Food Microbiology 34 (2013) 158–163

Contents lists available at SciVerse ScienceDirect

Food Microbiology

journal homepage: www.elsevier.com/locate/fm

Effect of the medium characteristics and the heating and cooling rates on the nonisothermal heat resistance of *Bacillus sporothermodurans* IC4 spores

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ARTICLE INFO

Article history:
Received 3 July 2012
Received in revised form
27 November 2012
Accepted 28 November 2012
Available online 17 December 2012

Keywords:
Heat resistance
Inactivation kinetics
Bacillus sporothermodurans
Predictive models
Spores

ABSTRACT

In recent years, highly thermo-resistant mesophilic spore-forming bacteria belonging to the species *Bacillus sporothermodurans* have caused non-sterility problems in industrial sterilization processes. The aim of this research was to evaluate the effect of the heating medium characteristics (pH and buffer/food) on the thermal inactivation of *B. sporothermodurans* spores when exposed to isothermal and non-isothermal heating and cooling treatments and the suitability of non-linear Weibull and Geeraaerd models to predict the survivors of these thermal treatments. Thermal treatments were carried out in pH 3, 5 and 7 McIlvaine buffer and in a courgette soup. Isothermal survival curves showed shoulders that were accurately characterized by means of both models. A clear effect of the pH of the heating medium was observed, decreasing the D_{120} value from pH 7 to pH 3 buffer down to one third. Differences in heat resistance were similar, regardless of the model used and were kept at all temperatures tested. The heat resistance in courgette soup was similar to that shown in pH 7 buffer. When the heat resistance values obtained under isothermal conditions were used to predict the survival in the non-isothermical experiments, the predictions estimated the experimental data quite accurately, both with Weibull and Geeraerd models.

1. Introduction

The traditional method for determining the kinetics of the death rate of microorganisms is based on the assumption that inactivation follows first-order kinetics and that microbial populations are homogeneous from the point of view of their heat resistance. Hence, D_T values are calculated from the survival curve by linear regression of log N versus time (Stumbo, 1973). However, many authors have found deviations from linearity of survival curves, such us shoulders or tails. Some researchers (Fernandez et al., 1999; Peleg and Cole, 1998) explain this behaviour assuming that at a given temperature, the time of exposure to heat, which causes the death of a microbial cell or a bacterial spore, is variable from one individual to the other, and that the dispersion of individual heat resistance is governed by a frequency distribution. This fact has leaded to propose alternative models that provide with a satisfactory goodness of fit for non linear survival curves showing shoulders or tails (Geeraerd et al., 2000; Mafart et al., 2002) or even sigmoidal survival curves showing both shoulder and tail (Geeraerd et al., 2000; Coroller et al., 2006).

Bacillus sporothermodurans is a heat-resistant mesophilic spore-forming bacterium causing non-sterility problems in canned foods (Huemer et al., 1998; Oomes et al., 2007). Van Zuijlen et al. (2010) observed the presence of shoulders in the survival curves of isothermal treatments of this microorganism and analysed the data obtained using Weibull and Geeraerd models. Both models characterized the heat inactivation of the spores of *B. sporothermodurans* better than the conventional log-linear model. Although these authors found *B. sporothermodurans* a suitable microorganism to evaluate and optimize commercially applied sterilization processes for low acid foods, they also pointed out the influence of the heating menstruum characteristics on its heat resistance.

Heat resistance of microorganisms is mostly influenced by time and temperature of treatment, but environmental factors such as pH, a_w, composition of menstruum, etc., may drastically influence its heat resistance, even during the heat treatment (Etoa and Michiels, 1998).

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0740-0020/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.fm.2012.11.020

The effect of these factors on the heat resistance of spore-forming bacteria has been extensively studied (Condón and Sala, 1992; López et al., 1996; Palop et al., 1999), however their effect on the thermal resistance of *B. sporothermodurans* spores is not so well known.

The effect of the heating and cooling rate has been scarcely studied. Still, some authors have used nonisothermal methods as an alternative to the study of microbial inactivation kinetics (Conesa et al., 2003; Fernández et al., 1999; Hassani et al., 2007; Peleg and Normand, 2004; Periago et al., 1998) and although differences between the heat resistance values obtained from isothermal and nonisothermal heating experiments have been found (De Cordt et al., 1992; Periago et al., 1998), the extraction of kinetic parameters from nonisothermal data has been successfully performed by several groups (Chen et al., 2007; Peleg and Normand, 2004; Valdramidis et al., 2008), who have obtained equivalent parameters using isothermal and non-isothermal data, unless adaptation of the microorganism due to the use of low heating rates (Valdramidis et al., 2006).

The aim of this research was to evaluate the effect of the heating medium characteristics (pH and buffer/food) on the thermal inactivation of *B. sporothermodurans* spores when exposed to isothermal and nonisothermal heating and cooling treatments and the suitability of Weibull and Geeraerd models to predict the survivors to these thermal treatments.

