pH^{j}$$

$$k_{max} = \exp\left(\sum_{i=0}^{n}\sum_{j=0}^{n}\sum_{k=0}^{n} G_{k_{max\ ijk}}\ a_w^{\,k}\, pH^{j}\ T^{i}\right)$$

$$L = \exp\left(\sum_{i=0}^{n}\sum_{j=0}^{n}\sum_{k=0}^{n} G_{L_{ijk}}\ a_w^{\,k}\, pH^{j}\ T^{i}\right)$$

pH = 6.2

a_w = 0.98

T = 52.5 ºC to 65.0 ºC

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

Model description

To predict the inactivation behaviour of *L. innocua* **under non-isothermal conditions**, a combined model including the **temperature effect** was used:

$$y_{dynamic} = \int_0^t \left[-k_{max}(T)\, e \exp\left(-\frac{k_{max}(T)e}{\log\left(\frac{N_{res}}{N_0}\right)} \left(L(T) - t'\right) + 1 \right) \exp\left(-\exp\left(-\frac{k_{max}(T)e}{\log\left(\frac{N_{res}}{N_0}\right)} \left(L(T) - t'\right) + 1 \right) \right) \right] dt'$$

Arrhenius type-equation

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

Ratkowsky inspired model

$$L(T) = \left[A_{Ratk_1} \left(T - A_{Ratk_2} \right) \right]^2$$

where k_{ref} is the inactivation rate at a finite reference absolute temperature (T_{ref}), Ea the activation energy, and R the universal gas constant; A_{Ratk1} and A_{Ratk2} are Ratkowsky models parameters.

The values of the parameters used in the simulation were the previously estimated considering inactivation experimental data of *L. innocua* obtained at different conditions in broth, by Miller *et al.* 2011.

Model description

T, pH and a_w effects
Non-isothermal conditions

The following equation expresses the variation of $\log(N/N_0)$ with time, for time-varying temperature conditions:

$$\log\left(\frac{N_{res}}{N_0}\right) = \sum_{i=0}^{n}\sum_{j=0}^{n} G_{Tail_{ij}}\, a_w^{\,k}\, pH^j$$

$$k_{max} = \exp\left(\sum_{i=0}^{n}\sum_{j=0}^{n}\sum_{k=0}^{n} G_{k_{max\,ijk}}\, a_w^{\,k}\, pH^j\, T^i\right)$$

$$L = \exp\left(\sum_{i=0}^{n}\sum_{j=0}^{n}\sum_{k=0}^{n} G_{L_{ijk}}\, a_w^{\,k}\, pH^j\, T^i\right)$$

pH = 6.2

a_w = 0.98

T = 21.0 + 1.9 t

$$\log\left(\frac{N}{N_0}\right) = \int_0^t \left[-k_{max}(T(t),a_w,pH)\, e \exp\left(-\frac{k_{max}(T(t),a_w,pH)\, e}{\log\left(\frac{N_{res}}{N_0}\right)(a_w,pH)}\left(L(T(t),a_w,pH) - t'\right) + 1\right) \times \exp\left(-\exp\left(-\frac{k_{max}(T(t),a_w,pH)\, e}{\log\left(\frac{N_{res}}{N_0}\right)(a_w,pH)}\left(L(T(t),a_w,pH) - t'\right) + 1\right)\right)\right] dt'$$

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Materials and Methods

- All microbial inactivation data was experimentally obtained by Miller *et al*. 2011.
- Since the model was developed for a specific range of pH (from 4.5 to 7.5) and water activity (from 0.95 to 0.99) values, a solid food with characteristics within these ranges was chosen.
- Parsley was the selected one ($pH_{parsley}$=6.2 and $a_{w_{parsley}}$=0.98).
- Moreover, parsley is an aromatic herb with a great potential of contamination. This re-enforces the interest on selecting parsley as a case to study.
- For non-isothermal conditions, the model developed was dependent on media temperature history.

Miller, FA, Ramos, BF, Gil, MM, Brandão, TRS, Teixeira, P, Silva, CLM. Heat inactivation of *Listeria innocua* in broth and food products under non-isothermal conditions. *Food Control* 2011: **22**: 20-26.

