Effect of Hyperosmotic NaCl and Storage Temperature on the Injury and Recovery of *Escherichia coli* after High Hydrostatic Pressure Treatment

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Abstract

Food safety is a critical concern in food preservation. In the food industry, intervention technologies are conventionally used to inactivate microbes, especially bacteria. Bacterial inactivation is not always lethal, and sublethal stress may induce bacterial injury. Injured bacteria may temporarily lose their proliferation ability, although they are still viable. In addition, injured bacteria may recover and regain the ability depending on the storage condition. Since bacterial injury and subsequent recovery result in overestimation of food safety, injured bacteria are potential risks in the food industry.

In this study, *Escherichia coli* ATCC25922 was adopted as a model bacterial strain, and two stresses, hyperosmotic NaCl and high hydrostatic pressure (HHP), were applied to the cells respectively or in combination to clarify their effect on the bacterial viability, injury, and recovery. Subsequently, colony formation of the HHP-injured cells was evaluated by image analysis to reveal the levels of bacterial injury.

In Chapter 1, background information was reviewed in terms of bacterial inactivation, injury and recovery, NaCl effects, HHP pasteurization, hypothesis on levels of bacterial injury, and colony formation of injured cells.

In Chapter 2, the effect of hyperosmotic NaCl and storage temperature on *E. coli* cells was studied. The cells were suspended in brines (phosphate-buffered solutions containing NaCl at 0.9, 3.5, 5.0, and 10.0 %) and stored at 5, 10, 15, 20, and 25 °C. It was demonstrated that the effect of NaCl on the cells was dependent on the storage temperature. Hyperosmotic NaCl slightly protected the cells at 5 and 10 °C, while it enhanced the inactivation with increased storage temperature and NaCl concentration. Viable cell counts decreased from ca. 9 log CFU/ml (hereafter, log) to nondetectable level in 10.0 % brine, and possible adaptation was observed at NaCl ≤ 5.0 % at 20 and 25 °C, leading to a final cell counts of ca. 6 log. Injured cells were observed limitedly in 5.0 and 10.0 % brines. The injured cells seemed recovered in 5.0 % brine by 2 log cycles during storage at 20 °C. Lethal effect was limitedly observed at 5.0 % and 10.0 %, indicating that high concentration of NaCl would be indispensable in food preservation against *E. coli*.

In Chapter 3, the effect of hyperosmotic NaCl and storage temperature on the injury and recovery of HHP-treated *E. coli* has been studied. After HHP treatment (400 MPa, 25 ºC, 10 min), healthy cells were not detected in 0.9 % brine, while the total viable cell counts decreased from ca. 9 log to ca. 2 - 4 log. It was indicated that all or most of the viable cells were judged to be injured after HHP treatment. Healthy cells were detected more in brined at higher NaCl concentration (0.47 log, 1.20 log, and 2.32 log in 3.5 %, 5.0 %, and 10.0 %

brines, respectively). It was indicated that NaCl slightly protected the cells from the HHP inactivation. On the other hand, recovery of injured cells to ca. 8 log was observed solely in 0.9 % brine during storage at 15, 20, and 25 ºC, indicating either refrigeration temperature (≤ 10 ºC) or hyperosmotic NaCl (≥ 3.5 %) suppressed the recovery. Furthermore, it was suggested that the combined treatments of HHP and NaCl (≥ 3.5 % NaCl) would inactivate *E. coli* cells efficiently.

Based on the past reports where colony size of HHP-treated *E. coli* cells was highly heterogeneous, it was hypothesized that levels of HHP-induced bacterial injury might also be heterogeneous and they affected the subsequent bacterial recovery. In Chapter 4, *E. coli* cells were treated with HHP of 200, 300, and 400 MPa to obtain injured cells at different stress levels, and colony formation were quantitatively evaluated by image analysis using a software "ImageJ®". In order to quantify the colony formation, parameters such as time distribution of colony emergence, equivalent circle diameter, and relative frequency of colony diameter were defined and analyzed. After HHP treatment, total viable / healthy cell counts of 10.34 ± 0.08 log / 10.38 ± 0.03 log decreased to 10.08 ± 0.04 log / 9.28 ± 0.02 log (200 MPa), 7.13 ± 0.01 log / 3.13 ± 0.04 log (300 MPa), and 4.69 ± 0.01 log / 1.32 ± 0.37 log (400 MPa), respectively. It was indicated that most of the survivors were injured. All the colonies of untreated cells emerged after 1 d incubation, whereas 17.9 % and 6.0 % of the 400 MPa-treated cell colonies emerged after 4 d and 5 d incubations, respectively. Average colony diameter decreased as HHP increased. However, the decreasing trend became obscure after 2 d incubation, probably due to increased colony density. Broad distribution curve of colony diameter after 400 MPa treatment possibly implicated heterogeneous levels of bacterial injury. As compared with colony diameter and relative frequency, time distribution of colony emergence clearly presented the bacterial injury.

The observations in this study indicated that both of hyperosmotic NaCl and HHP stresses in this study were found sublethal in the inactivation of *E. coli*, due to the possible cell adaptation to NaCl (≤ 5.0 %) and the recovery from injury after HHP treatment (in 0.9 % brine, 400 MPa). However, combined treatments of HHP and hyperosmotic NaCl resulted in lethal inactivation in some cases even at relatively low hyperosmotic conditions (e.g. 3.5 % NaCl). Therefore, HHP treatment, as an intervention hurdle technology in food processing, might contribute to NaCl reduction in salt preservation of foods via synergistic effect of HHP and low NaCl concentration. In addition, the image analysis of colony formation provided a new method for quantitative evaluation the levels of HHP-induced bacterial injury.

Key words: Injury; Recovery; Hyperosmotic NaCl; High hydrostatic pressure; Cold storage; Colony formation