THE IMPACT OF HIGH HYDROSTATIC PRESSURE ON INACTIVATION AND SUBLETHAL INJURY OF FOODBORNE PATHOGENS IN BEETROOT JUICE

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Summary

High hydrostatic pressure (HHP) is a well known method currently used for food preservation. Nevertheless this treatment can also cause sublethal injury of foodborne pathogen cells, which could repair and become potentially dangerous for consumers. The survival of *Listeria innocua* CIP80.11T*, Escherichia coli* ATCC 8739 and the wild strains isolated from beetroot juice after HHP treatment (200 MPA, 300 MPa and 400 MPa) as well as the level of sublethal injuries in the surviving cells were investigated in this study. Lethal effect was reported after treatment at 400 MPa for the most of strains. The maximum level of sublethal injuries was reported after 5 minutes under pressure 300 MPa (*L. innocua*) and 400 MPa (*E.coli*).

Key words: *Listeria innocua*, *Escherichia coli*, high hydrostatic pressure, beetroot juice, sublethal injury

INTRODUCTION

Fresh fruits and vegetables as well as fruit and vegetable juices that are consumed without any thermal treatment, and are contaminated of *Listeria* with more than 100 cfu/g, are considered to be a direct risk to human health. Exceeding this number is dangerous especially for people with reduced immunity, children, the elderly and pregnant women, causing listeriosis and even sepsis [Goulet et al. 2008]. Some serotypes of *E. coli* can cause serious food poisoning and be very dangerous for human health.

Because of the numerous health benefits, fresh beetroot juice became more and more fashionable nowadays. Root vegetable juices are the most contaminated among commercially available raw, freshly squeezed juices [Sapers 2003, Sokołowska et al. 2011, Sokołowska et al. 2012]. Sokołowska et al. (2011) showed that among investigated juices of this kind 41% contained *Listeria monocytogenes* and 57% of tested samples included *Escherichia coli*.

High Hydrostatic Pressure (HHP) is a nonthermal food preservation method which reduces the microbial counts, ensures microbiological safety and does not markedly change the sensory and nutritional attributes of products [Bayindirli et al. 2006, Buzrul et al. 2008, Marszałek et al. 2014, Żyngiel et al. 2009]. HHP is used worldwide for the preservation of several commercial products, including fruit juices.

The level of microbial injury by HHP treatment depends on microbial physiology factors such as growth phase, species type as well as pH. Presence of particles in product can also modify the effect of HPP on microorganisms. The mechanism of microbial inactivation by HHP is related to the cell morphological changes, cytoplasmic membrane modification, damage of genetic mechanism and adverse biochemical reaction [Hoover et al. 1989]. This damages can be reversible or irreversible, affecting the integrity and functionality of membrane and generate sublethally injured cells under some treatment conditions [Wesche et al. 2009]. An indirect way to evaluate the number of sublethally injured cells is the use of plating technique on selective medium with NaCl addition [Mackey 2000, Yuste et al. 2004], because immediately after HHP processing damaged cells have no or lower ability to grow on this medium.

The aim of this work was to investigate the effect of high hydrostatic pressure on inactivation and sublethal injury of foodborne pathogens, *Listeria innocua* and *Escherichia coli*, suspended in beetroot juice

MATERIALS AND METHODS

Microorganisms and growth conditions

E. coli ATCC 8739, *L. innocua* CIP80.11T and the wild strains isolated from beetroot juice (E. coli 61/14 and L. innocua 23/13) used in this study were stored in Cryobank at temperature -27ºC±3ºC. Broth subcultures were prepared by inoculating a tube containing 10 mL of sterile Brain – Heart Infusion medium (BHI) (bioMerieux) with a single culture immobilized on sterile bead. After inoculation, tubes were incubated in 37°C for 24 h and then each overnight culture was moved with 0.1 ml loop on Petri dish with Tryptic Soy agar (TSA) (Biocar Diagnostics) or Tryptic Soy Yeast Extract agar (TSYE) (Biocar Diagnostics) respectively. Next, culture from plate was added to 250 mL Erlenmeyer flasks containing 200 mL of Tryptic Soy Broth (TSB) (Biocar Diagnostics) or Tryptic Soy Broth Yeast Extract (TSBYE) (Biocar Diagnostics) to prepare the second subculture, and incubated at 37°C for 18 h to yield stationary phase culture. Then the cultures were harvested by centrifugation (4000 x g, 10 min., 4°C). The sedimented cells were aseptically re-suspended into phosphate-

