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Modelling the inactivation of *Listeria monocytogenes* by high hydrostatic pressure processing in foods: A review

Arícia Possas*, Fernando Pérez-Rodríguez, Antonio Valero, Rosa Maria García-Gimeno

Department of Food Science and Technology, International Campus of Excellence in the AgriFood Sector (CeiA3), University of Córdoba, C-1, 14014 Córdoba, Spain

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ABSTRACT

Background: The application of High Hydrostatic Pressure (HHP) processing technology as a non-thermal pasteurization method has been extensively investigated over the last two decades. *Listeria monocytogenes* is a relevant target for food safety due to its ability to grow and/or survive in a wide range of environmental conditions and be present at hazardous levels in food commodities where lethal treatments have not been carried out, such as some ready-to-eat foods (RTE).

Scope and approach: This review presents a compilation of modelling studies describing pressure-induced inactivation of *L. monocytogenes* in foods. The influence of a series of factors, including technological parameters, food matrix composition and the physiological state of bacterial cells on inactivation levels is also discussed, as it should be clearly understood and evaluated in order to set and optimize HHP processing conditions.

Key findings and conclusions: The use of mathematical models to predict the inactivation and probability of recovery of *L. monocytogenes* in foods during HHP application and subsequent storage can help food processors and managers to comply with the current microbiological regulations established for RTE foods, as well as optimize processing conditions.

1. Introduction

Listeria monocytogenes is a psychrotrophic bacteria, considered as a major safety concern in the food industry due to its ability to grow and survive in different types of foods under a wide range of environmental conditions (Das, Lalitha, Joseph, Kamalakanth, & Bindu, 2016). The contamination of ready-to-eat foods (RTE) by *L. monocytogenes* during processing operations such as slicing and packaging is particularly relevant, since these products are generally not submitted to lethal treatments before consumption (Bover-Cid, Belletti, Aymerich, & Garriga, 2015).

The European Commission Regulation No. 2073/2005 requires food operators to demonstrate that RTE foods that support the growth of *L. monocytogenes* do not exceed the limit of 100 cfu/g throughout their shelf-life (European Commission, 2005). In accordance with Codex Alimentarius guidelines, the amount of the pathogen should be limited to 100 cfu/g at the end of shelf life when storage conditions do not permit its growth (Luber, 2011). Otherwise, the absence of *L. monocytogenes* in 25 g of product must be guaranteed (Luber, 2011).

High hydrostatic pressure (HHP) processing is a non-thermal technology that has shown great potential to inactivate pathogenic and spoilage microorganisms, producing microbiologically safer products with extended shelf life and a non-severe impact on the nutritional and organoleptic characteristics of foods (Syed, Buffa, Guamis, & Saldo, 2016). This preservation technique basically consists of the application of isostatic pressures, uniformly and instantaneously transmitted to foods by air-driven pumps through a liquid, generally water (Hugas, Garriga, & Monfort, 2002).

The application of HHP processing has been proposed as a non-thermal pasteurization method to inactivate *L. monocytogenes* in RTE foods (Georget et al., 2015; Syed et al., 2016). Regarding pasteurization of RTE foods with novel technologies, the FDA requires processes that guarantee at least a 5-log reduction of the target microorganism (Saucedo-Reyes, Marco-Celdrán, Pina-Pérez, Rodrigo, & Martínez-López, 2009).

Over the last two decades much effort has been put into process optimization and understanding the inactivation kinetics of *L. monocytogenes* in HHP-processed foods. Mathematical models for predicting inactivation of pathogens constitute useful tools for food processors to select optimum HHP processing conditions (Bover-Cid, Belletti, Garriga, & Aymerich, 2011; Chen, 2007b). Several researchers have highlighted the need for databases containing kinetic model parameters

* Corresponding author. Department of Food Science and Technology, Faculty of Veterinary, University of Córdoba, Campus de Rabanales s/n. Edif. Darwin_anexo (C1) Crta, Madrid-Cádiz Km 396_A 14014, Córdoba, Spain.
E-mail address: ariciamp@gmail.com (A. Possas).

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for target microorganisms (Valdramidis, Taoukis, Stoforos, & Van Impe, 2012). Furthermore, predictive models can help food industries to comply with process criteria and current regulations for *L. monocytogenes* in RTE foods (Hereu, Dalgaard, Garriga, Aymerich, & Bover-Cid, 2012).

Microbial inactivation through the application of HHP processing has been modelled as a function of technological processing variables and food intrinsic factors/characteristics. This review presents a compilation of inactivation models of *L. monocytogenes* during HHP processing of foods, as well as logistic models of its behaviour during storage. First, an overview of inactivation kinetic models is presented, followed by a discussion on the different factors influencing the inactivation of *L. monocytogenes* induced by the application of HHP technology, which include technological parameters, food matrix characteristics and culture conditions.

2. *Listeria monocytogenes* inactivation kinetics by HHP

Various predictive models are available for HHP inactivation of *L. monocytogenes* or *Listeria innocua* (a *L. monocytogenes* surrogate for processing plant safety purposes) in food simulated systems (Ates, Rode, Skipnes, & Lekang, 2016; Doona, Feeherry, Ross, & Kustin, 2012), meat products (Bover-Cid et al., 2011, 2015;; Carlez, Rosec, Richard, & Cheftel, 1993; Hereu, Dalgaard, et al., 2012; Lerasle et al., 2014; Rubio, Possas, Rincón, García-Gímeno, & Martínez, 2018), fish (Ramaswamy, Zaman, & Smith, 2008), seafood (Das et al., 2016; Fletcher, Youssef, & Sravani, 2008), milk (Amina, Kodogiannis, Petrounias, Lygouras, & Nychas, 2012; Buzrul, Alpas, Largeteau, & Demazeau, 2008; Chen & Hoover, 2003, 2004), dairy products (Shao, Ramaswamy, & Zhu, 2007) and RTE vegetables (Jung, Lee, Kim, Cho, & Ahn, 2014; Muñoz, Ancos, Sa, & Cano, 2006).

