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ScienceDirect

Procedia Food Science 7 (2016) 145 – 148

Procedia Food Science

9th International Conference on Predictive Modeling in Food

Modeling the Enhanced Thermal Inactivation of *Cronobacter sakazakii* by Inclusion of "Parabens"

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Abstract

The goal of this study is to develop mathematical models that describe the enhanced thermal inactivation of *Cronobacter sakazakii* by the inclusion of "parabens". The key parameters include heating temperature, parabens concentration, and the length of parabens' alkyl side chains. The heating trials were conducted in a submerged coil apparatus using Brain Heart Infusion (BHI) as the model heating menstruum. The results clearly demonstrated that a significant enhancement of thermal inactivation is concentration dependent and increases with increasing alkyl chain length. Once data sets are complete, primary and secondary models will be developed for prediction of thermal inactivation parameters.

Peer-review under responsibility of Department of Food Science, Faculty of Food Engineering, University of Campinas.

Keywords: Enhancement; parabens; thermal inactivation; parameters; modeling

1. Introduction

Cronobacter sakazakii, is a gram-negative, rod-shaped pathogenic bacterium. It has been linked to fatal invasive infections in infants traced back to low-level contamination of powdered infant formula (PIF). While a rare foodborne disease, infected infants suffer from potentially life threatening bacteremia, meningitis and necrotizing colitis, which can include permanent neurological damage[3]. It is controlled through effective pasteurization of infant formula prior to dehydration. The thermal resistance of *Cronobacter sakazakii* strains in rehydrated PIF can vary

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Peer-review under responsibility of Department of Food Science, Faculty of Food Engineering, University of Campinas.
doi:10.1016/j.profoo.2016.06.003

substantially. Using a submerged coil apparatus, the D_{58}-values ranged from 30.5 to 591.9 sec, with *C. sakazakii* 607 being the most heat resistant strain[2]. Reducing the thermal resistance of *C. sakazakii* would allow PIF to be processed at lower temperatures and may provide a potential means of providing a post-final packaging pasteurization of PIF.

Parabens are a group of p-hydroxybenzoic acids, which possess both antioxidant and antimicrobial activity. They are widely used in foods, pharmaceuticals, and cosmetics, such as food colorings, antacids, toothpastes, mouthwash, hair care products, soaps, body washes, lotions, and eye drops. Past research has established that a shorter heat treatment achieved a desired log-reduction when propyl-paraben added[1]. In addition, the relative antimicrobial activity of the parabens increases with the length of alkyl side chain goes up (methyl, ethyl, propyl, butyl, heptyl)[4]. Moreover, parabens are active for their antimicrobial function over a pH range 4~8[4]. It makes parabens a potentially desirable antimicrobial agent in various food applications.

The overall goal of the current research is to determine if thermal inactivation can be enhanced through synergistic action of the heat treatment with the inclusion of parabens. The specific goals are (i) establish quantitatively the effects and interactions of heating temperature, parabens alkyl side chain length, and parabens concentration on the thermal resistance of *C. sakazakii* in a model system (brain heart infusion), and (ii) use these data to develop primary and secondary mathematical models that effectively describe these process parameters. The current manuscript provides the results and observations related to the first specific goal.

2. Materials and methods

***C. sakazakii* isolates.** *C. sakazakii* 607, one of twelve strains obtained from the Food and Drug Administration/Center for Food Safety and Applied Nutrition stock culture collection was used throughout the study. All cultures were stored in brain heart infusion broth (BHI) with 30% glycerol at -70°C.

Parabens. The parabens used in the study are methyl-paraben (MP), ethyl-paraben (EP), propyl-paraben (PP), butyl-paraben (BP) and heptyl-paraben (HP), which were obtained from Fisher Scientific, Pfaltz & Bauer, and MP Biomedicals. All of them were white powder/crystal stored at room temperature.

Sample preparation and inoculation. Approximately 24 h before the experiment, 10 ml of BHI was inoculated with *C. sakazakii* 607 strain. The culture was incubated at 37°C for 24 h and then concentrated by centrifugation. Before starting a thermal trial, 80 µl of a specific solubilized paraben with the desired concentration was added to a 19-ml BHI blank and then agitated thoroughly. A 1-ml portion of the concentrated culture was transferred to 19 ml of BHI broth and vortexed. This served as the paraben treatment zero-time sample. The initial level of *C. sakazakii* 607 in the zero-time sample was approximately 10^8 CFU/ml.

Determination of enhancement of thermal resistance with submerged coil apparatus. Thermal trials with the submerged coil apparatus were conducted using a modification of the techniques of Buchanan and Edelson[2]. Prior to the start of a heating trial, 9 vials, each with 3.6 ml 0.1% peptone water were placed on the carousel. Then, 10 ml of zero-time sample was injected into the heating coil apparatus that was pre-equilibrated to 52°C, 55°C, or 58°C and preprogrammed to deliver 400 ul aliquots at designated sampling time i.e. every 90 seconds. After being ejected by the submerged coil, the vials were immediately put in ice to halt any further thermal inactivation. The samples were then transferred to precooled dilution blanks, which contained 9.9 ml 0.1% peptone water.

Plating and enumeration. All the dilutions (10^{-1}, 10^{-3}, 10^{-5}) were surface plated onto Tryptic Soy Agar (TSA) and MacConkey Agar (MA) using a spiral plater. After allowing the plates to dry for 10 minutes, they were inverted and incubated at 37 °C for 18-24 h. Plates were enumerated with an automatic plate counter, and converted to $\log_{10}$ values.

Inactivation curves analysis. The collected data were imported into Excel spread sheets and inactivation curves were graphed. The survivor curves were then fitted to the survival models using IPMP software.

3. Results

Initial preliminary studies confirmed that *C. sakazakii* 607 was the most heat resistant among twelve strains of *C. sakazakii* available, and ethanol could be used to solubilize the parabens without confounding the assessment of thermal resistance.