buffered saline (PBS, pH 7.2) and again centrifuged. The washing procedure was repeated twice more. After that, model suspensions of *E. coli* and *L. innocua* were prepared in PBS. Just before HHP treatment commercial beetroot juice acidified with citric acid (pH from 3.98 to 4.17, produced by Victoria Cymes, Poland) was inoculated with bacteria cells in concentration ca. 6 log cfu/mL and transferred into sterile polyethylene tubes (Sarstedt) in 13 mL portions in duplicate.

HHP treatment

The samples were exposed to high pressure treatment at the Institute of High Pressure Physics, The Polish Academy of Science, using apparatus U 4000/65 (Unipress). The volume of the treatment chamber was 0.95 L and the maximum working pressure 600 MPa. The pressure-transmitting fluid used was distilled water and polypropylene glycol (1:1). The working temperature of the apparatus ranged from −10°C to +80°C. Samples were subjected to hydrostatic pressure of 200, 300 and 400 MPa at 20°C and held for 1, 5 and 10 min. The pressurization times reported do not include the come-up and come-down time. The temperature was measured in the chamber.

Analytical methods

HHP-treated samples were analyzed immediately after processing. The viability of each strain was assayed by counting colony-forming units. Ten-fold serial dilutions in Tryptone Salt broth (Biokar Dignostics) of each sample were prepared. Appropriate dilutions of samples were spread on agars. Counts of total viable cells were determined by spread plate on TSA/TSYE agar. TSA/TSYE agar with 5% NaCl (POCh) were used to determine non-injured cells in population. This was the maximum concentration of NaCl that caused no reduction in the colony count of unstressed cells of *E. coli* and *L. innocua* strains. The difference between the viable and non-injured cells was used to estimate the number of sublethaly injured survivors. As a control samples buffers and beetroot juice containing *E. coli* and *L. innocua*, without HHP treatment, were also analyzed. Plate with TSA/TSYE agar were incubated for 24 h at 37°C, and TSA/TSYE agar +5% NaCl for 48 h at 37°C. Plates containing less than 300 cfu/mL were selected for counting.

RESULTS AND DISCUSSION

Results of the experiment showed that inactivation and injury of *E. coli* and *L. innocua* cells in beetroot juice, subjected to HHP, depended on origin of strain and parameters of process. Survival of population for studied conditions for all media are presented in Figures from 1 to 4.

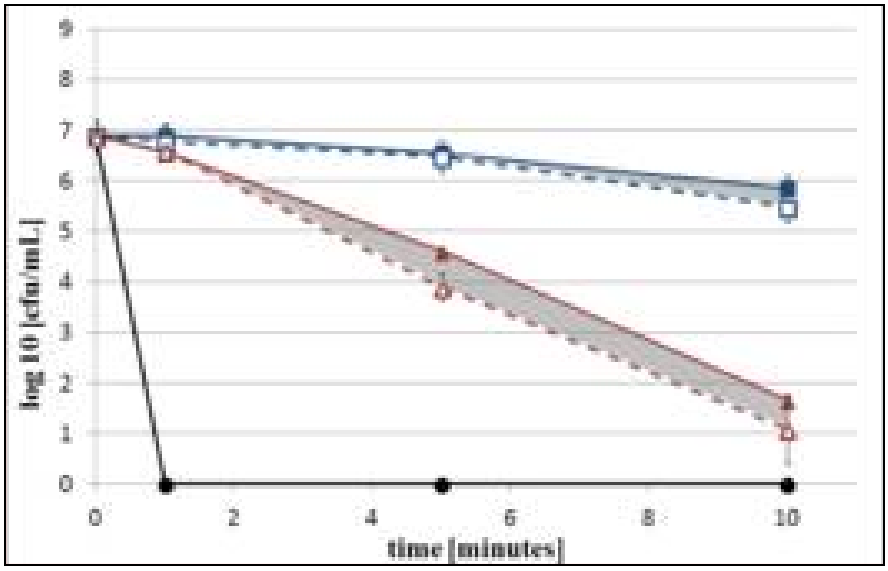

Figure 1. Effect of high hydrostatic pressure on the survival and sublethal injury of *L. innocua* CIP80.11T in beetroot juice. The level of all surviving cells (injured and uninjured) subjected to 200 MPa (■), 300 MPa (▲) and 400 MPa (●). The level of uninjured cells in population subjected to 200 MPa (□), 300 MPa (□) and 400 MPa (○).

