Available online at www.sciencedirect.com
ScienceDirect
International Journal of Food Microbiology 119 (2007) 300–307
INTERNATIONAL JOURNAL OF Food Microbiology
www.elsevier.com/locate/ijfoodmicro

Predictive modelling of the recovery of *Listeria monocytogenes* on sliced cooked ham after high pressure processing

Shigenobu Koseki *, Yasuko Mizuno, Kazutaka Yamamoto

National Food Research Institute, 2-1-12, Kannondai, Tsukuba, Ibaraki 305-8642, Japan

Received 31 May 2007; received in revised form 17 August 2007; accepted 20 August 2007

Abstract

This study examined bacterial recovery on sliced cooked ham that was inoculated with *Listeria monocytogenes,* treated by high pressure processing (HPP) and then stored at 10 °C for 70 days. The number of *L. monocytogenes* on the ham inoculated with 5 $\log_{10}$ CFU/g was initially reduced by HPP at 500 MPa for 10 min to below the detectable level (10 CFU/g). However, the bacterial count gradually increased during storage, and exceeded the initial inoculum level at the end of the 70-day period, having risen by 7–8 $\log_{10}$ CFU/g. A novel predictive model was therefore developed to estimate the recovery of *L. monocytogenes* during storage after HPP. Recovery of *L. monocytogenes* was defined as the detection of $>10^2$ CFU/g bacteria, in view of the relevant food safety objectives of *L. monocytogenes*. At each 14-day sampling session, the ham was scored as either 1 or 0 indicating bacterial recovery or no bacterial recovery, respectively. The data were then subjected to a simple linear logistic regression model, which provided a good fit as indicated by the performance statistics. Using this model, we estimated the minimum HPP conditions necessary for the required storage periods. Additionally, as the developed model was based on logistic regression, the probability of the recovery of *L. monocytogenes* during storage after HPP was estimated. Our model not only calculated the appropriate shelf life and process conditions, but also provided a method for evaluating the risk of the recovery of pathogenic bacteria during storage.

Keywords: High pressure processing; Recovery; Logistic regression; Interface model

1. Introduction

Previous studies have investigated the use of high pressure processing (HPP) for the non-thermal inactivation of various microorganisms in food products (Cheftel, 1995; Hendrickx and Knorr, 2001). However, so far, there have been few reports on the recovery of bacteria during storage after HPP treatment. Some studies have reported the recovery of damaged pathogenic and spoilage bacteria in nutrient broth, milk, phosphate buffer, and ground pork after treatment by HPP followed by storage for between 6 h and 4 weeks at various temperatures (Bozoglu et al., 2004; Bull et al., 2005; Chilton et al., 2001; Ellenberg and Hoover, 1999; Koseki and Yamamoto, 2006). The findings showed that HPP-injured cells could repair themselves within 1–15 days, indicating the potential for bacterial recovery on these food products (Bozoglu et al., 2004). Furthermore, we previously demonstrated that *Escherichia coli* cells injured by HPP could recover even in a nutrient-free environment (phosphate-buffered saline) at 25 °C within 48 h (Koseki and Yamamoto, 2006). These reports demonstrated that surviving bacterial cells could not be detected on a non-selective medium immediately after treatment; therefore the conventional procedure of detecting injured cells by comparing recovery on selective and non-selective media could not be applied to the assessment of cells injury immediately following HPP. Therefore, HPP-injured cells may recover during storage.

Most of the published reports on HPP-induced microbial inactivation have analyzed the number of surviving cells immediately after treatment, which does not take into account the recovery of injured cells. However, damaged cells lacking colony-forming ability on an agar plate might survive, but might not be detected immediately after HPP. Of course, surviving cells in numbers below the limit of detection of the method employed will also not be detected immediately after HPP. As a result, the microbial inactivation induced by HPP could be

* Corresponding author. Tel.: +81 29 838 7152; fax: +81 29 838 8122.
E-mail address: koseki@affrc.go.jp (S. Koseki).

0168-1605/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijfoodmicro.2007.08.025

overestimated in analyses that do not account for bacterial recovery. This inaccuracy could lead to an increased risk of food poisoning or spoilage, and might be critical for the safety of high-pressure-processed food. Hence, there is a need for a novel procedure to estimate the risk taking into account the potential microbial recovery after HPP. In the present study, the recovery cell number was defined as all cells detected on a non-selective medium without distinguishing recovery of injured cells from reproduction.

Predictive modelling is useful for the estimation of microbial behaviour, including growth and inactivation under various conditions. Predictive models of HPP-induced microbial inactivation have been developed with the aim of optimizing the processing conditions (Buzrul et al., 2005; Chen et al., 2004; Kilimann et al., 2005; Koseki and Yamamoto, in press-a; Reyns et al., 2000; Rodriguez et al., 2004; Yamamoto et al., 2005). However, these are generally kinetic models that only consider pressure and time as factors. Moreover, the published predictive models are based on survival immediately after HPP, and do not consider the recovery of injured bacteria during storage. Conventional modelling procedures might not be sufficient for developing a predictive model for microbial inactivation by HPP taking into account bacterial recovery. We therefore adapted a conventional modelling concept to create a novel predictive modelling procedure for bacterial recovery after HPP.

