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Effect of Hydrostatic Pressure on the Inactivation of *Salmonella* and *Campylobacter* Species at Low Temperature

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Salmonella typhimurium cells were treated with high hydrostatic pressure (<200 MPa) at 0, 5 and 10 °C in 0.9% NaCl solution. Inactivation efficiency by high hydrostatic pressure treatment increased with decrease in the treatment temperature. Two *Campylobacter* species were also treated with high hydrostatic pressure at 0 °C in 0.9% NaCl solution and poultry thighs. Both *Campylobacter* species were effectively inactivated in 0.9% NaCl solution compared to poultry thighs. The appearance of poultry thighs slightly discolored by the high hydrostatic pressure treatment at 200 MPa, 0 °C for 60 min.

INTRODUCTION

High hydrostatic pressure has recently been exploited as a new method for sterilizing and processing food (Shigehisa *et al.* 1991, Cheftel 1995, Yuste *et al.* 2001). Pressure induces several irreversible changes in food ingredients and concomitants depending on the magnitude of the pressure, as observed with heat. For example, protein including enzymes are denatured and inactivated (Bridgman 1914, Hayakawa *et al.* 1992, 1996), microorganisms are killed (Sale *et al.* 1970, Hayakawa *et al.* 1994). Shigehisa *et al.* (1991) reported that some gram–negative microorganisms could be inactivated by high hydrostatic pressure at >3000 atm (ca. 300 MPa) for 10 min at 25 °C in pork slurries. The pressure treatment at higher than 3000 atm, however, caused some coagulation and discoloration of the pork slurries. On the other hand, yeast was more inactivated at low temperature, especially at sub–zero temperature, than room temperature (Hashizume *et al.* 1995).

Considering these results, microorganisms may be inactivated at low temperature without any detrimental changes, such as coagulation and discoloration, in raw meat and poultry. Thus, in this study we investigated the effect of the high hydrostatic pressure below 200 MPa at low temperature on the inactivation of *Salmonella* and *Campylobacter* species.

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MATERIALS AND METHODS

Bacterial strains and growth conditions

Salmonella typhimurium IFO 13245 was grown to late exponential and/or stationary phase in nutrient broth (Eiken Chemical Co., Ltd. Tokyo Japan) at 30 °C for 18 hr. The cells were washed by centrifugation at 1,000×*g* and 4 °C for 15 min and resuspension with 0.9% sodium chloride solution for triplicate.

Campylobacter jejuni subsp. *jejuni* ATCC 33560, *Campylobacter jejuni* KG1 were grown on *Campylobacter* blood free selective agar base (CM739, Oxoid Ltd., Hampshire, UK) plates. The incubation was performed under microaerophilic conditions (5% O_2, 10% CO_2, and the balance, N_2) at 42 °C for 2 days. After the colonies growing on the plates were harvested with 0.9% sodium chloride solution, the cells were washed by centrifugation at 5,000×*g* and 4 °C for 10 min and resuspension with 0.9% sodium chloride solution for triplicate. *C. jejuni* KG1 was isolated from a patient using *Campylobacter* blood free selective agar base plates with CCDA selective supplement (SR155, Oxoid) under same condition as above. After isolation the single colony on the plates without CCDA selective supplement was used for confirming as *C. jejuni* by microscopic observation, catalase and hippuric acid tests.

Hydrostatic pressure treatment

The washed cells of *Salmonella* and *Campylobacter* were adjusted to 10^7 colony–forming–unit (CFU) per ml with 0.9% sodium chloride solution. The cell suspensions (1.5 ml) were sealed into a sterile screw–capped plastic tube (Greiner Labortechnik Co., Ltd., Frickenhausen, Germany). The sample plastic tubes were then pressurized using a prototype pressurization apparatus (Yamamoto Suiatsu Co. Osaka, Japan) to 50–200 MPa at 0, 5, and 10 °C. The come–up time to processing pressure was approximately 10 min per 50 MPa. After holding for 12, 30, 42, and 60 min at each pressure, the samples appropriately diluted and surviving cells were enumerated as below.