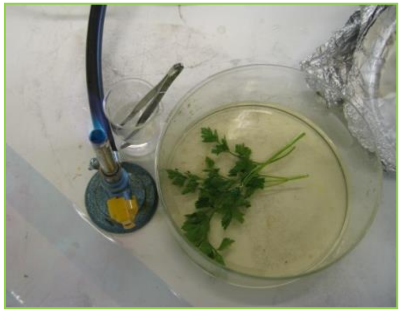
Materials and Methods

Isothermal conditions

Temperatures (ºC)

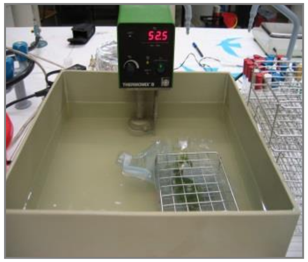
- 52.5
- 60.0
- 65.0

artificially inoculated by immersion



sealed and vacuum-packaged

immersed in a thermostatic agitated water bath



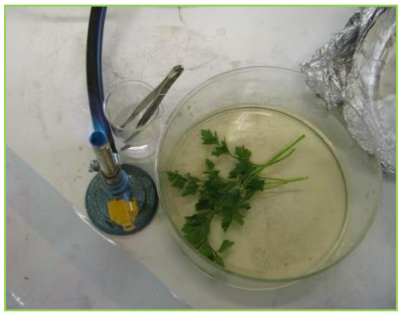
sterile peptone water was added

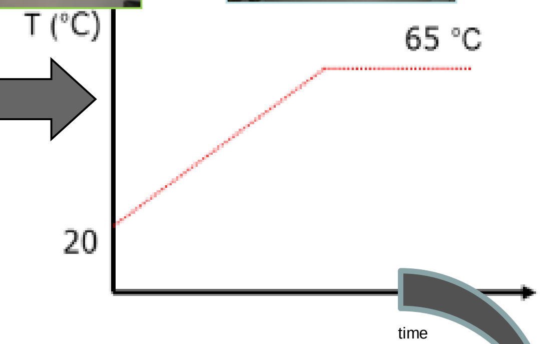
pummelled

diluted, plated and incubated

Materials and Methods

Non-isothermal conditions



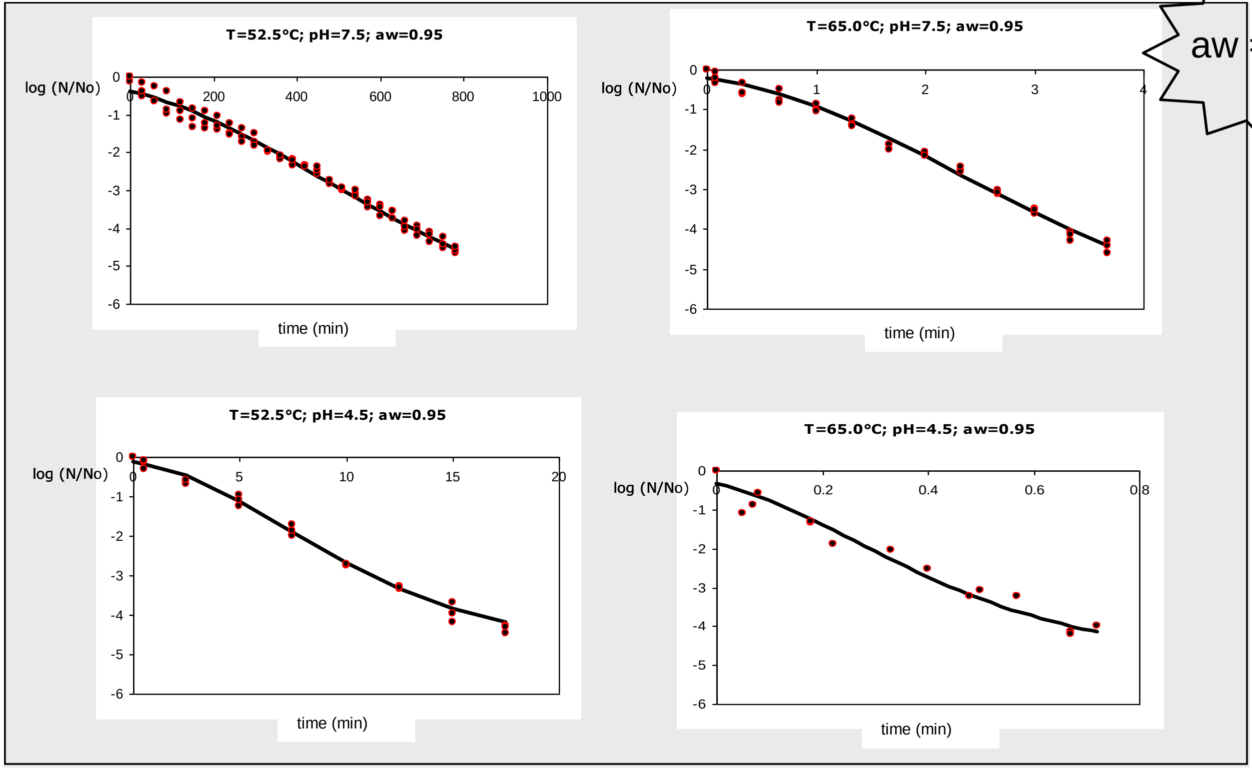


Results and Discussion

T, pH and a_w effects
Isothermal conditions

<u>Previous work</u>, reported by Gil et al. 2015, showed that the **Gompertz-inspired model**, expressed in terms of **temperature, pH and water activity,** was proven to be efficient in **describing microbial inactivation data of *Listeria innocua***, when the bacteria was in **broth,** for isothermal and non-isothermal conditions.

