# EFFECT OF PULSED ELECTRIC FIELD APPLICATION ON BIOACCUMULATION OF SELECTED METAL IONS IN LACTOBACILLUS RHAMNOSUS B 442 CELLS

# SUMMARY

 With the development of dietetics and nutrition science more and more attention is paid to correct balance of diet components, including bioelements necessary for correct functioning of living organisms. Ions of magnesium, zinc, calcium, selenium, as coenzymes, regulate cellular metabolism. In recent years symptoms of deficit of those elements are noted more and more often, which brings into focus bacteria which can be a source of not only protein, valuable in the diet, but also of the deficit bioelements. Bacteria absorb metal ions from the environment, and then incorporate them permanently into cellular structures. This may lead to the formation of stable complexes with proteins, referred to as bioplexes or metalloproteins. Studies conducted so far revealed that such forms of complexation of elemental ions with proteins are more easily assimilable by the human organism than mineral preparations. Lactic acid bacteria from the genus *Lactobacillus rhamnosus* are commonly used in food production, due to their capacity for fermenting products and their health-promoting properties. They are responsible for e.g. maintaining the correct intestinal flora, they prevent inflammations of intestines, and protect against disorders in the functioning of the alimentary tract. Thanks to their ability of capturing and binding metal ions in the processes of ionic exchange, complexation, chelating and microprecipitation, they can be used for enriching food products in essential micro- and macroelements. Bacterial biomass enriched in selected ions becomes an alternative for pharmacological supplementation used in deficits of those cations.

 We can distinguish several mechanisms for the intake and accumulation of metal ions by microorganisms. To stimulate the accumulation of selected metal ions in bacterial cells, electroporation was applied. The effect of pulsed electric field (PEF) consisted in the induction of short electric pulses within a specific time. In the process of reversible electroporation, PEF induces transitional permeability of cell membranes, as a result of which permanent structures are formed (usually called “pores” or “nanopores”), which facilitate the exchange of components with the environment of the cell. Suitably optimised parameters of PEF (electric field intensity, pulse duration, number of pulses applied and frequency) may cause specific effects in biological systems.

 Undertaking a study on this topic was stimulated by the lack of publications, both Polish and foreign, on the effect of pulsed electric field on the accumulation of selected ions in bacterial cells. The available literature data relate only to the survival rate of microorganisms or changes in enzyme activity under the effect of PEF, mainly in food products in liquid form. In recent years there appeared studies on the effect of pulsed electric field on the accumulation of metal ions in cells of *Saccharomyces cerevisiae*. Based on the conducted theoretical analysis, in the doctoral dissertation the author undertook a study on the effect of pulsed electric field in the bioaccumulation of selected ions in bacterial cells.

 The preliminary study consisted in the selection of a suitable bacterial strain for further analyses based on the dynamics of growth of the bacteria. Six selected strains of bacteria from the genera *Lactobacillus* and *Lactococcus* were subjected to incubation in the wells of a sterile microplate, to which ions of magnesium, zinc and selenium were added at various concentrations. The highest optical density, over the entire range of analysed concentrations of ions, was noted for the strain *Lactobacillus rhamnosus* B 442, which was selected for further analyses. Publications **PI**, **PIII** and **PVII** present the results concerning the effect of pulsed electric field on the bioaccumulation of magnesium, zinc and calcium ions in cells of *Lactobacillus rhamnosus* B 442. In cultures treated with pulsed electric field, over the entire range of applied concentrations (from 10 to 1000 μg Mg2+/ml of medium), higher accumulation of magnesium in cells was noted, relative to control samples that were not treated with PEF. The highest accumulation of magnesium (2.13 mg Mg2+/g DM) was characteristic of the culture of *L. rhamnosus* B 442 with an addition of ions Mg2+ at concentration of 400 μg/ml of medium, subjected to the effect of PEF. That concentration was adopted as optimal for the bioaccumulation of magnesium in bacterial cells and it was applied in further research. The highest accumulation of Mg2+ (4.28 mg/g DM) was noted after PEF treatment of a 20-hour culture and at the following process parameters: electric field intensity of 2.0 kV/cm, pulse width of 20 μs, and 15-minute time of exposure to the field (**PI**). In the next stage of the study, the effect of PEF on the bioaccumulation of zinc ions in cells of *L. rhamnosus* B 442 was analysed. The highest accumulation of that element (2.85 mg Zn2+/g DM) in the cells was noted at the concentration of 500 μg Zn2+/ml on medium, subjecting the samples to the effect of PEF with field intensity of 3.0 kV/cm, exposure time of 15 min, after 20 hours of culturing, and pulse width of 20 µs (**PIII**). Subsequently, attempts were made at enriching *L. rhamnosus* B 442 in ions of selenium. Concentrations above 40 μg/ml caused a significant inhibition of growth of the bacteria, below the threshold of detection of the ions in the technique applied. Taking into account also the viability of *L. rhamnosus* 442 in the presence of selenium, it was decided not to continue the experiments with the enrichment of cells with that element. Whereas, an analysis of the effect of pulsed electric field on the accumulation of calcium ions in cells of *L. rhamnosus* B 442 was performed. The application of optimised process parameters (field intensity of 3.0 kV/cm, exposure time of 10 min, pulse width of 75 μs), the 20-hour culture after which cells were treated with PEF and the concentration of 200 µg/ml of medium resulted in the highest concentration of the ions (7.30 mg/g DM) (**PVII**). At every stage of the study the survival rate of the bacteria was estimated – it was at a high level, which is described in publications **PI**, **PIII** and **PVII**. In addition, visualisation of the accumulation of ions in bacterial cells was performed. In combination with zinc ions, morin displayed a strong green fluorescence which was clearly observable in the distal and proximal parts of cells enriched in zinc by means of PEF. Whereas, in relation to calcium ions, distinct differences were observed between the analysed samples – bacterial cells enriched in calcium through the application of PEF displayed intensive fluorescence, while in the photo of cells from the control culture K2 fluorescence is observed only for individual bacteria (**PVII**).

