Korean J. Food Sci. Ani. Resour.
Vol. 29, No. 3, pp. 310~316(2009)

Effects of Pressure Assisted Mild Thermal Treatment on Inactivation of *Escherichia coli* ATCC 10536 in Milk Suspension

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Abstract

In this study, the influence of pressure assisted mild thermal inactivation (PAMTI) on *E. coli* ATCC 10536 was examined at 200 MPa and temperature range of 20-50°C. Inactivation rate significantly increased ($p<0.05$) as temperature and time increased at 200 MPa. The maximum inactivation (7.91 log reduction) was obtained at 50°C for 30 min under 200 MPa, which meant the complete inactivation of *E. coli* ATCC 10536. Inactivation kinetics were evaluated with the first order inactivation rate (k), activation energy (E_a), thermal death time (TDT), and z value. Kinetic parameters were significantly ($p<0.05$) influenced by variation temperature of PAMTI. In this study, the synergistic effect of pressure and temperature were found in the inactivation of *E. coli* ATCC 10536 through PAMTI.

Key words : high pressure, inactivation, kinetic, *Escherichia coli*, milk

Introduction

Recently, high pressure treatment has emerged as one of novel food processing technologies (O'Reilly *et al.*, 2001). High pressure effects are uniform and nearly instantaneous throughout food geometry and processing equipment (Antonio Torres and Velazquez, 2005). High pressure offers unique advantages over traditional thermal treatments, as it exerts antimicrobial effects without changing the sensory and nutritional quality of foods (Huppertz *et al.*, 2002) and some advantages in meat processing (Hong *et al.*, 2005; Park *et al.*, 2006). In these high pressure food processing technologies, main research interest has been focusing on high pressure microbial inactivation for the last 5 years. In conventional pasteurization and sterilization, the use of heat can destroy nutrients such as thermally labile vitamins and also components responsible (Deliza *et al.*, 2005). Whereas, high pressure microbial inactivation has been widely recognized its potential to inactivate vegetative microorganism and inhibit undesired activity of various food related enzymes with minimal changes in sensorial and nutritional properties (San Martin *et al.*, 2002; Trujillo *et al.*, 2002; Van Opstal *et al.*, 2005).

There are influential parameters and considerations in high pressure microbial inactivation: pressure level, pressurization time, processing temperature, bacterial species and development phase, rates in compression and decompression, and environmental conditions (Metrick *et al.*, 1989; Styles *et al.*, 1991; Patterson *et al.*, 1995; Kalchayanand *et al.*, 1998a; Kalchayanand *et al.*, 1998b; Patterson *et al.*, 1998; Alpas *et al.*, 1999; Benito *et al.*, 1999). Among these parameters, temperature may have a significant effect on high pressure microbial inactivation; a combination of high pressure with mild heat can be effective method of spore inactivation (Sale *et al.*, 1970; Gao *et al.*, 2006a). Inactivation of resistant strains by mild heat processes may depend on the use of combined "multiple-hurdle" system of food preservation (Benito *et al.*, 1999). High pressure alone cannot inactivate bacterial spores; a combination with other techniques, such as temperature elevation is required (Krebbers *et al.*, 2003; Polydera *et al.*, 2004). The process is described as pressure-assisted thermal processing or high-pressure thermal sterilization, and can increase the temperature of the test samples instantaneously resulting in the rapid pressurization of the sample (Ahn *et al.*, 2007).

In this study, the influence of high pressure microbial inactivation and its huddle effect on the stationary phase of *E. coli* ATCC 10536 were examined with pressure assisted mild thermal inactivation (PAMTI) and its

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kinetic evaluation. Stationary phase cells are less sensitive to pressure than exponential cells (Mackey *et al.*, 1995; Smelt, 1998). It could describe more exact inactivation kinetics in the experiment. *E. coli* strains are a major public health concern with a low infectious dose, high resistance, and severe disease symptoms ranging from a mild diarrhea to hemorrhagic colitis and the life threatening hemolytic-uremic syndrome (Kilimann *et al.*, 2005). High pressure alone could not sufficiently inactivate microorganism, thus it may be necessary to use hurdle type of approach by combining high pressure with one or more other factors that act synergistically (Hugas *et al.*, 2002). Accurate prediction of the effectiveness of high pressure processing against food-borne pathogens based on inactivation kinetics is essential to permit production safe products (Hashizume *et al.*, 1995; Chen and Hoover, 2003; Erkmen and Dogan, 2004a).

