High Pressure Inactivation Kinetics of *Escherichia coli* in Black Tiger Shrimp (*Penaeus Monodon*)

Srinivasa Rao Pavuluri, and Barjinder Pal Kaur

***Abstract*—**The aim of this study was to investigate the high pressure destruction kinetics of *Escherichia coli* in a black tiger shrimp (*Penaeus monodon)*. Pressure treatments in the range 300-600 MPa for 0-15 min was given at room temperature (27 ± 2 °C). Survival curves were established based on residual counts following high pressure treatment. Destruction kinetics were described as a dual effect, an initial destruction resulting from a pressure pulse (pulse effect) followed by a first order rate of destruction during the pressure holding time. During pressure-hold period, the associated D values (decimal reduction time) decreased with an increase in pressure. *E. coli* count in samples was significantly reduced with increased pressure and holding time with 3.70 log cycles reduction obtained at 600 MPa for 15 min.

***Keywords*—**High pressure processing, Black tiger shrimp, *Escherichia coli*, Inactivation kinetics.

I. Introduction

HIGH pressure processing (HPP) offers an attractive alternative to heat pasteurization as a means to produce preservative-free, microbiologically safe and stable foods. This technology is currently being used on items such as guacamole, salsa, fruits smoothies, oysters, jams and jellies in North America, Europe, Australia and Asia [1]. The total production of pressure treated food products is steadily growing. HPP targets foodborne pathogenic and spoilage microorganisms without altering the food quality [2]. HPP inactivates microorganisms by acting on multiple targets including intracellular and membrane-bound enzymes [3]. Earlier studies have demonstrated the efficacy of HPP in inactivating a wide spectrum of Gram-negative and Gram-positive bacteria in suspensions, as well as in various solid food items. Multi-dimensional HPP treatment incorporating a combination of time, pressure, heat, and antimicrobial compounds or performed consecutively with other decontamination methods to create "hurdle" effects that could significantly eliminate various pathogens [4].

The patterns of HPP inactivation kinetics, as reported with different micro-organisms are quite variable and influenced by pressure, temperature and medium composition [5]. The process conditions for the inactivation of microorganisms with regard to industrial applications should be considered for a broad use of high pressure technology in food processing [6]. To increase the safety and stability of HPP processed foods, the pressure treatment must ensure a satisfactory reduction in the initial microbial counts, thus kinetic analysis and the pressure dependency of microbial inactivation rates are needed. In general, pressures ranging from 300 to 600 MPa can inactivate most vegetative cells (pathogenic and spoilage), yeasts, and molds [7]. In the area of HPP of fish, some research has been carried out on inactivation kinetics of tuna meat and squid mantle flesh. No major study has been carried out on destruction kinetics of pathogens in shrimp using the HPP technique. *Escherichia coli (E. coli)* is a major food-borne pathogen and the risk of its infection is a particular problem in the food industry. A gram-negative bacteria *E. coli* was selected for this study as this food-borne pathogens have been reported to be resistant to pressure treatments [8]. The aim of this study was to investigate the high pressure destruction kinetics of *E. coli* in a black tiger shrimp within a range of 300 to 600 MPa treatments at oom temperature (27±2 °C).

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II. Materials And Methods

2.1 Sample preparation

Freshly harvested black tiger shrimp (*Penaeus monodon*) of uniform size (25-35 g) were procured from Shankarpur coast, West Bengal, India. Samples were transferred in insulated boxes with ice (ice: sample, mass/volume ratio of 1:1) to Indian Institute of Technology, Kharagpur within 4 h. The samples were washed with chilled water, deheaded and shelled manually. Then fish slurry was prepared by blending 10 g of shrimp sample in 90 ml of 0.1% peptone water using sterilized mortar pestle. The prepared slurry was sterilized, cooled and inoculated with the activated pathogens and test samples were prepared by taking 10 ml aliquots of the slurry stock in LDPE pouches.

