High hydrostatic pressure treatment as a new approach to extend the shelf-life of unfiltered beer

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Popularity and customer´s demand for unfiltered beer has been increasing. This phenomenon, which goes hand in hand with the growing number of craft breweries, can be explained by the consumer´s preference for natural, craft and unconventional products [1]. Furthermore, the consumption of unfiltered beer is frequently mentioned for its number of indisputable health benefits due to rich content of B vitamins, minerals and fiber, although the differences in nutritional aspects of beers containing the yeasts compared to filtered beers are not confirmed by sufficient scientific evidence [2]. However, the sensory and microbiological stability of unfiltered beer is limited and to maintain the fresh character as long as possible, particular storage conditions are required (low temperatures, prevention of light exposure). Therefore the new possibilities to prolong the shelf-life of unfiltered beer are currently being inquired. Pasteurization represents a conventional treatment method to assure the product stability and microbiological safety. Apart from that, the innovative approaches involve application of high hydrostatic pressure, or other non-thermal methods such as ultrasound treatment or use of pulsed electric fields [3].

So far, the high hydrostatic pressure is widely applied in food industry and represents an alternative to pasteurization [4]. The method is commercially applied in processing of fruits and legumes, juices, dairy and meat products [5]. Among the most frequently mentioned advantages of pressurized products are the retention of original flavour and nutritional values and thereby, an enhanced acceptance from the customer´s point of view in comparison to the thermal treatment. Pressure treatment is performed by „in batch“process, during which the product is placed in a chambre surrounded by liquid (most commonly water), which serves as a medium for transfer of the pressure [6]. The ranges of pressure are usually between 300 – 600 MPa and the holding time is 5 – 20 min to achieve the desired inactivation of spoilage microorganisms [4].

Several trials on the application of high hydrostatic pressure treatment in order to prolong the shelf-life of unfiltered beer have been already conducted. In a pilot study by Castellari et al., application of 600 MPa for 5 min was assesed. Basic analytical parameters of beer (OG, ethanol content, bitterness, pH and total polyphenols) were not affected and the microbiological stability was comparable to thermally pasteurized samples [7]. In trials with filtered beer, pressurization caused increase in turbidity during the storage time, which does not represent an issue in case of filtered beer [8,9]. Yin et al. examined the pressurization of unfiltered wheat beer and evaluated the characteristics of the product after 84 days of storage at 20 °C. No bacterial growth was detected in pressure treated samples. Furthermore, colour was not affected in contrast to thermally pasteurized control sample, where the colour slightly increased as a result of the thermal treatment. Foam stability was higher in pressurized sample compared to pasteurized sample. Concluded from sensory analysis, pressurized samples were characterized by higher fullness and more intensive fruity flavours in comparison to pasteurized beer [10]. As a result from our assessments conducted at the University of Chemistry and Technology, Prague, Department of Biotechnology, pressurization lead to increase in content of volatile compounds and after 2 months of storage, stale flavours were identified during the sensory evaluation. Therefore we assume, precise optimization of the pressure and length of the holding time is necessary to diminish the risk of flavour deterioration. In our following experiments, we will focus at the mechanisms of the formation of stale flavour upon the application of high hydrostatic pressure and the dynamics of changes in the profile of organic volatile compounds which affect the organoleptic properties of beer. Furthermore, we will evaluate the impact of pressurization on the oxidative stability of unfiltered beer. Simultaneously, we will examine the effects of high pressure on the yeast cell to eliminate the risk of autolysis and subsequent release of the yeast intracellular content which would have negative impact on the sensory characteristics. We are convinced our future research will sustain the freshness and contribute to the improvement of quality of unfiltered beer and broaden the possibilities of brewers regarding their unfiltered beer production.