These make it possible to reduce the pressure level in industrial high pressure food processing, which is important from the economical consideration (Corwin and Shellhammer, 2002). Data describing microbial inactivation mechanism and kinetics are required to ensure food safety (Erkmen and Dogan, 2004b). The primary aim of this study was to elucidate the huddle effect of pressure and temperature on microbial inactivation with PAMTI technique, and develop its kinetic under maximum pressure level of 200 MPa.

Materials and Methods

Preparation of *E. coli* ATCC 10536 cultures

A isolated colony from *Escherichia coli* ATCC 10536 on a nutrient agar (Difco Laboratories, Detroit, MI, USA) was inoculated in milk and incubated at 37°C for 24 h. Two mL of the *E. coli* ATCC 10536 suspension was placed in a sterile silicon tube (Ø10×25 mm), and then sealed with rubber stopper in order to eliminate any bubbles in the microbial suspension. Each *E. coli* ATCC 10536 suspensions was pre-cooled at 4°C for 1 h and initial number of *E. coli* ATCC 10536 was constantly controlled as 2.10×10^8 colony forming units (cfu)/mL.

High pressure lab-scale multi-vessel system

Pressure assisted mild thermal inactivation (PAMTI) was carried out in a high pressure multi-vessel equipment (Fig. 1), designed for laboratory scale experiment. It consists of four high pressure vessels (HPV-II, Hiptec, Busan, Korea), which were connected with a hydrostatic pump (HSF-300, Haskel, California, USA). Ethyl alcohol was used as pressure transmitting fluid from hydropneumatic pump. Coolant circulated through external cooling jacket connected with a cryostat (FP-80, Julabo, Seelbach, Germany) to control the temperature during PAMTI. Temperature profiles of *E. coli* ATCC 10536 suspensions were recorded by K-type thermocouple and data acquisition system (MV-100, YOKOGAWA, Tokyo, Japan) with

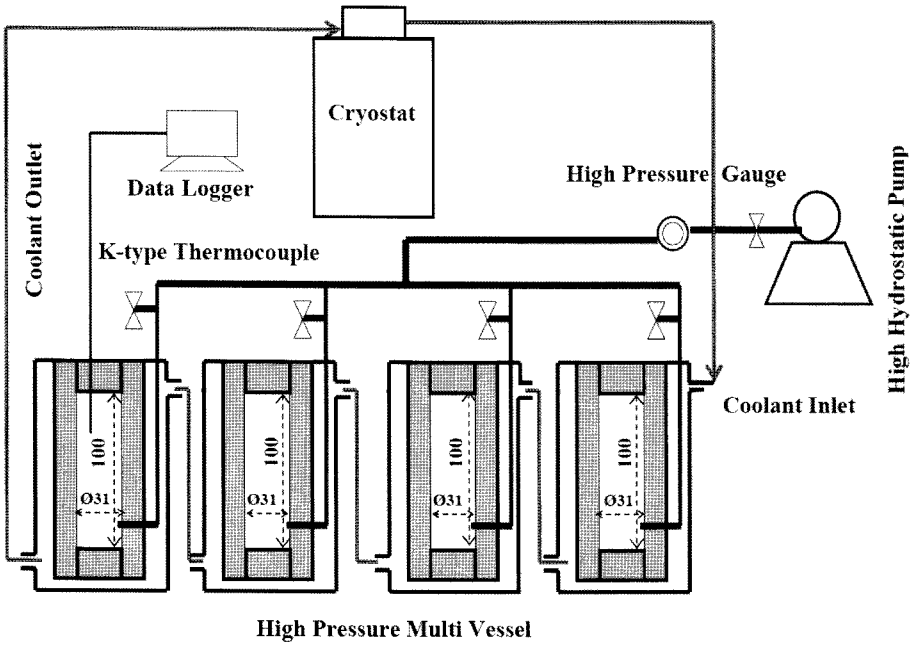

Fig. 1. Schematic diagram of high pressure lab-scale multi-vessel system.

a sampling interval of 1 s. The accuracy of temperature monitoring was better than ±0.2°C. All of thermocouples were previously calibrated using the reference thermocouple.