aw = 0.95

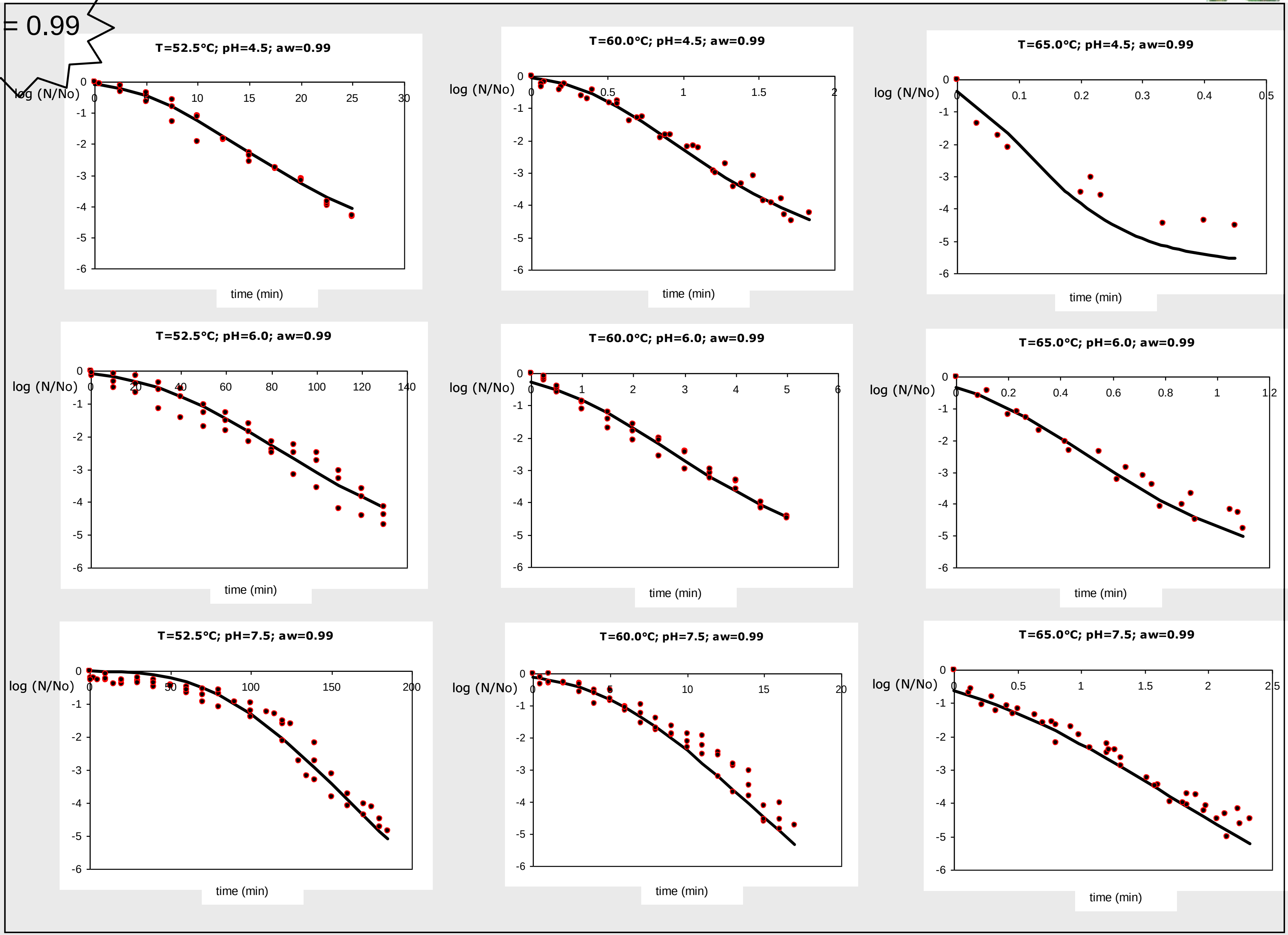


Gil, M.M.**,** Miller, F.A., Brandão, T.R.S. and Silva, C.L.M. (2015). Combined effects of temperature, pH and water activity on predictive ability of microbial kinetic inactivation model. Poster: "9th International Conference on Predictive Modeling in Foods", Rio de Janeiro, Brazil, 8 - 12 September.

Results and Discussion

T, pH and a_w effects
Isothermal conditions

aw = 0.99



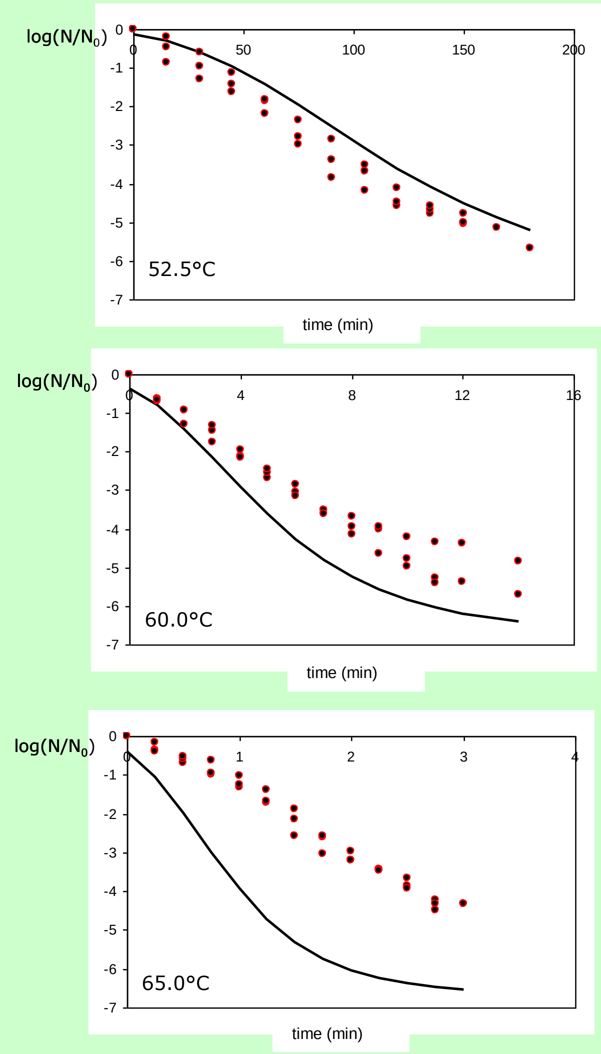
Results and Discussion

T, pH and a_w effects
Isothermal conditions

Experimental results and model predictions
Parsley surface

- Using the experimental temperature conditions, pH and water activity values of the parsley, the microbial load was predicted.
- It is obvious the inadequacy of model predictions, particularly for the higher temperatures tested.
- For 52.5 ° C, the model slightly overestimates the microbial inactivation.
- However, and as temperature increases from 60.0 to 65.0 ° C, dangerous underestimations were attained.
- This reveals that when *Listeria innocua* was at parsley's surface, additional thermal resistance was observed.