2. Materials and methods

2.1. Bacteria strain and sample preparation

The strain used in this study was *B. sporothermodurans* IC4 (kindly supplied by Unilever Netherlands Sourcing Unit Oss). Spores were prepared on Petri dishes of plate count agar (PCA) from (Scharlau, Barcelona, Spain) incubated at 30 °C for 24 h. Four pure colonies were taken from this agar plate and suspended in physiological salt solution. Then the agar plates containing Campden Sporulation Agar (CSA; Brown et al., 1984) were inoculated with 0.2 mL of this mixture. After 7 days of incubation at 37 °C more than 90% of sporulation rate was achieved, as determined by a phase contrast microscopy (Leica, Wetzlar, Germany).

Spores were collected by flooding the agar plates with 5 mL of distilled water, scratching the surface with a spatula. After harvesting, spores were washed four times by centrifugation at 3040×*g* for 15 min at 2–4 °C and resuspended in sterile distilled water. The concentration of spores in the final suspension was adjusted at 10^9 spores/mL with sterile distilled water. The spore suspensions were stored at 4 °C until used.

2.2. Determination of heat resistance

All heat treatments were carried out in a thermoresistometer Mastia (Conesa et al., 2009), which allows the performance of heating ramps at different rates. The temperatures of isothermal treatments were: 115, 120, 125 and 130 °C. For nonisothermal treatments, the thermoresistometer was programmed to perform linear temperature profiles, starting from an initial temperature of 110 °C at established rates of 1 °C/min and 10 °C/min.

Once the initial heat treatment temperature had attained stability the menstruum (400 mL of McIlvaine buffer of pH 3, 5 and 7 or a commercial courgette soup, pH 6.3) was inoculated with 0.2 mL of the spore suspension. At preset intervals, 1 mL samples for each treatment time were collected. Viable counts were based on duplicate counts of appropriate dilutions plated in Brain Heart Infusion Agar (BHIA) (Scharlau, Barcelona, Spain) and incubated at 37 °C for 24 h. Preliminary experiments showed that longer incubation times did not modify plate counts.

All resistance determinations were performed at least twice in independent experiments at different days.

2.3. Data analysis

Survival curves showed shoulder phenomena. Hence, Weibull and Geeraerd non lineal models were used to describe such survival curves.

2.3.1. Weibull model

The cumulative form of the Weibull distribution function, as proposed by Mafart et al. (2002) was used (Eq. (1)):

$$\log_{10} S = -\left(\frac{t}{\delta}\right)^p \tag{1}$$

where $S(t)$ is the survival ratio, i.e. N_t/N_0, δ represents the time for the first decimal reduction (min), which is a function of temperature (see Eq. (3)), and p is the shape parameter.

In our study, we used a rate model derived from Eq. (1), representing the momentary time-dependent isothermal logarithmic inactivation rate, which can be written as an ordinary differential equation as given by Eq. (2) (van Zuijlen et al., 2010):

$$\frac{dN}{dt} = -p \cdot \left(\frac{1}{\delta}\right)^p \cdot t^{p-1} \tag{2}$$

with the initial condition: $N(0) = N_0$.

A single p value for all survival curves obtained in the same heating media was used as proposed by Couvert et al. (2005). For the global optimisation, Eq. (3) was incorporated into Eq. (1) and the parameter p was fitted in common, whereas the initial states were fitted for each curve.

The prediction of survivors under nonisothermal experiments was based on the dependence of δ with respect to temperature, which can be described with the classic Bigelow model as given by Eq. (3) (Mafart et al., 2002):

$$\delta(T) = \delta_{T_{ref}} \times 10^{-\frac{T-T_{ref}}{z}} \tag{3}$$

where $\delta_{T_{ref}}$ is the $\delta(T)$ value at the reference temperature (T_{ref}), and z is the number of degrees Celsius change of temperature required to achieve a tenfold change in δ value.

2.3.2. Geeraerd model

The dynamic model of Geeraerd et al. (2000) was used. The parameters of this model were estimated with the GInaFIT

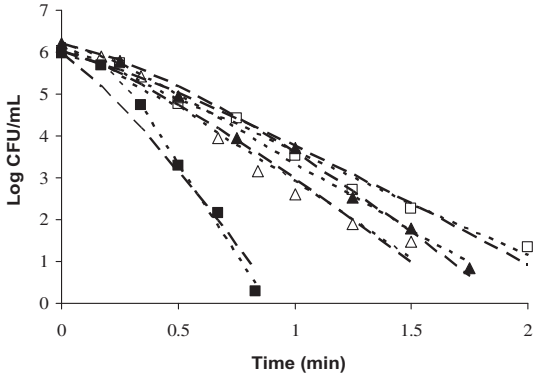

Fig. 1. Survival curves of *Bacillus sporothermodurans* IC4 at 125 °C in pH7 McIlvaine buffer (□) pH5 McIlvaine buffer (△) pH3 McIlvaine buffer (■) and in courgette soup (▲). Weibull model (dashed lines); Geeraerd model (dotted lines).