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Analysing and modelling the growth behaviour of *Listeria monocytogenes* on RTE cooked meat products after a high pressure treatment at 400 MPa

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ABSTRACT

Various predictive models are available for high pressure inactivation of *Listeria monocytogenes* in food, but currently available models do not consider the growth kinetics of surviving cells during the subsequent storage of products. Therefore, we characterised the growth of *L. monocytogenes* in sliced cooked meat products after a pressurization treatment. Two inoculum levels (10^7 or 10^4 CFU/g) and two physiological states before pressurization (freeze-stressed or cold-adapted) were evaluated. Samples of cooked ham and mortadella were inoculated, high pressure processed (400 MPa, 5 min) and subsequently stored at 4, 8 and 12 °C. The Logistic model with delay was used to estimate lag phase (λ) and maximum specific growth rate (μ_{max}) values from the obtained growth curves. The effect of storage temperature on μ_{max} and λ was modelled using the Ratkowsky square root model and the relative lag time (*RLT*) concept.

Compared with cold-adapted cells the freeze-stressed cells were more pressure-resistant and showed a much longer lag phase during growth after the pressure treatment. Interestingly, for high-pressure inactivation and subsequent growth, the time to achieve a concentration of *L. monocytogenes* 100-fold (2-log) higher than the cell concentration prior to the pressure treatment was similar for the two studied physiological states of the inoculum. Two secondary models were necessary to describe the different growth behaviour of *L. monocytogenes* on ready-to-eat cooked ham (lean product) and mortadella (fatty product). This supported the need of a product-oriented approach to assess growth after high pressure processing. The performance of the developed predictive models for the growth of *L. monocytogenes* in high-pressure processed cooked ham and mortadella was evaluated by comparison with available data from the literature and by using the Acceptable Simulation Zone approach. Overall, 91% of the relative errors fell into the Acceptable Simulation Zone.

1. Introduction

The occurrence of listeriosis, caused by foodborne *Listeria monocytogenes*, in the European Union has shown a fluctuating trend during the last five years, but with particularly high notification rates in some countries including Finland and Spain where the total number of cases increased by 97% and 90%, respectively, from 2005 to 2010 (EFSA, 2012). This foodborne disease shows a high fatality rate of around 20% and with high risk groups being neonates, pregnant women and immunocompromised individuals, especially elderly people. Epidemiological investigations have found a relation between this foodborne illness and the consumption of RTE foods with high numbers of *L. monocytogenes*. RTE meat products were reported as the first known source of listeriosis in Europe (FSAI, 2005). Due to the public relevance of *L. monocytogenes* in RTE foods, this foodborne pathogen is a priority of both regulatory agencies, *e.g.* when setting microbiological criteria, and of the food industry, which is responsible for producing safe products (Farber et al., 2011).

In RTE cooked meat products, such as cooked ham and mortadella, the thermal treatment of the manufacturing process eliminates *L. monocytogenes*, but recontamination can occur by cross-contamination during handling, slicing and packaging (Patterson et al., 2010). The physico-chemical characteristics of some RTE cooked meat products allow the growth of *L. monocytogenes* and it is a major safety concern if the pathogen reaches high concentrations during their distribution including extended refrigerated storage (Farber et al., 2011).

In order to eliminate *L. monocytogenes* or delay its growth in packed RTE cooked meat products, producers can apply specific control measures. High hydrostatic pressure (HHP) is a food preservation technology with interesting potential as a post-lethality treatment for RTE products (FSIS, 2012). As a non-thermal process the pressure-associated treatments minimize sensory changes, while causing microbial inactivation and related shelf-life increase and safety improvement of the processed products. The efficacy of HHP for inactivation of *L. monocytogenes* has been demonstrated by numerous studies on

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inoculated products (Simonin et al., 2012; Stollewerk et al., 2014) and by modelling the inactivation in laboratory broths or in selected foods, including RTE cooked meat products (Bover-Cid et al., 2011a; Chen and Hoover, 2004; Doona et al., 2012; Koseki and Yamamoto, 2007). However, HHP inactivation in combination with subsequent growth kinetics of the surviving cells remains little studied. This is unfortunate as the quantitative modelling of *L. monocytogenes* growth behaviour *e.g.* at different storage temperatures, would enable the optimization of the processing conditions to comply with food safety criteria. Concretely, Koseki et al. (2007) developed a probabilistic model for a 2-log increase in the concentration of *L. monocytogenes* in sliced cooked ham stored at 10 °C after HHP processing. Growth of *L. monocytogenes* after HHP processing may depend on its physiological state and possibly also on its concentration prior to the HHP treatment as well as on the food matrix (Bover-Cid et al., 2011b; Hugas et al., 2002). Importantly, the effect of these conditions on growth kinetics after HPP has not previously been quantified. The objective of the present study was to quantitatively characterize and mathematically model the growth of *L. monocytogenes* in two types of RTE sliced meat products (*i.e.* cooked ham and mortadella) during chilled (4 °C to 12 °C) storage and after a HHP treatment. Additionally, the effect of two inoculum levels and two different physiological states of *L. monocytogenes* before the HHP treatment was investigated.