 The results of analyses conducted for ice cream produced with the use of potentially probiotic bacteria enriched in ions of magnesium, zinc and calcium by means of pulsed electric field are described in publications **PII**, **PIV**, **PV** and **PVI**. Strains of bacteria *Lactobacillus rhamnosus* B 442, *Lactobacillus rhamnosus* 1937, *Lactococcus lactis* JBB 500 were enriched in the ions by means of pulsed electric field (PEF). The addition of bacteria enriched in magnesium ions did not cause any changes in the analysed chemical parameters of the ice cream, and had no effect on the process of its freezing, melt rate, and hardness. No statistically significant changes were noted in the colour parameters among the analysed samples. Assays of the viability of microorganisms showed an increase in the total number of microorganisms in the ice cream in relation to the start cultures (**PII**). In the next stage of the study, *Lactobacillus rhamnosus* B 442 were enriched in zinc ions with the use of pulsed electric field (PEF). The strain was added to the blend and used for the production of two kinds of ice cream: fermented and non-fermented. The pH of the ice cream was 6.38-6.41 (non-fermented samples) and 5.97-6.02 (fermented samples). Also, differences were observed in certain properties of the fermented and non-fermented ice cream. The fermented ice cream had a lower content of fat and a lower hardness, while the non-fermented ice cream was characterised by a higher melt rate and adhesion. The viability of the bacteria was at a high level. The fermented products were characterised by a higher total number of microorganisms (**PIV**). Subsequently, the ice cream analysed in publication **PIV** was subjected to further analyses in which the changes taking place in the course of its storage were estimated. The parameters under analysis were as follows: total number of microorganisms, dry matter, pH, melt rate and hardness after 1, 30, 60 and 90 days. A decrease was observed in the viability of microorganisms in ice cream subjected to the process of fermentation. Dry matter content decreased in all the samples already after 30 days of storage. Whereas, the first drop point and the total melt time of the ice cream increased proportionally to the extension of that time (**PV**). In the final stage of the study, PEF was applied to enrich bacteria of the strain *L. rhamnosus* B 442 in calcium ions. The level of calcium in bacterial cells and in the ice cream was determined, and after 24 hours from ice cream production, samples were analysed for their chemical composition, their pH was determined, and their melting parameters and texture were also analysed. The colour parameters and the total number of microorganisms were also determined. Significant differences were demonstrated in all physicochemical parameters dependent on the process of ice cream production. The use of PEF-modified bacteria *L.rhamnosus* B 442 for the fermentation of milk allowed to obtain ice cream with the highest content of dry matter, fat, protein and carbohydrates, characterised by the lowest melt rate. No differences were found in the colour parameters *a\** and *ΔH*. Ice cream with an addition of bacteria enriched in calcium ions with the use of PEF did not differ statistically significantly in terms of bacteria survival rate (**PVI**).

References to publication:

1. **Góral, M**., & Pankiewicz, U. (2017). Effect of Pulsed Electric Fields (PEF) on Accumulation of Magnesium in *Lactobacillus rhamnosus* B 442 Cells. The Journal of Membrane Biology, 250(5), 565-572.
2. **Góral, M**., Kozłowicz, K., Pankiewicz, U., & Góral, D. (2018). Magnesium enriched lactic acid bacteria as a carrier for probiotic ice cream production. Food Chemistry, 239, 1151-1159.
3. **Góral, M**., Pankiewicz, U., Sujka, M., & Kowalski, R. (2019). Bioaccumulation of zinc ions in *Lactobacillus rhamnosus* B 442 cells under treatment of the culture with pulsed electric field. European Food Research and Technology, 245, 817-824.
4. Pankiewicz, U., **Góral, M**., Kozłowicz, K., & Góral, D. (2019). Novel method of zinc ions supplementing with fermented and unfermented ice cream with using PEF. International Journal of Food Science & Technology.
5. Kozłowicz, K., **Góral, M**., Góral, D., Pankiewicz, U., & Bronowicka-Mielniczuk, U. (2019). Effect of ice cream storage on the physicochemical properties and survival of probiotic bacteria supplemented with zinc ions. LWT, 108562.
6. Pankiewicz, U., **Góral, M.,** Kozłowicz, K., & Góral, D. (2020). Application of pulsed electric field in production of ice cream enriched with probiotic bacteria (*L. rhamnosus* B 442) containing intracellular calcium ions. Journal of Food Engineering, 275, 109876.
7. **Góral, M**., Pankiewicz, U., Sujka, M., Kowalski, R., Góral, D., & Kozłowicz, K. (2019). Influence of pulsed electric field on accumulation of calcium in *Lactobacillus rhamnosus* B 442. Journal of Microbiology and Biotechnology, 30, 44-53.