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Sigmoidal thermal inactivation kinetics of *Listeria innocua* in broth: Influence of strain and growth phase

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ABSTRACT

Listeria innocua inactivation was studied within the temperature range 52.5–65.0 °C, comparing two different strains (10528 and 2030c) and two growth phases (exponential and stationary). Survival curves may present a sigmoidal behaviour, with an initial shoulder (L), followed by a maximum inactivation rate (k_{max}) period and a final tailing tendency. A Gompertz-inspired model was used to fit experimental data, and kinetic parameters (L, k_{max} and tail) were estimated by non-linear regression analysis. The influence of temperature, growth phase and strain on kinetic parameters was studied using a 2^3 factorial experimental design. Results showed that temperature and growth phase were the most significant variables affecting the kinetic parameters. *Listeria* thermal inactivation varied from a log-linear tendency till a pronounced sigmoidal behaviour, depending on the studied factors.

1. Introduction

Listeria monocytogenes is a major concern for food industries and consumers, because of its association with foodborne diseases. The organism is ubiquitous in the environment and its occurrence in foods, such as in fresh and processed meat and seafood products, in raw and pasteurised milk and milk products, is well documented (Bell & Kyriakides, 1998; Farber & Peterkin, 1991; Mena et al., 2004). The presence of *Listeria* spp. in pasteurised products makes its heat resistance assessment an important topic.

It has been concluded in several works (Kamat & Nair, 1996; Margolles, Mayo, & Reyes-Gavilán, 2000; Piyasena, Liou, & McKellar, 1998) that *Listeria innocua* can be used as a biological indicator of *L. monocytogenes* in the food industry, since it provides for the majority of strains, a margin of safety. Besides *L. innocua* exhibits most of the characteristics of *L. monocytogenes*, it shares the same natural environments and can be frequently isolated in the same food products, making it a good surrogate for *L. monocytogenes*.

Most of food processes are developed and applied with the purpose of controlling spoilage and/or pathogenic microorganisms' survival, temperature being one of their major stressing factors. Thermal processes, such as pasteurisation and sterilisation, when conveniently applied, are efficient in reducing/eliminating hazardous pathogenic bacteria and viruses that may be present in the raw products (Orta-Ramirez & Smith, 2002). However, the extent of this impact will depend on a number of factors, including: (i) properties of the organism, (ii) strains heat susceptibility, (iii) organism physiological state prior to treatment and (iv) food chemical composition (Ray, 2004).

The knowledge of the kinetic behaviour of a microorganism suffering a thermal treatment, as well as the influence of the affecting factors, is important for design, assessment and optimization of the process.

For almost one century, it has been assumed that the logarithmic of the number of viable microorganisms decreases linearly with time, when unfavourable high temperatures (or other stressing factors) are imposed. However, deviations from linearity are often referred (Huang, 2009; McKellar & Lu, 2004; Xiong, Xie, Edmondson, Linton, & Sheard, 1999). Generally, the inactivation behaviour may exhibit a delayed initial period prior to the exponential phase, often referred to as *shoulder* (or *lag*; this designation more commonly applied for bacteria growth) and/or a tailing phenomenon. Besides this sigmoidal behaviour is often observed, *D*-value (decimal reduction time, or time required to inactivate 90% of the population) and *z*-value (temperature necessary to reduce *D*-value by 10-fold) are frequently calculated, assuming first-order kinetics.

The use of convenient mathematical models to describe microbial kinetic behaviour is commonly referred as predictive microbiology, being this designation firstly suggested by McMeekin and Olley (1986). If models are appropriate in kinetics description, and if models' parameters include the effect of environmental factors, one can extract the best from predictive microbiology. Experimental design (i.e. planning sampling conditions according to statistical background) plays an important role in achieving such quality inference with minimal experimental effort. Juneja and Eblen (1999) and Juneja, Marmer, and Eblen (1999) applied a factorial experimental design (Box, Hunter, & Hunter, 1978) when

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studying the most significant environmental effects (temperature, pH, NaCl and sodium pyrophosphate) in thermal inactivation of *L. monocytogenes* and *Escherichia coli*, respectively.

Temperature is the most studied factor influencing microorganisms' survival. However, the microbial thermal inactivation behaviour may be affected by the strains of the specie under study. Quite often, mathematical models are developed on the basis of only one strain and, erroneously, are assumed for the entire specie without validation.

The growth phase (i.e. exponential or stationary phase) of the microorganisms, selected for the thermal inactivation studies, also affects significantly their heat resistance. Consequently, this should be cautiously taken into consideration when a mathematical model for predicting purposes is being developed.

All efforts must be done in gathering experimental data that can help to clarify microorganims' performance, controlling intrinsic characteristics and environmental factors.

Thus, the objectives of this study were: (i) to evaluate the influence of temperature on the inactivation of *L. innocua*, (ii) comparing two strains and (iii) microbial growth phase, based on a convenient experimental design.