2. Materials and methods

2.1. Product manufacture and characterization

Two different cooked meat products were studied: cooked ham (a lean product) and mortadella (a fatty product). They were produced *ad-hoc* by a local manufacturer using minced pork meat, with no fat added for cooked ham and with 20.5% fat added for mortadella. The following ingredients and additives were used (g/kg): water, 120; salt, 19.3; sodium tripolyphosphate, 5.0; dextrose, 5.0; sodium erythorbate, 0.5 and 0.85 of curing salt (sodium chloride containing 12% of sodium nitrite). Meat was minced in a cutter to a particle size of 6 mm. Ingredients were homogenized in a mixer for 30 min, stuffed into an impermeable plastic film, and cooked in an oven at 68 °C for 5 h resulting in a product core temperature of 65 °C. Once cooled, the products were immediately transported to the laboratory and stored at 1 °C until use within one week.

The products were characterised physico-chemically in triplicate. The a_w was measured with an AquaLab™ instrument (Series 3; Decagon Devices Inc., Pullman, WA, USA). The pH was measured by direct measurement with a penetration probe (52-32; Crison Instruments SA, Alella, Spain) connected to a portable pH-metre (PH 25; Crison Instruments). Lactic acid concentration was determined by HPLC using an ion exclusion column (Transgenomic ICSepICE-ORH-801, Chrom Tech. Inc., MN, USA) with a refractive index (RI) detector. Nitrites were determined by spectrofluorometry according to the Spanish official methods (Anonymous, 1979). The fat, protein, water, and sodium chloride contents were determined according to the AOAC official method 2007.04 (Anderson, 2007) with a FoodScan™ device (FOSS Analytic, Hillerod, Denmark). The *t*-test was used to compare the obtained results between the two types of product.

2.2. L. monocytogenes strain and inoculum preparation

The *L. monocytogenes* CTC1034 strain was used in this work. It was originally isolated from a meat product and later shown to have a high resistance to HHP treatments (Bover-Cid et al., 2011a) and the fastest recovery when compared to ten different strains examined in preliminary experiments (data not shown). A stock culture of the strain was kept at −80 °C in Brain Hearth Infusion (BHI) broth (Beckon Dickinson, Sparks, Md., USA) with 2.5% NaCl (Merck, Darmstadt, Germany) and 20% glycerol.

One loopful of the stock culture was grown in salted BHI (with 2.5% NaCl) at 37 °C for 7 h. This fresh culture was used to prepare two different inoculum cultures in stationary phase reaching two different physiological states:

i) One was grown in salted BHI under refrigeration at 8 °C for 90 h to obtain a cold-adapted culture.
ii) The other was grown in salted BHI at 37 °C overnight (18 h) and subsequently freezed (in 20% glycerol) at −80 °C during less than one week to obtain a freeze-stressed culture.

These two physiological states are relevant from two perspectives. The cold-adaptation mimics the chilled conditions usually found in clean rooms for production of RTE products (i.e. slicing and packaging). Freezing conditions expose cells to concentrated solutes, which cause an osmotic stress similar to that caused by dry environments occurring in the food industry (e.g. clean and dry food contact surfaces). Additionally, some industrial processes to prepare sliced RTE products include a pre-freezing step to facilitate the slicing process.

2.3. Sample preparation

Cooked ham and mortadella were aseptically sliced, placed into plastic bags and inoculated with the pre-cultures described above at two different inoculum levels, *ca.* 10^7 or 10^4 CFU/g obtained by diluting appropriately in saline solution (0.85% NaCl (Merck) and 0.1% Bacto Peptone (Beckton Dickinson)). The inoculation procedure was followed according to Mejlholm and Dalgaard (2007). In brief each type of sliced product was inoculated with 1% (vol/wt) of a *L. monocytogenes* pre-culture as four portions of 0.25% (vol/wt). After each addition, the products were manually tumbled to distribute *L. monocytogenes* on the slices. After inoculation, 25–28 g portions of each product were vacuum packaged (EV-15-2-CD; Tecnotrip, Terrassa, Spain) in PET/PE bags (oxygen permeability <50 $cm^3/m^2/24$ h and low water vapour permeability <15 $mg/m^2/24$ h; Sacoliva S.L., Barcelona, Spain).

2.4. High hydrostatic pressure treatment and storage

Samples were pressurised at 400 MPa for 5 min using commercial high pressure processing equipment (Wave 6000; Hiperbaric, Burgos, Spain) at an initial fluid temperature of 15 °C. The come-up rate was 220 MPa/min and the pressure release almost instantaneous (<6 s). The HHP conditions applied were selected on the basis of a previous work (Hereu et al., 2012) to avoid the complete inactivation of *L. monocytogenes*, thus enabling quantification of the pathogen in most of the analysed samples. The inactivation results and the influence of the physiological state and inoculum level of *L. monocytogenes* and the type of product were statistically assessed through the ANOVA test.

A representative part of the samples inoculated with the lower inoculum were not pressure treated and used as a control. Pressurised and non-pressurised samples were stored at 4, 8 and 12 °C for not less than 40, 20 and 16 days, respectively. In this way, a total of 36 growth curves were obtained including 24 for pressurised treatments (two inoculum levels, two physiological states, two products and three storage temperatures) and 12 for non-pressurised treatments (one inoculum level, two physiological states, two products and three storage temperatures). For each combination of conditions, the growth of *L. monocytogenes* was monitored in triplicate or duplicate as described below.

2.5. Monitoring L. monocytogenes

Samples from all treatments were periodically analysed with a total of 9 to 23 sampling times distributed all along the refrigerated