Isothermal



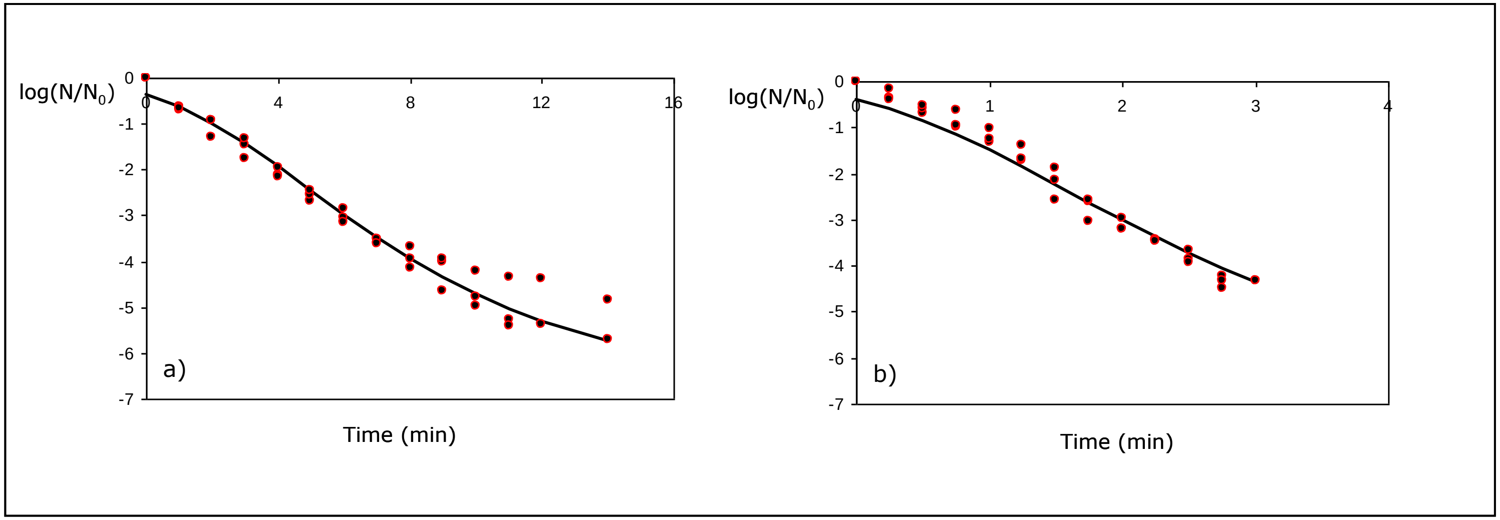
Results and Discussion

….a clear understanding of this:

(a) L. innocua in **parsley processed at 60 ºC** presented a behaviour equivalent to *L. innocua* in **broth processed at 59 ºC** (with the same pH and a_w values);

(b) L. innocua in **parsley processed at 65 ºC** presented a behaviour equivalent to *L. innocua* in **broth processed at 62 ºC** (with the same pH and a_w values);

…..Milder process required in broth, for the same target inactivation.



a) experimental data (dots) at 60ºC and model predictions (line) at 59ºC
b) experimental data (dots) at 65ºC and model predictions (line) at 62ºC