The cells of *Campylobacter* were also tested with poultry thigh purchased from a local supermarket. The raw poultry thighs (ca. 10 g) were dipped in the cell suspension of *Campylobacter* for 10 min at room temperature, resulting that the cell density was about 10^6 CFU/g. The raw thigh was vacuum–sealed in polyethylene bag and subjected to high hydrostatic pressure treatment. After pressurization the thigh was placed into a stomacher bag (Oxoid) with 0.9% sodium chloride solution (10 ml) and homogenized by Stomacher Lab–Blender 400 (Organo Co. Tokyo, Japan) for 2 min, and the surviving cells was enumerated.

Enumeration of viable cells

After treatments, serial dilutions in 0.9% sodium chloride solution were performed and surviving cells were enumerated by colony–count method. *S. typhimurium* was grown on nutrient agar (Eiken Chemical Co., Ltd.) at 30 °C for 24 h and *C. jejuni* was grown on *Campylobacter* blood free selective agar base plates at 42 °C for 48 h under microaerophilic condition. Each measurement was done for triplicate, and arithmetic average was used in figures. Surviving cells were represented as a surviving rate, log (N/N_0), where N is colony counts of the surviving cells and N_0 is the initial colony counts.

RESULTS AND DISCUSSION

Figure 1 shows surviving curves of *S. typhimurium* IFO 13245 after high pressure treatments. The surviving cells decreased linearly with increase of treatment time, except for the treatment of 50 MPa at 0 °C, 50 and 100 MPa at 5 °C, and 50, 100 and 125 MPa at 10 °C at which conditions the cells were hardly inactivated. On the other hand, the cells might be completely inactivated during the come-up time to 200 MPa at each treatment temperature. When using higher treatment pressure, *S. typhimurium* cells were inactivated at higher inactivation rate. The inactivation rate was also higher at 0 °C compared to 5 and 10 °C. From the surviving curves, the D-values, which is defined as the time required to kill 90% of microorganisms at certain temperature and pressure, was calculated. When the treatment pressure was 100 MPa, the D-values were 20.6, 111, and 250 min at 0, 5, and 10 °C, respectively. Furthermore, the D-values increased to 5.47, 15.8, and 17.6 min at 0, 5, and 10 °C after the treatment of 150 MPa, respectively.

Vegetative cells are inactivated more easily by the combined use of low temperature and high pressure than by pressurization at room temperature (Sonoike *et al.* 1992; Hashizume *et al.* 1995). In our results, *S. typhimurium* cells were also inactivated effec-