Experimental design

PAMTI was conducted with pressure, time, and temperature dependency at temperature of 20, 30, 40, and 50°C for 15-30 min under 200 MPa. Compression and decompression rates were controlled as 4 and 6.67 MPa/s, respectively. *E. coli* ATCC 10536 inoculated milk suspension was previously equilibrated to each designated temperature of 20-50°C before pressurization. Temperature equilibrated samples were subjected to aimed pressure and temperature for designated time.

Enumeration of *E. coli* ATCC 10536 inactivation

After high pressure treatment, *E. coli* ATCC 10536 suspensions were serially diluted with a sterile saline solution (0.9% (w/v) NaCl) and plated on nutrient agar (Difco Laboratories, Detroit, MI, USA). Each plate was incubated at 37°C for 24 h before colony counting. Plates containing 30 and 300 colonies were counted, and then the inactivation ratios were calculated as the logarithmic proportion of survived bacterial suspensions: log (N_0/N), N_0 as the initial number of *E. coli* ATCC 10536 (cfu/mL) and N as the number of survived *E. coli* ATCC 10536 (cfu/mL) after PAMTI.

Determination of inactivation kinetic parameters

First order kinetics of Eq. (1) was used to describe the microbial inactivation of PAMTI at each temperature as some previous studies carried out (Ludwig *et al.*, 1992; Tang and Sokhansanj, 1993; Chen and Tseng, 1997; Metwalli *et al.*, 1998; Ludikhuyze *et al.*, 2002; Wang *et al.*, 2002)

$$\ln\left(\frac{N_t}{N_0}\right) = -kt + c \quad (1)$$

where N_0 is initial number of *E. coli* ATCC 10536 (cfu/mL) and N_t is *E. coli* ATCC 10536 (cfu/mL) cell counts at time t after PAMTI. A linear regression represented the slope of k (inactivate rate constant) and intercept of c.

To describe temperature effect on inactivation rate constant at 200 MPa, Arrhenius equation Eq. (2) was used, and activation energy (E_a) was calculated with the equation presented in previous studies (Weemaes *et al.*, 1999; Wang *et al.*, 2002).

$$\ln k = \ln k_{ref} - \left[\frac{E_a}{R}\left(\frac{1}{T_p} - \frac{1}{T_{ref}}\right)\right] \quad (2)$$

where T_p is the pressurization temperature (K), k_{ref} is inactivation rate constant for reference temperature of pressurization at T_{ref}(K), E_a is the activation energy (J/mol), and R is the universal gas constant (8.314 J/mol·K).

Thermal death time (TDT) was calculated with 12 decimal reduction (12 D) concept for commercial sterilization through Eq. (1) and N_t as 10^{-12}.

z value was calculated to estimate the temperature increase for one log reduction of decimal reduction time from Eq. (3) presented by Tang *et al.* (2000) and Wang *et al.* (2002).

$$z = \frac{2.303 \cdot R \cdot T_p \cdot T_{ref}}{E_a} \quad (3)$$

Statistical analysis

The experimental data obtained from 5 replications were analyzed by ANOVA using the SAS statistical program (V 9.1, SAS Institute, Cary, NC, USA), and differences among the means were compared using Duncan's multiple range test ($p<0.05$).

Results and Discussion

Temperature evolution during PAMTI

Fig. 2 illustrates the changes in temperature of *E. coli* ATCC 10536 inoculated milk suspension and pressure transmitting fluid during PAMTI. The temperature rapidly increased after compression and the thermal profiles indicated more temperature increase with higher initial temperature. Maximum temperature increase recorded 67.2°C from the initial temperature of 50°C at 200 MPa. Each increased temperature of microbial suspension decreased to initially designated temperature within 5 min, representing the thermal gradient with externally circulating coolant. The effect of compression heating on microbial inactivation was discussed from many researches (Meyer *et al.*, 2000; Balasubramanian and Balasubramaniam, 2003; de Heij *et al.*, 2003; Matser *et al.*, 2004). Compression heating could influence microbial death kinetics data and should be documented in the experiment (Balasubramaniam *et al.*, 2004). Generation of high pressure is often accompanied with compression heat and subsequent heat transfer among pressure medium, pressure chamber, and samples (Guan *et al.*, 2006), and then the temperature of the content falls below the initial starting temperature of the test (Balasubramanian and Bala-