Results and Discussion

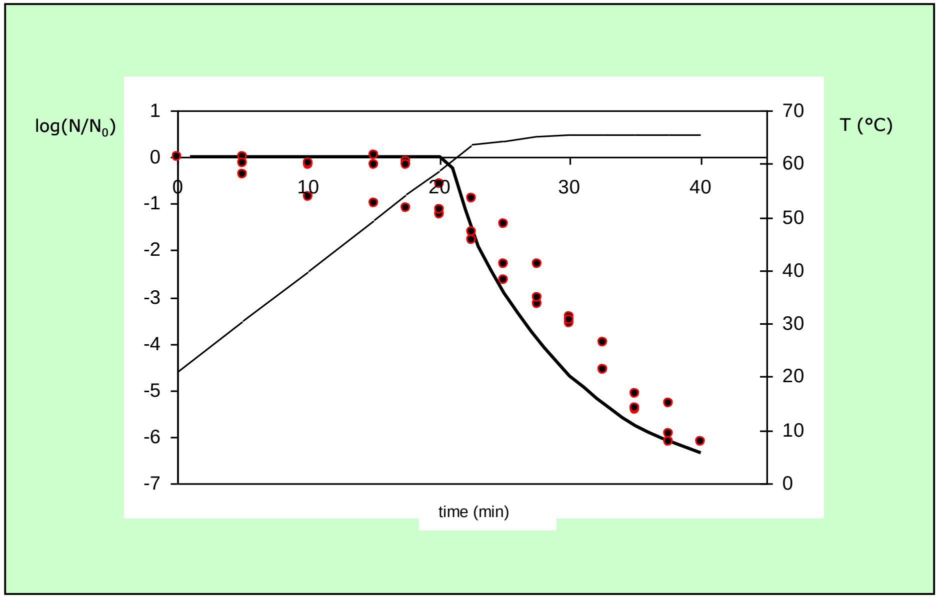
- **This clearly illustrates that broth-data-based models should be used with caution if the bacteria is on solid food surfaces.**

- **Crucial importance should be devoted to pathogens' behaviour, since potential hazards should not be underestimated.**

Results and Discussion

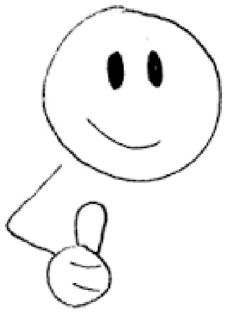
Using the experimental time-varying temperature conditions, the microbial load was predicted:

Non-isothermal



Results and Discussion

T, pH and a_w effects
Non-isothermal conditions



- Accurate predictions were attained (contrarily to isothermal situations) and results showed the ability of the Gompertz-inspired model in estimating microbial response in food, under non-isothermal conditions.

- It was found that no microbial growth was verified during the come-up-time and there was a limit of temperature below which no inactivation was observed: *the minimum temperature for inactivation was approximately 60.0 ºC for a heating rate of 1.9 ºC/min, which corresponds to the minimum temperature for inactivation in broth (around 60.5 ºC for the same heating rate).*

- Microbial hypothesis concerning initialization of thermal inactivation in broth was validated in parsley.

Results and Discussion

- Microorganisms, after exposed to a slow temperature evolution, would have time to adapt their plasma membranes and might increase their resistance to subsequent lethal heat treatments.

- Consequently, microbiological behaviour in broth or in food should be similar due to a similar heat resistance.

- Moreover, the heat resistance greatly depends on the physiological state (and therefore the previous environment). This fact can explain the predictive ability of the model, only under non-isothermal conditions.

Conclusions

- One important issue in food microbiology, is the extent to which results obtained in broth can be applied in predicting microbial responses on solid foods.

- For isothermal conditions, the Gompertz-inspired model expressed in terms of temperature, pH and water activity and developed on the basis of microbial inactivation data in broth, was not adequate in predicting microbial response on a food surface.

- The higher the temperature, the worse under-predictions attained. Additional important factors should be included when dealing with a “real” solid food processing.

Conclusions

- The kinetic behaviour may change when the microorganisms are on a solid food surface and, consequently, for any given binomial microorganism/food, the model should be validated.

- However, for non-isothermal conditions satisfactory model predictions were attained.

- Besides the required assessment when dealing with food systems, the model developed and expressed in terms of the most relevant variables studied (i.e. temperature, pH and water activity) allowed predictions that will certainly contribute to improvements of the preliminary designs of adequate thermal treatments.

Thank you!

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