The most interesting knowledge we received during comparison of several technique for measurement of ratio dead and live cells after treatment of high hydrostatic pressure. The effect of HHP on the viability of yeast was determined by flow cytometry, methylene blue dead yeast cell stain test [11] and by counting of colony forming units (CFU) [11].The ratio of living/dead yeast cells was evaluated by the CFlow program and the results were compared with the results from microscopy (with microscope OLYMPUS BX51) and CFU counting. For the CFU counting, samples of the model beer were

diluted 10 or 100 times, and 0.1 mL was put on agar plates. The cells were grown at room temperature for 3 days and then the CFU were counted.

Flow cytometry is a fluorescence optical method used to measure the size and number of cells, watching cell cycle and changes in growth rate yeast population or changes in population induced by mutations. The sample in flow is entrained in a carrier liquid. In the measuring cell occurs a collision of light from the excitation source and the cells passing individually through the capillary. The light is then either dispersed, reflected or can produce fluorescence which is spread in all directions from its source. Light scattered at a certain angle is detected by a “forward scatter detector” and “side scatter detector”. Using the forward scatter detector determines the size of cells, while the side scatter detector signal corresponds to the granularity of cells [12].

Samples for the flow cytometry were prepared by concentrating the stabilized model beer. In the first step, 25 mL of beer was centrifuged (6000 G), 5 minutes), the sediment was washed by 5 mL of physiological saline solution, centrifuged (6000 G, 5 minutes), washed again by 2 mL of physiological saline solution and centrifuged (<1400 G, 5 minutes). Then, the sedimented cells were resuspended in 1 mL of physiological saline solution, and 125 μL were mixed with 875 μL of physiological saline solution (optical density between 0.4 and 0.8, λ= 600 nm). Finally, 10 μL of propidium iodide was added to the suspension. After 10 minutes, 960 μL of demineralized water was added to 40 μL of this mixture and put into the flow cytometer. It was demonstrated that HHP inactivates yeast cells very effectively too. In samples subjected to a pressure of 350 MPa for 5 minutes, no cell growth on the nutrient agar was observed, however, flow cytometry and dead yeast cell stain method showed around 70 % rel. of viable cells (Figure 1, Figure 2).

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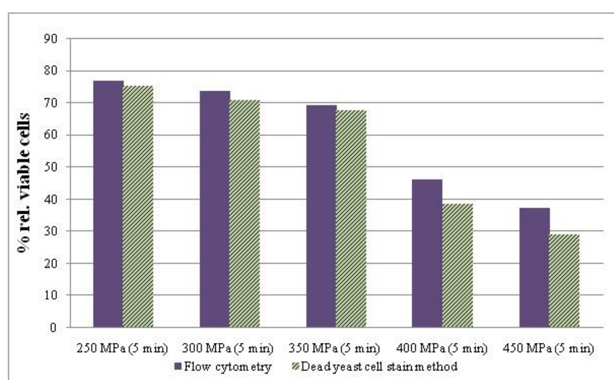

Figure 1. Comparison of analysis of yeast viability by flow cytometry and dead yeast cell stain method

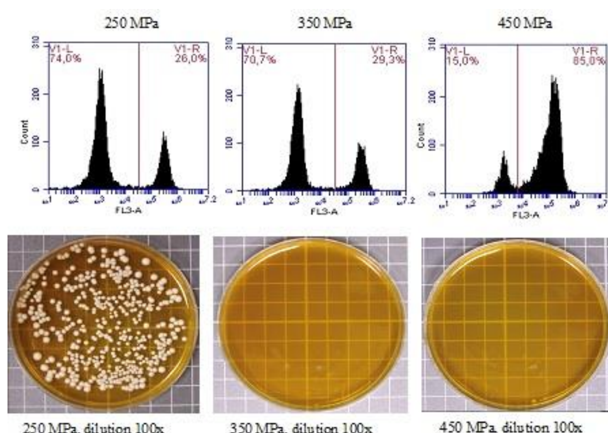

Figure 2. Comparison of histograms (on the left side of dividing line are living cells, on the right side of dividing line are dead cells) and growth on Petri dishes for beers treated by HHP (250, 350 and 450 MPa)