2. Materials and methods

2.1. The experimental design

A 2^3 factorial design (Box et al., 1978) was applied to assess the effect of (i) temperature, (ii) strain of *L. innocua* and (iii) growth phase, on the inactivation behaviour evaluated by shoulder, maximum inactivation rate and tail parameters of the Gompertz-inspired model (Eq. (1); Section 2.3.1). The levels assumed for the variables were: (i) 52.5 and 65.0 °C for temperature, (ii) *L. innocua* NCTC 10528 and *L. innocua* 2030c strains, and (iii) exponential and stationary phase (totalling eight cases).

2.2. Experimental procedures

2.2.1. Cultures

L. innocua NCTC 10528 and *L. innocua* 2030c, obtained by Public Health Laboratory Service – PHLS (Colindale, UK) private collection, were subcultured (30 °C, 24 h) in Tryptic Soy Broth – TSB (Lab M, Lancashire, UK) containing 0.6% yeast extract – TSBYE (Lab M). Working cultures were maintained at 7 °C on Tryptic Soy Agar – TSA (Lab M) supplemented with 0.6% yeast extract – TSAYE.

2.2.2. Preparation of cultures

The second subculture of *L. innocua* was incubated at 30 °C for 9 or 20 h to yield exponential or stationary phase cultures, respectively. These times were selected from the experimental growth curves of both strains (data not shown).

The culture was centrifuged (4000 rpm for 10 min), the pellet was washed twice and re-suspended in TSBYE. Cells in each cellular suspension were enumerated by plating appropriate dilutions, in duplicate, on TSAYE.

2.2.3. Heat treatments

Heat treatments were carried out (of both strains and growth phases) in an agitated water bath at temperatures defined according to the experimental design (52.5 and 65.0 °C). Four more temperatures were also considered (55.0, 57.5, 60.0 and 62.5 °C). Two covered Erlenmeyer flask with 99 mL of TBSYE, used as heating medium, were immersed in the water bath at the desired temperature. One of the flasks was used for the microbial inactivation experiments while the other was used for temperature control. Once the heating medium temperature had attained stability, it was inoculated with 1 mL of *L. innocua* cell suspension. Samples were taken at different times and placed in a mixture of ice-water.

There was a control for each experiment, which consisted of another 99 mL of TSBYE inoculated with 1 mL of the same cellular suspension and incubated at 30 °C for the same time. This control was used to ensure that the observed death was only due to the temperature applied.

Three replicates of all these experiments were performed.

The initial concentration of *L. innocua* was determined to be approximately 10^7 cfu/mL for all conditions tested.

2.2.4. Enumeration

Samples were serially diluted and plated in duplicate onto TSAYE. Plates were incubated at 30 °C and counted each 24 h during 5 days, or until the number of colony formation units (cfu) no longer increased.

Mean values of bacterial counts, from duplicate plate samples, were converted to log numbers for each strain, temperature and growth phase.

2.3. Modelling procedures

2.3.1. The inactivation model

Assuming that the microbial thermal inactivation follows a sigmoidal behaviour, experimental data can be mathematically described by a Gompertz-inspired model (Bhaduri et al., 1991; Char, Guerrero, & Alzamora, 2009; Gil, Brandão, & Silva, 2006; Huang, 2009; Linton, Carter, Pierson, & Hackney, 1995):

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

where N is the microbial cell density at a particular process time, t. The indexes 0 and res indicate initial and residual (or tail) microbial cell density, respectively; L is the initial shoulder and k_{max} the maximum inactivation rate.

The versatility of fitting linear data and those that contain shoulder and/or tailing effects makes Gompertz one attractive model (Zwietering, Jongenburger, Rombouts, & Vantriet, 1990).

2.3.2. Temperature effects

The parameters k_{max} and L are temperature dependent.

The maximum inactivation rate is the reciprocal of the D-value (i.e. the time required for 1-log reduction in microbial load, at a given temperature), being this parameter often preferred by microbiologists. The Bigelow model can be used to express the dependence of k_{max} (or D-value) on temperature (Mafart, 2000; Valdramidis, Geeraerd, Bernaerts, & Van Impe, 2006):

$$\log\left(\frac{1}{k_{max}}\right) = \log(D) = \log(D_{ref}) - \frac{(T-T_{ref})}{z} \quad (2)$$

herein D_{ref} is the D-value at a reference temperature, T_{ref} and z is the temperature required for a 10-fold reduction of D-value.

The shoulder parameter can also be related to temperature using a Bigelow-type relation:

$$L = L_{ref}10^{-\frac{(T-T_{ref})}{z'}} \quad (3)$$

where L_{ref} is the shoulder at a reference temperature and z' is the temperature required for a 10-fold reduction of L.

2.3.3. Data analysis

The parameters of the Gompertz-inspired inactivation model, i.e. L, k_{max} and $\log(N_{res}/N_0)$, were estimated by non-linear regression analysis, fitting Eq. (1) to experimental inactivation data at the temperatures studied.