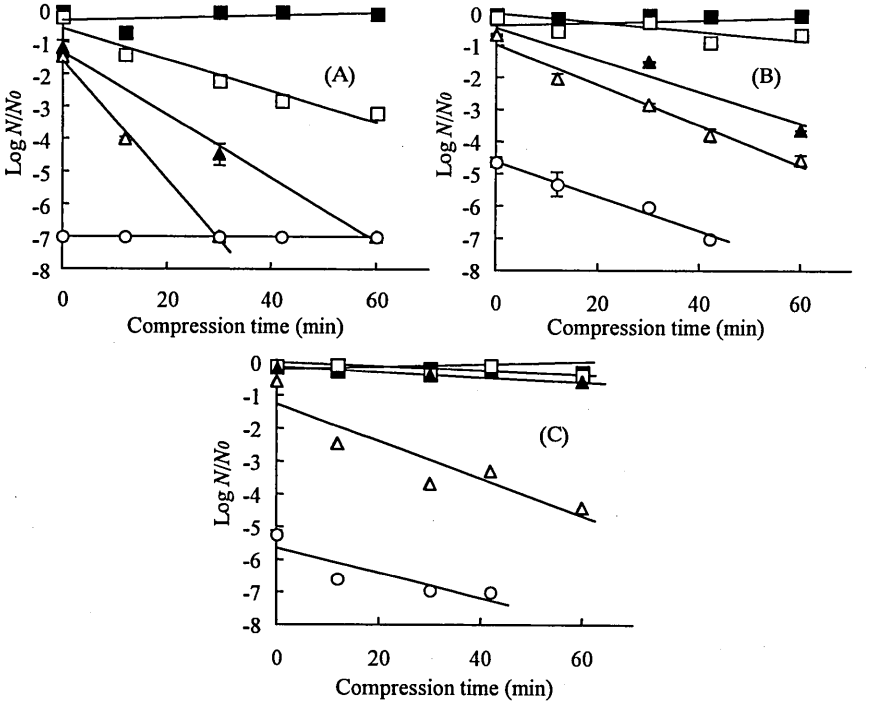

Fig. 1. Surviving curves of *S. typhimurium* after high hydrostatic pressure treatment at various temperatures, (A) 0 °C, (B) 5 °C, and (C) 10 °C. Treatment pressures were (■) 50, (□) 100, (▲) 125, (△) 150, and (○) 200 MPa. *N*, colony counts of the surviving cells; N_0, initial colony counts.

tively at low temperature, and the inactivation rate followed a first–order inactivation. It is considered that the inactivation rate might increased by using lower temperature such as −10 and −20 °C, according to the study of Hashizume *et al.* (1995). However, the appearance of raw poultry meat may discolor at these sub–zero temperature, and moreover the processing cost may be more expensive under such these sub–zero temperature. Thus, we selected 0 °C as a treatment temperature for the following experiments.

Inactivation curves of *C. jejuni* KG1 and ATCC 33560 suspended in 0.9% sodium chloride solution after high pressure treatments at 0 °C were shown in Fig. 2. The inactivation rate increased with the increase of treatment pressure, and both *Campylobacter* species was completely inactivated after first 12 min holding time at 200 MPa, 0 °C. *C. jejuni* ATCC 33560 showed slightly higher resistance to high hydrostatic pressure compared to *C. jejuni* KG1. As observed with the high hydrostatic pressure treatment of *S. typhimurium* cell suspension, the inactivation rate of *C. jejuni* cell suspension by the treatment also followed first–order kinetics. The D–values of *C. jejuni* ATCC 33560 were 24.0 and 9.0 min, and those of *C. jejuni* KG1 were 22.9 and 7.1 min after the treatment at 100 and 150 MPa, and 0 °C, respectively.

Each *Campylobacter* species with chicken thighs was also subjected to high hydrostatic pressure (Fig. 3). The inactivation rates of *Campylobacter* with thicken thighs decreased at any high hydrostatic pressure used. For instance, a 60–min treatment of 150 MPa at 0 °C resulted in an approximate 7.0–log cycles reduction of *C. jejuni* ATCC 33560 in 0.9% sodium chloride solution (Fig. 2), whereas only a 2.5–log cycles reduction with chicken thigh. In fact, certain company reported that the shelf life of poultry meat could be extended from 2 days to 4 days in refrigerator by the high hydrostatic pressure treatment at 200 MPa and 0 °C. Patterson *et al.* (1995) and Patterson and Kilpatrick (1998) investigated the effect of the high hydrostatic pressure on the inactivation of vegetative bacteria in several substrates, such as buffer, milk, and poultry meat, suggesting that the sensitivities to pressure treatment in different substrates depended on the variety of bacteria. For example, *Listeria monocytogenes* was more sensitive in