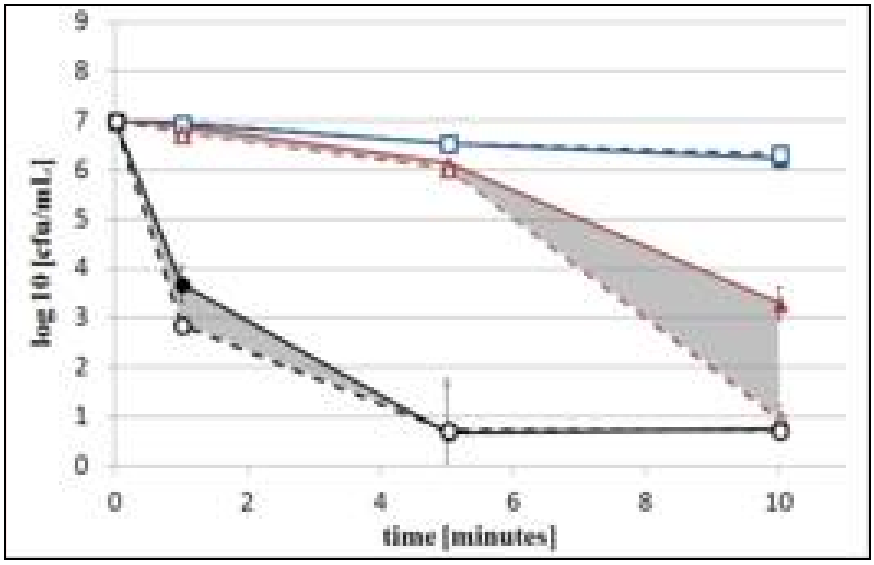

Figure 2. Effect of high hydrostatic pressure on the survival and sublethal injury of *L. innocua* wild type strain in beetroot juice**.** The level of all surviving cells (injured and uninjured) subjected to 200 MPa (■), 300 MPa (▲) and 400 MPa (●). The level of uninjured cells in population subjected to 200 MPa (□), 300 MPa (□) and 400 MPa (○).

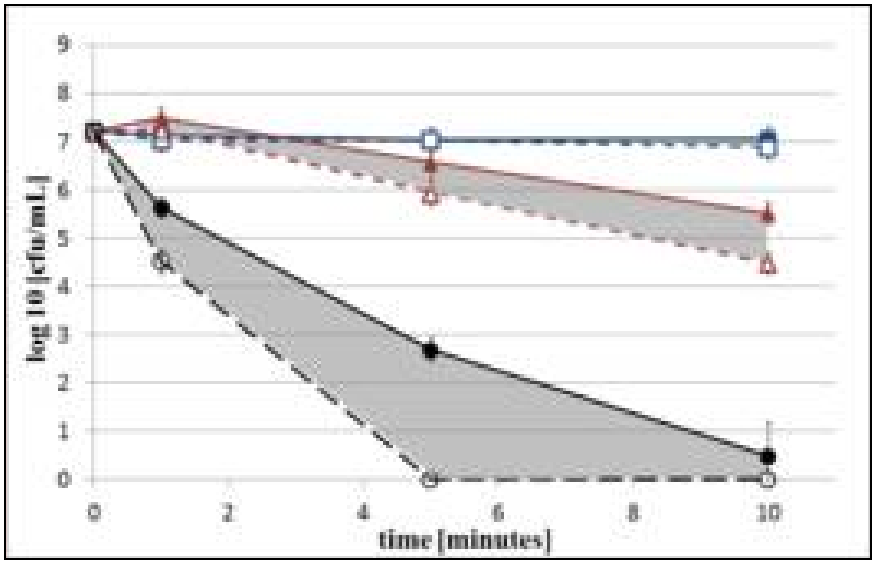

Figure 3. Effect of high hydrostatic pressure on the survival and sublethal injury of *E. coli* ATCC 8739 in beetroot juice. The level of all surviving cells (injured and uninjured) subjected to 200 MPa (■), 300 MPa (▲) and 400 MPa (●). The level of uninjured cells in population subjected to 200 MPa (□),300 MPa (□) and 400 MPa (○).