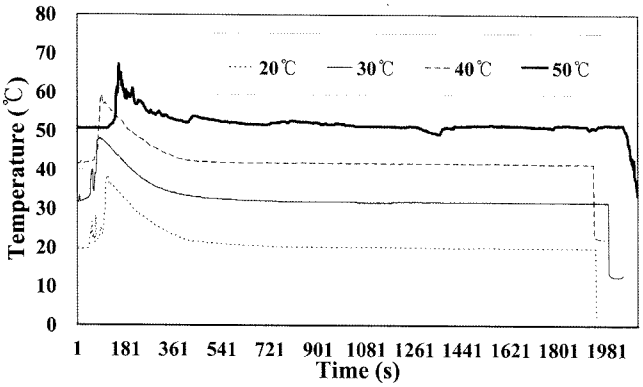

Fig. 2. Temperature profiles of *E. coli* ATCC 10536 in UHT milk suspension during PAMTI at 20, 30, 40, and 50°C under 200 MPa.

subramaniam, 2003). In these view points, there should be careful consideration for compression heating in high pressure pasteurization and sterilization process.

Influence of PAMTI on *E. coli* ATCC 10536

Table 1 describes the microbial viability of *E. coli* ATCC 10536 influenced by different pressurization time and temperature at 200 MPa. The inactivation efficacy significantly increased as exposure time increased at the same pressure level ($p<0.05$), and elevated pressure also induced the significantly increased microbial inactivation ($p<0.05$). Several scientists reported that counts of microorganism exponentially decreased with the elapsed holding-time in high pressure microbial inactivation (Erkmen and Karatas, 1997; Mallidis *et al.*, 2003; Erkmen and Dogan, 2004a; Van Opstal *et al.*, 2005). The most microbial inactivation was observed with 1.24 log reduction at 200 MPa for 30 min. However, this value could not meet the current standards (>5 log reduction) of *E. coli* inactivation for food safety (Kilimann *et al.*, 2005). Therefore, the other processing condition was required to achieve the qualifications of microbial safety.

Table 2 presented the time and temperature dependency of PAMTI on *E. coli* ATCC 10536 inactivation at the constant pressure of 200 MPa. Both elevated exposure time and temperature increased the microbial inactivation ($p<0.05$). The maximum microbial inactivation was observed as 7.91 log reduction after PAMTI at 50°C for 30 min. The efficacy of inactivation was also obtained with 5.07 log reduction from PAMTI at 50°C for 15 min. Several scientists reported the similar results to our experiment, which presented the increased efficacy of inactivation at the elevated temperature than room temperature (20-25°C). Van Opstal *et al.* (2005) pointed out that temperature is another important factor, which affects the inactivation level of vegetative bacteria in high pressure microbial inactivation. Patterson *et al.* (1995) reported that low and room temperature (-20 and 25°C) did not affect the microorganism, but *Staphylococcus aureus* became much more pressure sensitive with increasing temperature (40-50°C). Elevated temperature promoted the pressure induced inactivation of microorganisms, but the effect of low temperature is less clear as Patterson and Kilpatrick (1998) presented the similar results to our study. Fig. 3 represented the first-order kinetic of PAMTI between 20 and 50°C through linear regression of eq.(1). Inactivation rate constant (k), intercept (c), thermal death time (TDT), and activation energy (Ea) were presented in

Table 1. Microbial inactivation ($\log N_0/N$) of *E. coli* ATCC 10536 after PSTTDI at 200 MPa

Time (min)	Temperature (°C)			
	20	30	40	50
5	$0.26\pm0.02^{c,C}$	$0.93\pm0.02^{c,B}$	$3.24\pm0.17^{c,A}$	$3.27\pm0.15^{c,A}$
15	$0.52\pm0.02^{b,D}$	$1.14\pm0.07^{b,C}$	$3.54\pm0.15^{b,B}$	$5.07\pm0.15^{b,A}$
30	$0.56\pm0.01^{a,D}$	$1.36\pm0.13^{a,C}$	$4.23\pm0.06^{a,B}$	$7.91\pm0.01^{a,A}$

$^{a-c}$ Means within same row with different superscript letters are significantly different ($p<0.05$)
$^{A-D}$ Means within same column with different superscript letters are significantly different ($p<0.05$).