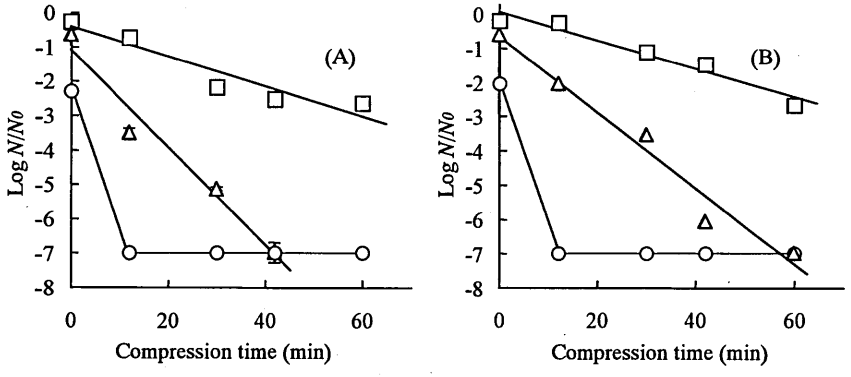

Fig. 2. Surviving curves of (A) *C. jejuni* KG1 and (B) *C. jejuni* ATCC 33560 after high hydrostatic pressure treatment in 0.9% sodium chloride solution at 0 °C. Treatment pressures were (□) 100, (△) 150, and (○) 200 MPa.

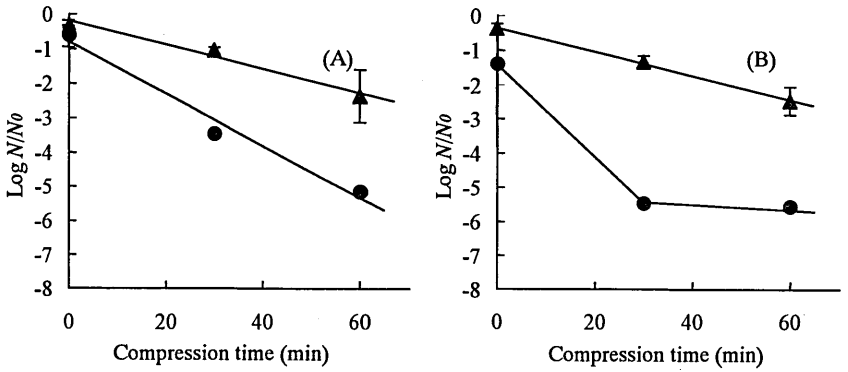

Fig. 3. Surviving curves of (A) *C. jejuni* KG1 and (B) *C. jejuni* ATCC 33560 after high hydrostatic pressure treatment with poultry thighs at 0 °C. Treatment pressures were (▲) 150 and (●) 200 MPa.

poultry meat than phosphate buffer and milk. In contrast, *Escherichia coli* was more sensitive in phosphate buffer than poultry meat and milk. They also reported that vegetative bacteria in poultry meat were more inactivated at higher treatment temperature than room temperature. However they did not mentioned the appearance of the poultry meat after the high hydrostatic pressure treatment combined with mild heat. The appearance of poultry meat might significantly discolor under such a high treatment temperature.

The high hydrostatic pressure treatment, however, slightly affected the appearance of poultry thighs (illustrate was not shown) under low temperature we used. By the treatment at 200 MPa, 0 °C for 60 min, which is a condition enough to kill more than 5–log cycles of *Campylobacter*, the surface color of thighs turned whitish, while the inside of thighs was almost same as untreated thighs. Okamoto *et al.* (1990) studied pressure–induced gels of food proteins, such as an egg, carp actomyosin, and rabbit meat. They reported that gels standing under their own weight maintaining their shapes were obtained at 200 MPa for 30 min for the carp actomyosin and the rabbit meat paste. From their results, it is considered that the treatment pressure of 200 MPa may be too high to sterilize raw poultry meats without discoloration. To minimize the discoloration, it is required that reduction of treatment time and it may be effective with combination of other procedure, such as an addition of antibiotics (Yuste *et al.* 1998) and rapid decompression method (Noma *et al.* 2003).

In conclusion, the use of high hydrostatic pressure under low temperature was effective to inactivate *Salmonella* and *Campylobacter* species. Nevertheless the efficiency of inactivation decreased when treated with poultry thighs. In addition, the appearance of poultry thighs slightly discolored due to the denaturation of proteins.

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