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Dynamic kinetic analysis of growth of *Listeria monocytogenes* in a simulated comminuted, non-cured cooked pork product☆

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ABSTRACT

The objective of this study was to directly construct a tertiary growth model for *Listeria monocytogenes* in a simulated comminuted, non-cured cooked pork product and simultaneously determine the kinetic parameters using a combination of dynamic and isothermal growth curves. Growth studies were conducted using a cocktail of 5 strains of *L. monocytogenes* in cooked pork under both dynamic and isothermal temperature profiles designed to examine the effect of temperature on bacterial growth.

A direct kinetic analysis method was used to construct the growth models and determine the kinetic parameters. The bacterial growth was simulated by a set of differential equations, and the temperature effect was evaluated by the cardinal parameters model. A numerical analysis and optimization method was used to simultaneously solve the different equations and search for the best fits of kinetic parameters for the growth curves and models. The estimated minimum, optimum, and maximum growth temperatures were 0, 33.0, and 42.6 °C, matching well with typical growth characteristics of this microorganism. The root-mean-square error (RMSE) of curve-fitting was 0.42 log CFU/g.

The growth models and kinetic parameters were validated using both independent dynamic and isothermal growth curves to check the accuracy of the models. The results showed that the RMSE of predicted growth was 0.49 log CFU/g. The residual errors of predictions follow a Laplace distribution, with 80.3% of the residual errors falling within ±0.5 log CFU/g of the observations. This study proves that the one-step dynamic analysis with both dynamic and isothermal temperature profiles can be an effective approach for simultaneously constructing a tertiary model and determining the kinetic parameters.

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1. Introduction

Listeria monocytogenes is a potentially fatal foodborne pathogen frequently associated with ready-to-eat (RTE) meat and poultry products. As a psychrotroph, *L. monocytogenes* has a minimum growth temperature around 0 °C, and can survive well in cold and wet environments. This microorganism can contaminate many of food contact surfaces in the food industry and at home. *L. monocytogenes* is easily destroyed by heat during cooking. However, cross-contamination can occur in the post-lethality areas, allowing *L. monocytogenes* to enter and re-contaminate fully cooked products prior to packaging. *L. monocytogenes* is relatively salt-tolerant, and can survive and multiply in contaminated RTE meat and poultry products even during refrigerated storage. The unique ability of *L. monocytogenes* to grow at refrigerated temperatures and its presence in RTE meat and poultry products makes it a serious public health hazard, as RTE products do not require a kill step prior to consumption. The populations with compromised immune systems, pregnancy, and poor health, and others such as cancer survivors are particularly vulnerable to the infections of *L. monocytogenes*. Foodborne listeriosis, although generally rare and usually sporadic, can cause serious public health consequences if occurring. The Centers for Disease Control and Prevention (CDC) estimated that *L. monocytogenes* causes approximately 1600 illnesses, 1500 hospitalizations, and 260 deaths annually in the United States (CDC, 2011). The mortality rate can be as high as 20% among the at-risk population. In the U.S., a zero-tolerance policy is enforced for RTE meats to control and prevent foodborne listeriosis (FDA & USDA-FSIS, 2003). It is worthwhile to note that "zero

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tolerance" does not mean no *L. monocytogenes* in a product. It means the absence of *L. monocytogenes* in a product at the time of detection according to a sampling plan. In the U.S., zero *L. monocytogenes* means no detection of the bacteria in either two of the 25 g samples tested. Under a Codex definition, zero *L. monocytogenes* means absence of *L. monocytogenes* in RTE products in 5 25-g samples using the 2-class attributes sampling plan (Codex Alimentarius, 2007).

Predictive microbiology is a useful tool for evaluating the potential growth of *L. monocytogenes* in contaminated RTE products and conducting risk assessment to estimate its effect on public health. The traditional methods in predictive microbiology usually require a three-step process to develop models for predictive applications (Huang, 2015). The first step is to conduct a series of isothermal studies to investigate the growth kinetics under constant temperature conditions and develop suitable primary models. This step identifies the most suitable primary model and estimates the associated kinetic parameters (growth rate and lag time, for example). The second step is to evaluate the effect of environmental factors such as temperature on the kinetic parameters of the primary models and choose an appropriate secondary model. In the third step, both primary and secondary models are combined to form a tertiary model that is used to predict the microbial growth after proper validation. The first two steps are commonly known as the inverse problems, using data to find kinetic parameters for the most suitable kinetic models. The last step in the traditional approach is a direct problem, using the kinetic parameters obtained in the first two steps and kinetic models to make predictions. While these steps are necessary in the traditional methods, they are very time-consuming and labor-intensive.

Recently, Huang (2015) developed a one-step dynamic method to directly construct a tertiary model to predict the growth of *Clostridium perfringens* in cooked beef during cooling. Instead of using the traditional methods, multiple dynamically cooling temperature profiles and growth curves were used to determine the kinetic parameters directly from the tertiary model, which included both primary and secondary models. This approach produced a very accurate model for predicting the growth of *C. perfringens* in cooked beef during cooling and under isothermal conditions. For dynamic cooling growth curves used in validation, this model produced a mean residual error of predictions of −0.02 log CFU/g, with a standard deviation only 0.23 log CFU/g. Overall, 74% of the residuals of predictions are <0.2 log CFU/g, 7.7% are >0.4 log CFU/g, while only 1.5% are >0.8 log CFU/g (Huang, 2015).