Although bacterial resistance to HHP has been reported to be higher in solid foods than in culture media and liquid foods (Ates et al., 2016; Bover-Cid et al., 2015), a substantial number of modelling approaches developed in buffered solution and culture media is available in literature (Muñoz-Cuevas et al., 2013). Despite some limitations, the development of predictive models in model systems may offer certain advantages, such as high reproducibility, rigorous control of environmental factors and the absence of interfering background microbiota, but, prior to application, their validation on target foods is highly recommended (Baka, Noriega, Van Langendonck, & Van Impe, 2016).

Pressure inactivation models can be classified into primary, secondary and tertiary models in the same way as traditional predictive models (Whiting & Buchanan, 1993).

2.1. Primary models

Primary models in HHP technology are mathematical equations describing changes in microbial counts induced by pressure as a function of treatment times. The most frequently applied equations are described in sections 2.1.1 and 2.1.2. These models are useful when evaluating the inactivation of *L. monocytogenes* at fixed conditions, such as at a specific temperature and pressure level.

2.1.1. Linear models

Studies have shown that the pressure destruction kinetics of *L. monocytogenes* in foods as a function of pressure-holding times may follow a first order process in which the number of viable cells inactivated decreases proportionally depending on treatment time (Phua & Davey, 2007). This relationship is represented in Equation (1). Studies where L. *monocytogenes* behaviour followed a linear trend during high pressure treatments are shown in Table 1.

$$\log\left(N/N_0\right) = -kt = -\frac{t}{D_P} \quad (1)$$

where, assuming static conditions of pressure and temperature, N refers to the number of survivals in samples after pressure treatments; N_0 is the number of viable cells just before application of a pressure level set in the experimental design; t is the pressure-holding time; k is the inactivation rate constant of bacteria number at pressure P due to HHP treatments; and D_P is the time required for one log reduction of bacteria number due to HHP treatments.

2.1.2. Non-linear models

Despite the increasing number of published studies in which linear inactivation kinetics have been observed, patterns of microbial inactivation during HHP are frequently non-linear. Non-linear behaviour during pressure treatments is attributed to cumulative damage to microbial cells, which simultaneously affects a combination of processes or functions (Tay, Shellhammer, Yousef, & Chism, 2003).

The non-linear functions most commonly applied to describe *L. monocytogenes* inactivation kinetics under HHP are the Weibull model, the log-logistic function, the modified Gompertz equation and the Baranyi model (Table 2). Although these sigmoidal functions were originally developed for fitting growth curves, they have been restructured by authors and used to describe microbial survival curves after thermal treatments (Cole, Davies, Munro, Holyoak, & Kilsby, 1993; Linton, Carter, Pierson, & Hackney, 1995).

A tail-shaped pattern is frequent in non-linear inactivation models (Buzrul & Alpas, 2004; Hereu, Dalgaard, et al., 2012; Muñoz-Cuevas et al., 2013). The most accepted hypothesis to explain the tailing effect is the presence of subpopulations within a microbial population that are more resistant to pressure treatments and remain viable even after prolonged pressure holding times (Gayán, Torres, & Paredes-Sabja, 2012). The presence of a shoulder on inactivation curves has also been reported, characterized by a low rate of cell inactivation at the beginning of pressure treatments (Doona et al., 2012; Fletcher et al., 2008). Some hypotheses have been put forward to explain the shoulder in inactivation curves, such as non-uniform delivery of pressure into the product and different pressure sensitivities of the target microorganism (Bermúdez-Aguirre & Barbosa-Cánovas, 2011). The mechanism of tailing and shouldering needs to be elucidated in future studies to enable effective pressure treatments to be established.

Among the non-linear models, the Weibull model is the one most popularly applied to describe HHP-induced inactivation, due to its flexibility and simplicity (Bermúdez-Aguirre & Barbosa-Cánovas, 2011; Serment-Moreno, Barbosa-Cánovas, Torres, & Welti-Chanes, 2014). The Weibull distribution in pressure-induced inactivation events can be interpreted as a cumulative function that determines the exposure time at which the bacterial cells fail to resist pressures and become inactivated. This distribution assumes that the resistance of microorganisms present in a population differs from cell to cell (Chen & Hoover, 2004; Serment-Moreno et al., 2014).

Drawbacks have been reported in the application of the Weibull model to describe microbial inactivation by HHP, such as the fact that its parameters (n = shape parameter and δ = scale parameter) are dependent or strongly correlated, leading to instability in their estimations (Buzrul, Alpas, et al., 2008; Chen & Hoover, 2004; Doona et al., 2012). Mafart, Couvert, Gaillard, and Leguerinel (2002) found it worthwhile to fix the n parameter value at a probability-averaged characteristic of a strain, thereby enabling the δ values to be estimated from a linear regression. This leads to better stability of the δ values and increases the robustness of the model (Couvert, Gaillard, Savy, Mafart, & Leguérinel, 2005). Many authors have followed this procedure of fixing the n-value to obtain δ parameter estimates (Buzrul & Alpas, 2004; Chen & Hoover, 2004; Lerasle et al., 2014).

The log-logistic model assumes that bacterial cells in a population have different pressure resistances and that these differences are permanent (Chen, 2007b). Chen, Joerger, Kingsley, and Hoover (2004), Chen and Hoover (2003) and Muñoz-Cuevas et al. (2013) compared the application of the Weibull, log-logistic and Gompertz models to fit the same sets of pressure inactivation data. The Gompertz model was the