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The effect of human milk preservation methods on selected bioactive components and microbiological safety

Abstract

Thanks to the nutrients it contains, breast milk plays a key role in a child's healthy growth and development. A special function is assigned to its bioactive factors that stimulate, among others, the immune and digestive systems. If it is not possible to feed premature babies with their own mother's milk, the second choice recommended by World Health Organisation is using milk from donors from human milk banks. These banks aim to prepare a microbiologically safe portion of milk to administer to prematurely born infants with low birth weight. Typically, donor milk is preserved using a holder pasteurisation (HoP) method and then stored in cold storage conditions. Implementing these two stages by human milk banks in their operations may contribute to the decline in milk quality, and as a result, reduce its therapeutic properties.

The study aimed to optimise an alternative method of preserving donor human milk, high-pressure processing (HPP), and to improve the method of storing donor human milk in human milk banks by freeze-drying it. The impact of these methods on milk microbiological safety and its bioactive components was tested.

The microbiological quality assessment of milk samples donated to human milk banks was based on statistical data from the first years of operation of a Regional Human Milk Bank in Warsaw (publication 1). The effect of high-pressure processing on milk bactericidal properties was determined by analysing the growth of *Escherichia coli* NCTC 9111 in processed milk (publication 2). In the next stage of optimisation, the impact of high-pressure and freeze-drying techniques on the microbiological purity and bioactive components of milk was assessed. Using enzyme-linked immunosorbent assays (ELISA), the concentrations of bioactive components such as lactoferrin, insulin, leptin, adiponectin, hepatocyte growth factor, and immunoglobulin G were determined, while the lipase activity in milk subjected to the analysed processes was examined using fluorescence spectroscopy method. By performing microbiological analyses, it was checked whether alternative preservation methods effectively eliminate the native milk microbiota and whether they are effective against potentially pathogenic strains inoculated into milk samples in laboratory conditions: *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 33862, *Listeria monocytogenes*

ATCC 7644, *Cronobacter sakazakii* ATCC 51329 and *Bacillus cereus* ATCC 14579. The high-pressure processing was optimised by testing the following parameters: 600 MPa, 200 MPa + 400 MPa, 100 MPa + 600 MPa, 200 MPa + 600 MPa, and 450 MPa. Each stage of the research was also performed for milk subjected to HoP (publications 3a, 3b, 4). In the final phase of the research, the impact of the time and method of milk storage on the survival of *Bacillus cereus* was determined by analysing various variants of milk storage (4 °C and 21 °C) before and after holder pasteurisation. Quantitative analysis of vegetative forms of bacteria was performed using culture methods on selective and differential growth medium, while microscopic analysis of samples stained with malachite green and crystal violet was used to visualise spores (publication 5).

Microbiological testing of milk samples donated by potential donors showed that the milk donated to the Regional Human Milk Bank in Warsaw is of high microbiological quality. However, few samples showed the presence of potentially pathogenic bacteria such as *Staphylococcus aureus*, *Bacillus cereus*, and *Escherichia coli*, therefore pasteurisation of donor milk before administering it to patients is justified (publication 1). Special attention should be paid to detecting milk samples contaminated with spore-forming bacteria. The presence of *Bacillus cereus* endospores was observed during storage of unpasteurised and pasteurised milk in cold storage conditions (publication 5).

Analyses have shown that high-pressure processing is a good, alternative technique for ensuring milk microbiological purity. Pressurised milk showed bactericidal activity against an *E. coli* strain at the level of 29.6-50.34%, and it was higher than for milk after HoP: 12.1% (publication 2). No bacterial growth was recorded in samples after processing (HPP, HoP). At the same time, the concentrations of most of the analysed bioactive factors were maintained at a much higher level after high-pressure processing than in milk subjected to holder pasteurisation. The best parameters were obtained for the following HPP variants: 200 MPa + 400 MPa, and 450 MPa. Moreover, it has been shown that freeze-drying milk subjected to HPP is the best way to handle milk, significantly facilitating sample storage (publications 3a, 3b, 4).

Conducting this research may significantly improve the work of human milk banks in the future. The use of high-pressure processing as a preservation technique will ensure that donor milk administered to premature infants meets high standards of microbiological safety and has a more favourable composition. The high-pressure technique is a good alternative to currently

used methods of preservation, and freeze-drying of the final pasteurisation product may facilitate the storage of breast milk in human milk banks.