While this direct approach can produce a very accurate tertiary model, it is not efficient in determining the growth boundaries (temperature) of *C. perfringens*. As the response of *C. perfringens* was not sufficiently sensitive to temperature changes as it approached the upper and lower growth limits, the dynamic method was not effective in determining the upper and lower temperature limits of the bacterium. Therefore, the minimum and maximum temperatures were fixed to well-defined values during data analysis in Huang (2015). For well-characterized microorganisms such as *C. perfringens*, this approach does not affect the accuracy of the model. However, for other microorganisms, this approach may not be able to determine the growth limits. Grijspeerdt and De Reu (2005) used a dynamic method in combination of a step change near the minimum temperature of *Bacillus cereus* and *Enterobacter cloacae* to estimate the parameters of the Ratkowsky square-root model. Van Derlinden, Bernaerts, & Van Impe (2008) also applied a dynamic method to estimate cardinal growth temperatures of *Escherichia coli,* but relied on the dynamic temperature changes optimized near the cardinal growth parameters. However, the methods reported by Grijspeerdt and De Reu (2005) and Van Derlinden, Bernaerts, & Van Impe (2008) require prior knowledge of microorganisms to design the dynamic temperature profiles in order to estimate the parameters in the secondary model. This study was conducted to overcome the shortcoming of the methodology proposed in Grijspeerdt and De Reu (2005), Huang (2015), and Van Derlinden, Bernaerts, & Van Impe (2008). Instead of using all dynamic and pre-optimized temperature profiles, this study attempts to use an arbitrarily changing dynamic profile that simulates random changes in temperature in combination with isothermal growth curves to construct growth models and determine the associated kinetic parameters. Isothermal growth curves were strategically located near the minimum, optimum, and maximum growth temperatures of *L. monocytogenes*, while the dynamic profile was designed to cover the entire temperature range and different growth phase of the microorganism. This approach, if proven effective and suitable, may become a new time-saving and cost-effective methodology for directly constructing tertiary models and determining kinetic parameters of microorganisms in foods.

2. Materials and methods

2.1. Sample preparation

Fresh pork loin, purchased from a local grocery store, was cut and ground through ~3.2 mm holes in a meat grinder. Ground pork was mixed with 15% ice, 1.5% salt, 2% sucrose, 0.3% sodium tripolyphosphate, 2.5% sodium lactate, and 0.468% sodium ascorbate in a Stephan Universal Food Processor (Model UMC 5, Hameln, Germany) to prepare a simulated meat product. The product was mixed at high speed (1500 rpm) for 2 min, and then at low speed (300 rpm) for another 2 min. The simulated meat product was divided into small portions (150–200 g), packaged in plastic bags, and vacuum-sealed. The product (~1 cm in thickness) was cooked in a hot water bath at 90 °C for 30 min. Once cooked, the products were immediately submerged under iced water and cooled. After cooling, the cooked meat was subdivided into 4 ± 0.05 g portions and packaged in filter bags (Whirl-Pak®, 7 oz., 95 mm × 180 mm × 0.08 mm, NASCO - Fort Atkinson, Fort Atkinson, WI). Fifty (50) filter bags with meat samples were placed in a larger plastic bag, and vacuum-sealed. After which, the samples were transferred to a −80 °C freezer and kept frozen until ready for use.

2.2. Bacterial cultures and sample inoculation

Five rifampicin (rif)-resistant strains of bacteria, including *L. monocytogenes* 4b (F2365, H7858, and ATCC 19115), *L. monocytogenes* 1/2b (F4260), and *L. monocytogenes* 1/2a (V7), were used in this study. These strains were the same as the strains used in previous studies (Fang & Huang, 2014; Fang, Liu, & Huang, 2013). These bacterial strains were induced to resist 100 mg/L of rifampicin (Sigma, R 3501-5G, Sigma-Aldrich Co., MO) in Brain Heart Infusion (BHI) broth (BD/Difco Laboratories, Sparks, MD) and kept frozen at −80 °C. The stock cultures were prepared by inoculating the thawed culture of each strain to 10 ml BHI broth containing 100 mg/L of rifampicin (BHI/R) and incubated aerobically at 37 °C overnight with mild agitation (~100 rpm) on an orbital shaker. The overnight culture of each strain was streaked onto Tryptic Soy agar (TSA, BD/Difco Laboratories) plates that were supplemented with 100 mg/L rifampicin (TSA/R). The viability of the rif-resistant *L. monocytogenes* cultures was regularly propagated and maintained on TSA/R plates at 4 °C.

One day prior to each experiment, a loopful of each culture was individually inoculated into 10 ml BHI/R and incubated for 18–20 h at 37 °C with agitation (~100 rpm). The cultures were