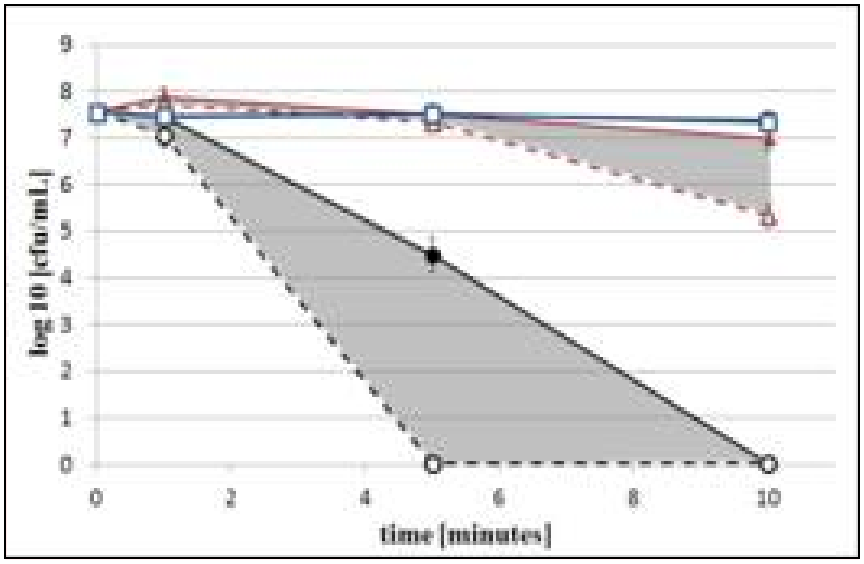

Figure 4. Effect of high hydrostatic pressure on the survival and sublethal injury of *E. coli* wild type strain in beetroot juice**.** The level of all surviving cells (injured and uninjured) subjected to 200 MPa (■), 300 MPa (▲) and 400 MPa (●). The level of uninjured cells in population subjected to 200 MPa (□), 300 MPa (□) and 400 MPa (○).

No pronounced effect on reduction of number of pathogens was observed after HHP treatment at 200 MPa. Similar observation was reported by Jordan et al [2001] in earlier studies carried out on orange (pH 3.8) and tomato juice (pH 4.1) for *L. monocytogenes* NCTC11994 under 200 MPa at 20°C for 5 min, although inactivation in apple juice (pH 3.5) was more successful and achieved 4.0 $\log_{10}$. The differences of inactivation between collection and wild type strains of the same species were observed under 300 MPa. For *L. innocua* after 5 min treatment collection strain demonstrated 2.3 $\log_{10}$ reduction, while under the same conditions the reduction for wild type strain was 0.9 $\log_{10}$. For *E. coli* after 10 min treatment collection strain demonstrated 1.7 log reduction while under the same conditions the reduction for wild type strain was only 0.6 log.

Lethal effect was reported after treatment at 400 MPa for the most of strains. For the collection strain of *L. innocua* it was reported after 1 minute of HHP. In the case of wild type strain under the same condition reduction was 3.3 $\log_{10}$. Increasing time of exposure up to 10 minutes resulted in the decrease of the number of population of wild type strain under 1 $\log_{10}$ but did not provide complete injury. The wild type strain of *E. coli* was inactivated after 10 min HHP treatment, but for the collection strain there was no complete injury even at the most severe conditions used.

The maximum level of sublethal injuries was reported under pressure 300 MPa for *L. innocua* and reached 0.7 and 2.3 $\log_{10}$ for collection strain (after 10 minutes) and wild type (after 5 minutes), respectively. Exposure to 400 MPa for 5 minutes allowed to reach 2.7 and 4.5 $\log_{10}$ sublethal injuries for collection strain and wild type respectively.

CONCLUSION

Our study confirmed that HHP is a good method for beetroot juice preservation and ensures consumer safety. Collection strains of both pathogens were easier to inactivate in beetroot juice than the strains isolated from natural environment.

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