Table 2. Inactivation kinetic parameters of *E. coli* ATCC 10536 through PAMTI

Temperature (°C)	Inactivation kinetic parameters				
	k	C	TDT (min)	*Ea* (kJ/mol)	*z* (°C)
20	0.040 ± 0.000^{d}	-0.270 ± 0.025^{a}	681.60 ± 5.67^{a}	68.97	26.3
30	0.086 ± 0.010^{c}	-0.896 ± 0.001^{b}	312.66 ± 36.84^{b}		
40	0.257 ± 0.001^{b}	-3.127 ± 0.211^{d}	95.39 ± 0.73^{c}		
50	0.555 ± 0.003^{a}	-2.415 ± 0.210^{c}	45.42 ± 0.51^{d}		

$^{a-c}$ Means within same row with different superscript letters are significantly different ($p<0.05$).

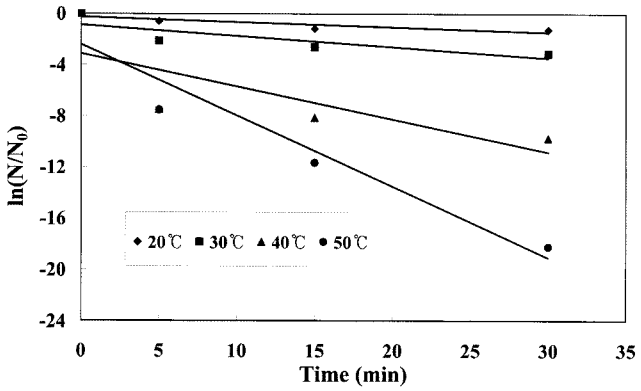

Fig. 3. First order kinetics of PAMTI on *E. coli* ATCC 10536 in UHT milk suspension at the temperature of 20, 30, 40, and 50°C under 200 MPa.

Table 2. Elevated temperature of PAMTI resulted in increased inactivation rate constant (k) with higher inactivation efficacy ($p<0.05$). TDT was estimated with the 12 D concept for commercial sterility, k, c, and Eq. (1). TDT significantly decreased with temperature increase ($p<0.05$). Minimum TDT for commercial sterilization was calculated as 45.42 min at 50°C under 200 MPa. In the temperature dependency, activation energy (Ea) was calculated as the reference temperature of 20°C and determined with 68.97 kJ/mol (Eq. (2)). z value was obtained with 26.3°C through activation energy of 68.97 kJ/mol and Eq. (3). This value is slightly higher than that of commercial sterilization process, however, this experiment was conducted at mild heat temperature of 20-50°C which meant the difficulty of direct comparison sterilization commercial process above 100°C. There should be careful consideration about efficient temperature and pressure, TDT, z value and their influence on nutritional and sensorial properties. The kinetic study also indicated that *E. coli* inactivation in buffer and carrot juice depended on the process temperature: the lowest were in the range of 20-30.8°C in buffer, but they increased with temperature in the range of 5-45.8°C in carrot juice (Guan *et al.*, 2006). Combinations of high pressure and moderate temperatures are potentially useful alternative retorting for the production of shelf-stable egg products (Rajan *et al.*, 2006). In contrast, Mallidis *et al.* (2003) could not find the synergistic effect of pressure and temperature for antimicrobial kinetic of *L. plantarum* at 550 MPa and 39°C. The microbial inactivation through non-isothermal effects during combined pressure-thermal treatment was not well characterized, and this is a main research interest of high pressure processing (Ting *et al.*, 2002; Rajan *et al.*, 2006). Various components of bacterial cell (nucleic acids, membranes, and ribosome) are known to be influenced by both pressure and heat, but the exact mechanisms of lethal effect have not been elucidated (Hoover *et al.*, 1989; Bartlett, 1992; Cheftel, 1995; Benito *et al.*, 1999). There should be further researches about synergistic effect of high pressure and mild heat processing on microorganism and its kinetics for the development of efficient process.

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(Received 2009.4.20/Revised 2009.6.14/Accepted 2009.6.16)