2.2 Preparation of inoculums

Frozen pure cultures stock *of E. coli* ATCC 11775 were maintained in the Agricultural and Food Engineering Department, IIT Kharagpur. The culture was maintained on tryptone soy broth supplemented with 0.6% yeast extract. The cultures were transferred on a weekly basis to ensure their viability. *E. coli* was incubated at 37 °C for 24 h to obtain

stock suspension containing 10^8 cfu/ml and this was used to establish the high pressure destruction kinetics.

2.3 High pressure processing

Packed samples were treated in a batch model high pressure food processor (Model: S-1L-100-250-09-W; Make: Stansted Fluid Power Systems, UK) having 2 L vessel capacity (cylindrical vessel with 100 mm inner diameter and 250 mm depth). Thirty percent aqueous solution of monopropylene glycol was used as the pressure transmitting medium. The samples were loaded into the vessel and treated at 300, 400, 500 and 600 MPa pressures for a holding time of 1 sec, 3, 6, 9, 12 and 15 min at room temperature (27 ± 2 °C) at a rate of 300 MPa/min. The adiabatic heating of pressure transmitting fluid was 2–3 °C for every 100 MPa increase in pressure. Samples were stored at 2 ± 0.5 °C after processing until further analysis. Untreated samples were used as controls.

2.4 *Enumeration*

Pressure treated samples were enumerated to determine the surviving microbial populations. Considering ease of handling, the direct pour plate method was adopted in this study. *E. coli* was enumerated on tryptone soy agar and the plates were incubated for 24 h at 37 °C. Initial counts were obtained from untreated control samples. The colonies counts were taken with 20–300 colonies.

2.5 Data analysis and microbial kinetics estimation

Microbiological analyses were conducted in duplicates and all other analyses were replicated thrice. Results were reported as their mean values ± standard deviation. Two-way analysis of variance (ANOVA) of the data was carried out using the SPSS 17 for Windows (SPSS Statistical Software, Inc., Chicago, IL, USA) software package. The difference between the pairs of means was evaluated by the Tukey's test at a confidence interval of 95%.

The high pressure-hold destruction of microorganisms was analyzed based on the combination of pressure inactivation with a pressure pulse effect (PE) and pressure-hold effect [9]. The pulse effect represents the destruction achieved during a pressure pulse, which consists of pressurization to the desired level followed by immediate release of pressure (with no holding time).

The PE values were calculated by determining the logarithmic difference in the microbial counts between the control samples and pressure treated samples subjected to a pressure pulse.

$$PE = Log\ (N_o) - Log\ (N_{PE})$$

The pressure destruction kinetics of microorganisms during the pressure-hold time phase were analyzed based on a first order reaction indicating a logarithmic order of death, and expressed as:

$$Log\ (N_t/N_{PE}) = -kt$$

Where, N_t is the number of surviving microorganisms following pressure treatment for time t (min)

N_0 is the initial number of microorganisms with no pressure treatment

N_{PE} is the number of survival cells after single pulse pressure

k is the reaction rate constant (min^{-1}).

The treatment time at any given pressure resulting in 90% destruction of the existing microbial population, i.e. resulting in one decimal reduction of the surviving population, is referred to as the decimal reduction time or D_p value. This was calculated as follows

$$D_P = 2.303/k$$

The pressure sensitivity parameter, z_p values was determined as the negative reciprocal of the slope Log D_p vs. pressure plots. Mathematically,

$$z_P = (P_2\text{-}P_1)/Log\ (D_1/D_2)$$

III. Results And Discussion

3.1 Destruction kinetics of microorganisms

The survival curve for *E. coli* (Fig. 1) indicate that the destruction was influenced by both pressure level and holding time. As expected survivor curves for higher pressure levels were steeper than at lower pressures, confirming higher K-values at higher pressures. The figures also demonstrate a good fit of data for the first order model, suggesting that the pressure destruction of *E. coli* followed the semi-logarithmic model during the pressure hold period. Computed D-values and other kinetic parameters are tabulated in Table 1. The Dp values for *E. coli* ranged between 2.20-6.19 for pressurization range of 300-600 MPa. Dp values of 1.66, 1.22, 0.68 and 1.35 min at 600 MPa for *E. coli* in raw milk, peach juice, orange juice and BHI broth, respectively were reported earlier [10].