We recently reported the development of a predictive bacterial survival/death interface model for estimating the inactivation effect of HPP, which was adapted from a conventional model of bacterial growth limits, known as the growth/no growth interface model (Koseki and Yamamoto, in press-b). This survival/death model can evaluate the inactivation effect, taking into account the period of recovery, and can include numerous relevant parameters in addition to pressure and time. As this predictive model is derived from a logistic regression procedure and evaluates survival/death in terms of probability, it could be used in risk assessment. We therefore hypothesized that a predictive model for the recovery of bacteria after HPP could be developed by applying the survival/death interface modelling procedure.

The present study used vacuum-packed sliced cooked ham to investigate the application of HPP to food products. High-pressure processed ham is usually distributed chilled and is believed to be at high risk from the pathogenic bacterium *Listeria monocytogenes* (ICMSF, 1996). The objective of this study was to develop a probabilistic predictive model, using a logistic regression procedure, for the recovery of *L. monocytogenes* on sliced cooked ham after treatment with HPP and storage. The model was intended to predict the recovery of *L. monocytogenes* after HPP and storage, and to determine the appropriate processing conditions to deliver the required shelf life.

2. Materials and methods

2.1. Sample preparation

L. monocytogenes ATCC 19117 was used in this study, as it showed a greater baroresistance than other reported strains (ATCC 19111, ATCC 19118, ATCC 13932, ATCC 15313 and ATCC 35152) (Koseki and Yamamoto, 2007). The strain was maintained at −85 °C in brain heart infusion (BHI) broth (Merck, Darmstadt, Germany) containing 10% glycerol. A sterile disposable plastic loop was used to transfer the frozen bacterial cultures to 10 ml BHI broth in a glass tube. The cultures were incubated without agitation at 37 °C, and were transferred using loop inocula at three successive 24-h intervals. The bacterial cell suspension was diluted by serial 10-fold dilutions in 0.1% peptone water, and then inoculated onto sliced cooked ham samples (~5 g). The inoculum levels on the ham were adjusted to ~3 or ~5 $\log_{10}$ CFU/g. The sliced cooked ham (pH=6.3, water activity a_w=0.98), which had been pasteurized by heat treatment, was obtained from a local manufacturer on the day of production. Results of standard plate counts for uninoculated pasteurized ham, which was determined by incubation at 35 °C for 48 h on plate count agar (TSA; Merck) plates, were <10 CFU/g for ham used in all trials.

2.2. High-hydrostatic pressure treatment

The inoculated sliced cooked ham samples were placed into a sterile polyethylene bag and heat-sealed under a vacuum. The bag was then placed into the pressure chamber (3 cm in diameter and 15 cm in height) of a HIGHPREX R7K-3-15 hydrostatic pressurization unit (Yamamoto Suiatsu Kogyosho, Osaka, Japan). HPP was performed at constant pressures ranging from 400 to 600 MPa at 50 MPa intervals and a temperature of 25± 0.5 °C for 1, 5, 10, 20, 30, 45, and 60 min using water as the pressure medium. The compression rate was ~250 MPa/min, and the pressure-release time was <20 s (note that the HPP time reported here does not include the compression and pressure-release times). The temperature in the pressure vessel rose from 25 to 33 °C during coming-up to 600 MPa by compression heating. Then the temperature decreased to 25 °C for about 5 min during pressure holding. Following the HPP, the samples were stored in an incubator at 10 °C for up to 70 days. In total, 70 experimental sets with different combinations of pressure (400, 450, 500, 550, and 600 MPa), pressure-holding time (1, 5, 10, 20, 30, 45, and 60 min), and inoculum level (3 and 5 $\log_{10}$ CFU/g) were examined, and triplicate trials were conducted for each combination.

2.3. Enumeration of L. monocytogenes on ham during storage

Sampling was carried out at 14-day intervals for 70 days. Each ham sample was mixed with 45 ml of 0.1% peptone water in a stomacher bag, and then pummelled for 2 min in a TC82 blender (CDC, Italy). The undiluted sample solution (0.25 ml) was plated onto tryptic soy agar (TSA; Merck) in quadruplicate, and the samples that were serially diluted in 0.1% peptone water (0.1 ml) were plated onto TSA in duplicate. The plates were then incubated at 37 °C for 48 h, and the number of colonies was enumerated. The detection limit was 10 CFU/g. In the present study, the recovery cell number was defined as all cells detected on non-selective medium (TSA) without distinguishing recovery of injured cells from reproduction. Several typical colonies were taken from the plates using a sterile loop, and the