Food Research International 107 (2018) 267–274

Contents lists available at ScienceDirect

Food Research International

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

Optimal characterization of thermal microbial inactivation simulating non-isothermal processes

Alberto Garre[a], Gerardo González-Tejedor[a], Jose Lucas Peñalver-Soto[b], Pablo S. Fernández[a], Jose A. Egea[b,*]

[a] Departamento de Ingeniería de Alimentos y del Equipamiento Agrícola, Instituto de Biotecnología Vegetal, Universidad Politécnica de Cartagena (ETSIA), Paseo Alfonso XIII, 48, 30203 Cartagena, Spain
[b] Departamento de Matemática Aplicada y Estadística, Universidad Politécnica de Cartagena, Antiguo Hospital de Marina (ETSII), Av. Dr. Fleming S/N, 30202 Cartagena, Spain

ARTICLE INFO

Keywords:
Optimal experimental design
Microbial inactivation
Non-isothermal processes
bioOED

ABSTRACT

In this paper, optimal experimental design is applied for the characterization of the microbial inactivation of *Listeria monocytogenes* under non-isothermal conditions, modelled by the Bigelow model. These conditions simulate industrial processes, where different temperature profiles can be applied for food processing. Here, the aim was to find the best time nodes for measurements given a fixed number of observations and a final time process. Results show that, compared to a "classic" uniform time distribution for the observations, the optimal distribution of observation times results in a more accurate description of the response of the microbial population. The OED resulted in a reduction in the relative standard errors for the estimated D and z-values of 25% and 80%, respectively.

Prediction intervals of the microbial counts were calculated based on the parameters estimated with both designs (i.e., uniform and optimal design). The prediction interval generated using the parameters estimated from the optimal design is notably narrower than the one obtained when the "uniform" experiment is considered, thus providing a more accurate description of the thermal resistance of the microorganism. Although the Bigelow model has been used in this particular case, the functions developed have been wrapped in an R package (bioOED), which is freely available and can be used for any other type of microorganism and/or inactivation model.

1. Introduction

The 21th century has seen a major step forward regarding food safety. The proliferation of the refrigeration systems for food preservation, as well as the development of pasteurization techniques has improved the safety and nutritional value of food products. Nonetheless, new challenges have arisen during the last years for food producers. Firstly, modern consumers are demanding minimally processed products (fresh-like) which, at the same time, have to remain free of foodborne pathogens and toxins. Secondly, a new concern has grown identifying food waste as an ethical, economic and environmental issue. In this respect, studies to evaluate impact on safety of shelf life extension of food products have been published by institutions such as EFSA (EFSA Panel on Biological Hazards (BIOHAZ), 2014). Hence, food producers are pushed toward optimizing their production lines in order to both reduce its impact on the product quality and improve their predictions of the shelf life. This challenge can only be faced through a detailed mathematical description of the response of the microbial populations during the different phases of the life cycle of the food product (Baranyi & Roberts, 1995; Ferrer, Prats, López, & Vives-Rego, 2009).

Predictive microbiology uses mathematical modelling to describe the response of the microbial populations in the food product (i.e. the microbial count), as well as to estimate the probability that a contaminated product may cause illness to the consumer (Perez-Rodriguez & Valero, 2012). Usually, these mathematical models contain a set of model parameters which characterize the response of the microorganism modelled to the environmental conditions studied. Currently, most of these model parameters are unknown and can only be estimated from data obtained in the laboratory through experiments. These experiments require microbiological analysis which need specific equipment and reagents, highly trained personnel and are time-consuming. Furthermore, the model parameters depend on the microorganism, as well as on the environmental conditions (pH, water

* Corresponding author.
E-mail address: josea.egea@upct.es (J.A. Egea).

https://doi.org/10.1016/j.foodres.2018.02.040
Received 13 July 2017; Received in revised form 7 February 2018; Accepted 13 February 2018
Available online 16 February 2018

activity…), thus, experiments must be repeated for different conditions. Consequently, the characterization of the microbial response is an expensive process. The importance of the parameter estimation process (also known as "inverse problem") in food science has been recently reviewed by Dolan and Mishra (2013) and Vilas, Arias-Mendez, Garcia, Alonso, and Balsa-Canto (2018).

The way of collecting data (i.e., designing the experiments) has a significant influence in the estimated values of the parameters as well as in the uncertainty associated to them (Franceschini & Macchietto, 2008). Experiments for the characterization of microbial inactivation are typically designed using sample points uniformly distributed in time (e.g. (Corradini & Peleg, 2004; Franco-Vega, Ramírez-Corona, López-Malo, & Palou, 2015; Huang, 2013; Valdramidis, Geeraerd, Bernaerts, & Van Impe, 2008)). However, when considering non-linear models, this design does not provide the maximum information given a fixed number of observations. Optimal Experiment Design (OED) aims to find the most informative experimental design for a given model, type of experiment and (possibly fixed) number of observations; in other words OED seeks the experiment(s) which provides the most accurate characterization of the model parameters under some constraints.

In the field of microbiology applied to food engineering and science, OED has proved to be an efficient tool to optimize the amount of retrieved information and the lab resources when carrying out experimental work. Although some studies have compared different experimental designs without taking into account the dynamic conditions (e.g., (Mertens, Van, & Van Impe, 2012)), the most important efforts have been focused on them, trying to reproduce the most usual cases in industrial environments and in predictive microbiology (Balsa-Canto, Alonso, & Banga, 2008). In this regard, most of the works have been applied to microbial growth. For example, Grijspeerdt and Vanrolleghem (1999) applied OED to estimate and reduce the parameter uncertainty in the Baranyi model (Baranyi, Roberts, & McClure, 1993). Bernaerts, Versyck, and Van (2000), García et al. (2015), Grijspeerdt and De Reu (2005) and Longhi et al. (2017) applied dynamic optimal experimental design to estimate the parameters of a square root secondary model (Ratkowsky, Olley, McMeekin, & Ball, 1982). A different secondary model, the Cardinal Temperature Model with Inflection (CTMI) (Rosso, Lobry, Bajard, & Flandrois, 1995) was also considered from the OED point of view (Van Derlinden, Bernaerts, & Van Impe, 2008). Recently, Stamati, Akkermans, Logist, Noriega, and Van (2016) used OED to discriminate among bacterial growth models.

