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Inactivation of *Salmonella typhimurium* DT 104 in UHT whole milk by high hydrostatic pressure

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Abstract

Cell suspensions of *Salmonella typhimurium* DT 104 in ultra-high temperature (UHT) whole milk were exposed to high hydrostatic pressure at 350, 400, 450, 500, 550, and 600 MPa at ambient temperature (ca. 21 °C). Tailing was observed in all survival curves, and sigmoidal survival curves were observed at relatively high pressure (500–600 MPa). Four modeling methods (linear and nonlinear including Weibull, modified Gompertz, and log-logistic models) were fitted to these data at 500, 550, and 600 MPa. Performances of the modeling methods were compared using mean square error (MSE). The linear regression model at these three pressure levels had a mean square error (MSE) of 1.260–2.263. Nonlinear regressions using Weibull, modified Gompertz, and log-logistic models had MSE values in the range of 0.334–0.764, 0.601–1.479, and 0.359–0.523, respectively. Modeling results indicated that first-order kinetics could not accurately describe pressure inactivation of *S. typhimurium* DT 104 in UHT milk; the log-logistic model produced the best fit to data.

Keywords: High hydrostatic pressure; Inactivation; *Salmonella typhimurium*; Modeling; UHT milk

1. Introduction

Applications in food preservation employing high hydrostatic pressure processing (HPP) now have a well-established base of knowledge. Available food products incorporating HPP are proliferating in the global marketplace. The primary reason for use of HPP in food processing is its inactivation of microorganisms in foods that result in safe products, with sensory and nutrient qualities more closely resembling untreated products than the thermally processed counterparts (Hoover, 1993).

It is commonly accepted in the canning industry that thermal inactivation of microorganisms follows first-order kinetics (Stumbo, 1973). This is based on the assumption that all bacterial spores or cells in a population have identical resistances to heat. In reality, significant deviations from linearity have been reported (Cerf, 1977; Peleg and Cole, 1998; Van Boekel, 2002). Consequently, a strategy of overprocessing has been used to ensure the microbiological

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safety of canned foods at the cost of sensory and nutritional qualities. It is also true that first-order kinetics is not generally applicable for prediction of pressure inactivation of microorganisms. Survival curves featuring shoulders, curves with tailings, and sigmoid curves are not uncommon. Chen and Hoover (2003a) exposed *Yersinia enterocolitica* ATCC 35669 to 300–450 MPa in sodium phosphate buffer (0.1 M, pH 7.0) and 350–500 MPa in UHT whole milk. Tailing was observed in all cases. A linear model and three nonlinear models were fitted to these data, and residual plots strongly suggested that a linear regression function was not appropriate. Instead, nonlinear models including log-logistic and Weibull models better described the pressure inactivation kinetics of *Y. enterocolitica* in milk and buffer. Nonlinear models were also more appropriate for depicting the survival curves of *Listeria monocytogenes* Scott A in UHT whole milk at 400 and 500 MPa in the temperature range of 22–50 °C (Chen and Hoover, 2003b). In this case, the log-logistic model consistently produced the best fits for all survival curves, although the log-logistic model was inferior to Weibull model at predicting pressure inactivation. Tay et al. (2003) investigated the inactivation kinetics of *L. monocytogenes* Scott A (pressure-sensitive strain) and OSY-8578 (pressure-resistant strain) at 350 and 800 MPa. First-order kinetics was not suitable to describe the inactivation, and extended pressure treatment did not eliminate the tailing phenomenon. In order to optimize HPP and guarantee the safety of pressure-treated products while effectively delivering the best sensory quality, an accurate description of the inactivation of the relevant foodborne pathogens is essential.

Salmonella enterica serovar *typhimurium* Definitive Phage Type 104 (DT 104) is resistant to multiple antibiotics such as ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline (Low et al., 1996; Threlfall et al., 1996). Foodborne transmission of *Salmonella typhimurium* DT 104 has been documented in the UK in several outbreaks, with the suspected vehicles being roast beef, ham, pork sausage, salami sticks, chicken legs, and unpasteurized milk (http://www.wisc.edu/fri/briefs/dt104.htm; Anonymous, 1996). In the United States, this serotype of *Salmonella* is the second most commonly reported in foodborne salmonellosis (http://vm.cfsan.fda.gov/~mow/chap1.html). In this study, ultra-high temperature (UHT) milk was used as a sterile suspension medium for the *Salmonella* cells. UHT milk is made from fresh milk. After UHT treatment (i.e., heated to more than 135 °C for at least 1 s), all bacteria in the milk are completely inactivated while the flavor, taste, and nutritional value are reasonably well preserved. The objective of this study was to investigate the pressure inactivation of *S. typhimurium* DT 104 in UHT whole milk. Appropriate modeling methods, including linear and nonlinear ones, were used to evaluate inactivation data and the goodness-of-fit of these models were compared. We hope that the kinetic study involving the inactivation of *S. typhimurium* DT 104 in UHT milk can assist in the understanding of HPP application for dairy products.

2. Materials and methods

2.1. Culture preparation and viability determination

S. typhimurium DT 104 was grown in 9.0 ml of tryptic soy broth (TSB) at 37 °C for 18 h. After incubation, 0.1 ml of culture was transferred to 50 ml of TSB and incubated at 37 °C for 18 h. Cells were pelleted by centrifugation (2400×*g*) at room temperature (22–23 °C) for 15 min with an IEC Centra-4B Centrifuge (International Equipment Co., Needham Heights, MA). The supernatant was discarded and the pellet was washed in 0.1% (wt/vol) Bacto-peptone water (Difco Laboratories, Detroit, MI). The cells were resuspended in 50 ml of UHT milk obtained from a local grocery store for pressure treatment.

To determine viable cell counts after pressure treatment, milk samples were serially diluted in 0.1% (wt/vol) peptone water and spread-plated in duplicate on tryptic soy agar (TSA; Difco). Plates were incubated overnight at 37 °C and colonies counted manually.

2.2. High hydrostatic pressure equipment

Two high hydrostatic pressure units were used in this study. The Daniels 600 pressure unit was custom-designed by W.B. Daniels and built at the University of Delaware (Fig. 1). Its maximum pressure was 600 MPa and it featured multiple pressure chambers, each with a total volume of 7.5 ml. Samples were contained