It has been reported that HPP could reduce the populations of *E. coli* by 5 to 6 logs (at treatments up to 345 MPa) and 5 logs (300 MPa), respectively [8], [11]. A 6 log reduction in *E. coli* was reported in 6% fat bovine milk after the 5 min treatment at 450 MPa but the results obtained in the present study did not corroborate with their findings [12]. Pressure treatments reduced the populations of *E. coli* by 3.7 log cfu/ml, which is most likely because of the differences in test substrates used in our study.

3.2 Pulse effect

The PE values ranged from 1.16 log cycle reduction at 300 MPa to 2.17 log cycle reduction at 600 MPa. Riahi et al. (2003) [13]reported complete destruction of *Z. bailii*, *P. membranaefaciens* and *L. mesenteroides* with a single pulse at 400 and 300 MPa, respectively, in apple juice. Basak et al. reported about a 3 to 4 log cycle reduction in the population of *L. mesenteroides* and *S. cerevisiae*, respectively [14]. It is believed that rapid decompression invokes cavitations in the cell that result in physical disruption and death.

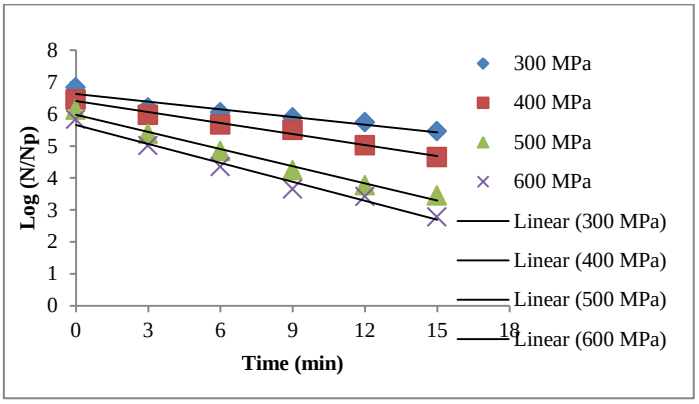

Fig. 1 High pressure survival curves of *E. coli* in fish slurry

3.3 Pressure sensitivity of D values

The zp values indicate the pressure difference that will result in ten-fold change in D values. The D_P value curves are shown in Fig. 2. The high R^2 value (0.94) indicates that the pressure death time approach is applicable to describe the pressure sensitivity parameters. The associated zp value for E coli was 555 MPa. However, much lower zp values for spoilage and pathogenic microorganisms were observed by Hiremath and Ramaswamy (2011) [15] in mango juice (zp values of 84, 84, 72, 204 and 121 MPa) for *Z. bailii*, *P. membranaefaciens*, *L. mesenteroides*, *E. coli* O157:H7 and *L. monocytogenes*, respectively).

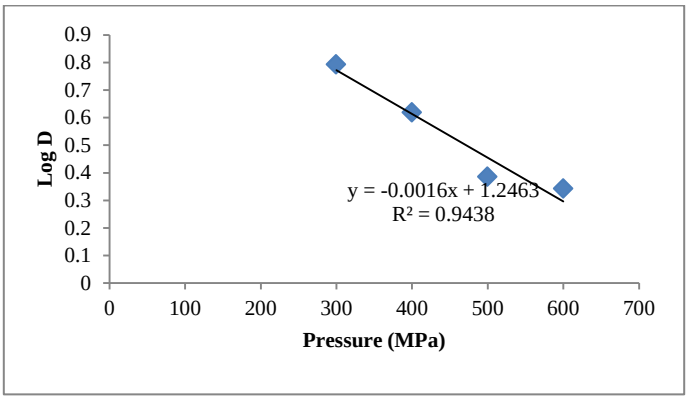

Fig. 2 High pressure decimal reduction time curves for *E. coli* in fish slurry

IV. Conclusion

This study showed dual destruction behaviour of *E. Coli* in shrimp with a step change in the number of survivors due to the application of pressure pulse and a first-order rate of destruction during the pressure hold. In addition to evaluation of destruction kinetics, it is always useful to carry out the storage study of pressure treated sample & developing the growth kinetics.

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