Optimal experimental design has not been as frequently applied to microbial inactivation as to growth. There are, however, some examples in the literature: Versyck, Bernaerts, Geeraerd, and Van (1999) introduced the dynamic optimal experimental design in predictive microbiology through an example related to microbial inactivation, van Derlinden, Balsa-Canto, and van Impe (2010) illustrated the OED methodology also in the field of microbial inactivation. In a recent work, Gil, Miller, Silva, and Brandão (2014) applied OED to a microbial inactivation process described by a Gompertz-based model under isothermal and non-isothermal conditions.

Some works have been devoted to the mathematical analysis of the optimal experimental design using particular inactivation models rather than focusing in the process. For example, Cunha, Oliveira, Brandão, and Oliveira (1997) analyzed the Bigelow model (Bigelow, 1921), whereas Cunha, Oliveira, and Oliveira (1998) focused on the Weibull probability distribution function, in which are based other typical models of microbial inactivation like the Peleg (Peleg & Cole, 1998) and Mafart (Mafart, Couvert, Gaillard, & Leguerinel, 2002) models. In a recent work, Paquet-Durand, Zettel, and Hitzmann (2015) performed a rigorous analysis of OED when using the Peleg model.

In this paper, the inactivation of *Listeria monocytogenes* under non-isothermal conditions has been characterized using an optimal experimental design. In order to generate a feasible experiment, a penalty function has been included. The model parameters estimated using the OED have been compared against those estimated using the "classic", uniform experiment design. Furthermore, the prediction intervals calculated from both model fits have been compared.

The mathematical algorithms used for the OED are included in the *bioOED* package (Garre, Penalver, Fernandez, & Egea, 2017) for the R programming language (R Core Team, 2016). It has been made publicly available through the Comprehensive R Archive (CRAN). Although in this paper the OED has been performed for the estimation of the model parameters of the Bigelow model, the functions in the package are ready to be used for other inactivation models commonly used in the scientific literature: Bigelow (1921), Peleg (Peleg & Cole, 1998), Geeraerd (Geeraerd, Herremans, & Van Impe, 2000) and Mafart (Mafart et al., 2002). The use of this package is straightforward for scientific and/or industrial practitioners, as shown in the supplementary material accompanying this paper.

2. Materials and methods

2.1. Bacterial strain and culture conditions

L. monocytogenes CECT 4032 was supplied by the Spanish Type Culture Collection. For rehydration, it was transferred to 10 mL of Tryptic Soy Broth (TSB) (ScharlabChemie S.A., Barcelona, Spain). After 30 min, 5 mL of culture were inoculated in 500 mL of TSB and incubated at 37 °C with constant agitation (200 rpm) for 21 h to obtain cells in the stationary growth stage. The cells were centrifuged twice at 4000 × *g* for 15 min at 4 °C and resuspended in 20 mL of TSB. They were then placed in 2 mL sterile plastic cryogenic vials containing TSB supplemented with 20% glycerol in a relation of 1:1. The 2 mL samples, with an approximate concentration of 5×10^9 CFU/mL, were immediately stored at −80 °C until use for the microbiological studies.

Every two months, one of the vials was opened and the stock culture grown in TSB for 24 h at 35 °C and streaked onto Palcam agar plates for purity check (Merck, Darmstadt, Germany). *L. monocytogenes* 4032 was prepared by transferring a colony obtained in Palcam agar to TSB that was incubated for 24 h at 37 °C before being stored at −20 °C in a solution of 40% TSB and 60% glycerol until use. The fresh cultures for the experiments were made by incubating one sample of pure culture in TSB for 24 h at 37 °C. It was then transferred to TSA, incubated at 37 °C for 48 h and individual colonies were used as inoculum for TSB broth incubated at 37 °C overnight. The inoculum was standardized by dilution in TSB until obtaining a bacterial concentration of 10^9 CFU/mL. The bacterial populations were estimated by spreading suitable diluted aliquots onto Trypticase Soy Agar (TSA) plates, followed by incubation at 37 °C for 24 h. Growth was checked again after 48 h.

2.2. Thermal treatments and enumeration of survivors

Thermal inactivation kinetics of *L. monocytogenes* was studied in peptone water using a thermoresistometer Mastia (Conesa, Andreu, Fernández, Esnoz, & Palop, 2009). Isothermal heat treatments were conducted at 55, 57.5, 60, 62.5 and 65 °C. Furthermore, the non-isothermal linear inactivation profile illustrated in Fig. 1 was performed. Once heating medium temperature had attained stability (T ± 0.05 °C), it was automatically inoculated with 0.2 mL of *Listeria* inoculum (approximately 10^9 cells mL^{-1}).

Serial decimal dilutions of the different samples, immediately after inoculation and at pre-established sampling times, were performed in sterile peptone water (Scharlab Chemie S.A.). The enumeration medium used for viable cells was Tryptic Soy Agar (TSA). The selected dilutions (plates) were incubated at 37 °C for 24 h and checked after 48 h; subsequently the plates were counted. Counts were expressed as the decimal logarithm (log10 of CFU/mL) of the subsequent dilutions. Two replicates were made for each sample, and the average was reported. Each treatment was assayed by triplicate.