

Effect of *Lactobacillus brevis* cells growth phase on inactivation and sublethal injury caused by high pressure

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Beer represents hostile environment for the most of bacteria. The growth of bacteria in beer is restricted by alcohol, lack of oxygen and low nutrient availability, the presence of antibiotic hop acids (alpha acids) and a low pH. Indeed, only limited species of micro-organisms have been reported to spoil beer [2]. Specifically, *Lactobacillus* (*L.*) *brevis* is very crucial and prominent contaminant during the production and storage of beer.

High Hydrostatic Pressure (HHP) technology was successfully applied to inactivation of *Lactobacillus plantarum* [5] and *Saccharomyces cerevisiae* ascospores [6] in beer and, what is also important, HHP did not affect on the main chemical constituents and colour [3, 4] and flavour [6] of the beer.

Cells exposed to different physical and chemical treatments suffer from injury that could be reversible in food materials during storage. It has been observed that many bacterias suffered from HHP injury [1]. The injured cells can be repaired in a medium containing the necessary nutrients, and the cells can potentially grow, after repairing the site of injury, during storage.

Generally, microorganisms possess higher pressure resistance during the stationary-phase, rather than during the exponential phase. This is because microorganisms in the exponential-phase undergo continuous cell division and synthesis, and the stress tolerance of cells in an adverse environment is lower. Microorganisms in the stationary-phase have complete cell structures and they are protected by cell membranes. Thus, they can resist more severe levels of stress. It has also been reported that the high resistance of stationary-phase cells to HPP is partly due to the synthesis of proteins which protect against a range of adverse conditions, such as: high salt concentrations, elevated temperatures, and oxidative stress [7, 8, 9].

The aim of this study was to explore the effect of growth phases on the inactivation and sublethal injury of *Lactobacillus brevis* 3/16/2 strain by high pressure

In this study HHP technology was tested on *Lactobacillus brevis* 3/16/2 strain isolated from spoiled beer. The cells were harvested by centrifugation from the 26 h cultures (exponential-phase) and 40 h cultures (stationary-phase) in MRS broth. Cells were washed in phosphate buffered saline PBS (pH 7.2) three times, then they were inoculated, in 10% wort and two commercial beers at the level of 8 - 9 log (cfu/mL). "Pomorzanin" beer from small brewery: unfiltered full beer, 13.8% original extract, 5.9% (v/v) ethanol content. "V.I.P." beer

from company brewery: light beer, 10.5% original extract, 5.4% (v/v) ethanol content was used in this study. Each of the analyses were performed in accordance with standard methods of the European Brewer Convention (EBC).

Pressurization was conducted by the high pressure single vessel apparatus U4000/65 (Unipress equipment, Warsaw, Poland) with a maximum operating pressure of 600 MPa, a volume of approximately 0.95 L and a theoretically operable temperature range of -10 °C to +80 °C.

The inactivations and sublethal injury obtained at 300 MPa applied for 5 minutes at temp. 20°C were tested just after the treatment, after a week and after two weeks of refrigerated storage.

Counts of viable cells were determined by spread plate on MRS agar (incubated at 30°C for 72 hours). MRS agar with 2 % NaCl was used to determine non-injured cells in population. The difference between the viable and non-injured cells was used to estimate the number of injured survivors.

Inactivation of *Lactobacillus brevis* 3/16/2 cells was affected by growth phase of cells and type of pressurized medium. The lowest inactivation was observed in 10% wort for cells from stationary-phase. It was 0.52 log cfu/mL While inactivation of 1.78 log cfu/mL for cells from exponential-phase was observed in this medium. The inactivation in unfiltered full beer "Pomorzanin" reached 1.08 cfu/mL for cells from stationary-phase and 3.98 cfu/mL for cells from exponential-phase. (Fig. 1). In light beer "V.I.P." the inactivation was the highest and reached 2.96 log cfu/mL for cells from stationary-phase and 6.73 log cfu/mL for cells from exponential-phase. In "Pomorzanin" beer there was more alcohol but the inactivation was lower because the baroprotective effect of unfiltered beer ingredients.

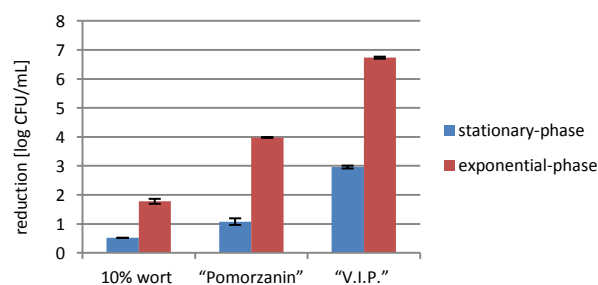


Figure 1. Reduction of *L. brevis* 3/16/2 after pressurization at 300 MPa / 5 min / 20 °C.

Sublethal injury of *Lactobacillus brevis* 3/16/2 cells were affected by growth phase of cells and type of pressurized medium. No significant number of injured survivors for cells from stationary-phase was determined in wort and in both beers. However sublethally injured cells from exponential-phase was observed in wort - 1.46 log cfu/mL and in unfiltered full beer "Pomorzanin" in an amount 1.00 log cfu/mL (Fig. 2). No injured cells from exponential-phase were observed in beer "V.I.P." after pressurization.

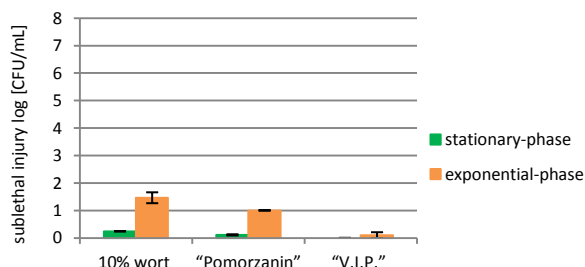


Figure 2. Sublethal injury of *L. brevis* 3/16/2 after pressurization at 300 MPa / 5 min / 20 °C.

The number of *Lactobacillus brevis* 3/16/2 cells surviving the pressurization, decreased during two weeks of refrigerated storage. The inactivation reached 1.39 cfu/mL in wort, 1.14 cfu/mL in "Pomorzanin" beer and 0.53 cfu/mL in "V.I.P." beer for cells from stationary-phase after two weeks of refrigerated storage. For cells from exponential-phase the inactivation obtained, depending on the medium: 0.91 log cfu/mL (wort), 2.6 log cfu/mL ("Pomorzanin" beer) and 2.5 log cfu/mL ("V.I.P." beer).

No significant number of injured survivors of *Lactobacillus brevis* 3/16/2 cells from stationary-phase was determined in wort and in "Pomorzanin" beer during refrigerated storage, while they were found in "V.I.P." beer and reached 0.9 log cfu/mL after two weeks of refrigerated storage.

Number of injured *Lactobacillus brevis* 3/16/2 cells in exponential-phase was 2.13 log cfu/mL and 2.3 log cfu/mL in wort and "Pomorzanin" respectively, during refrigerated storage. No significant number of injured survivors for cells from exponential-phase was determined in "V.I.P." beer.

These results demonstrate that HHP may be a useful technique for the inactivation of *Lactobacillus brevis* in beer. The baroprotective effect of wort and unfiltered beer compounds on *Lactobacillus brevis* 3/16/2 cells, during high pressure processing was observed. Light filtered beer can be relatively easily preserved with the usage of HHP. The cells of *L. brevis* from exponential-phase were more sensitive to HHP than cells from stationary-phase. The results may be helpful for designing appropriate nonthermal HHP conditions to pasteurize different kind of beers.

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