

The evolution of science has provided knowledge about the food compounds that bring beneficial effects to health, which was a reason for the appearance of food supplements. The latest trends emphasize the importance of the natural way of micronutrients delivery. Thus, the social request to get more from food causes the concept of "functional food". Functional food implies the fortification of meals with microelements, vitamins, flavonoids, or functional proteins to enhance the antioxidant, anticarcinogenic, immunostimulating, etc., properties. Dairy products are considered safe and healthy. Hence, value-added goods production based on milk components attracts considerable attention in science and industry. Milk is a source of highly valuable and bioactive proteins and peptides. For instance, peptides derived from β -lactoglobulin exhibit antioxidant, antitumor, opioid, and antibacterial activity. Moreover, antibacterial and antitumor activity was observed for peptides derived from α -lactalbumin and lactoferrin (LTF).

LTF is one of the most valuable proteins in milk, which exhibits a broad spectrum of biological activity. Apart from its antibacterial properties, LTF also showed immunomodulation activity. LTF also promotes the absorption of microelements, enhances cognitive functions, and accelerates tissues' reparation (bones, skin, etc.). Additionally, it was shown that LTF could promote the growth of probiotic bacteria while suppressing the pathogenic ones. Thus, the utilization of LTF as an additive in dairy products can be a novel solution in the food industry, which will simultaneously deal with several tasks, namely preventing spoilage, bringing beneficial effects on health, and supporting probiotics. However, a previous study has shown that LTF biological activity is dependent on its glycosylation level. Moreover, the natural concentration of LTF in milk and its glycosylation level can be influenced by the infection caused by bacteria. Thus, there is a need to investigate the correlation of microorganisms diversity in raw milk with LTF content and its glycosylation level. Such knowledge will be helpful not only in the dairy industry for the prediction of changes, which may occur during the production, but also in veterinary as LTF can be treated as a biomarker for infectious diseases. The development of the highly specific, reproducible, and precise method for LTF quantification (based on utilization of immunosorbents) is one of the goals of the present study.

It is noteworthy to mention that probiotics themselves are the factors that support the organism's normal functioning. Probiotics are defined as live commensal microorganisms (e.g., bacteria or yeasts), which can naturally improve your health when administered in adequate amounts. The probiotics act through several mechanisms. Probiotics can produce vitamins, biologically active peptides and turn saturated fatty acids into more healthy unsaturated ones. Moreover, by occupying different compartments of the organism, they prevent the spreading of pathogenic bacteria. Additionally, the constant presence of probiotic bacteria in the colon induces the organism's immune response, which naturally improves the protection of the host against infections, allergies, and cancer. Raw milk is a natural source of probiotic bacteria. However, milk can also contain pathogenic and environmental bacteria, which can provoke infections and, in the case of mammary glands, the appearance of mastitis. Both the searching of new probiotic strains and monitoring of infections caused by bacteria require fast, cheap and easy to perform, as well as accurate and repeatable methods for microorganisms differentiation.

MALDI-TOF MS (matrix-assisted laser desorption/ionization–time-of-flight mass spectrometry) is a relatively new technology, which has revolutionized the world of microbiological diagnostics. The technique is based on comparing bacterial protein profiles with the reference spectra placed in the database. The method offers species-level identification within minutes with accuracy that in some cases outperforms the identification by PCR analysis of 16S rDNA, which is considered as the "gold standard" in microorganisms identification. However, the limited amount of spectra for environmental bacteria, including probiotic ones, complicates the microbiological monitoring of milk samples. Thus, there is a need for the expansion of databases, which is also the goal of the study. Moreover, the studies on lactic acid bacteria, which were provided in our laboratory, showed that investigation of the bacteria lipid profiles might be helpful for the discrimination of closely related bacteria. Instead, the application of nanostructure-assisted laser desorption/ionization time-of-flight mass spectrometry (NALDI-TOF MS) will allow improving the ionization of the proteins and lipids. The nanostructures can change the transfer of the energy from the laser to an analyte, thus enhancing the ionization rate.

MALDI-TOF MS technique also is the technique that is often utilized in proteomics. The study of intact protein in linear mode allows careful investigation of protein masses. Instead, the analysis of protein digests (usually is obtained by digestion with trypsin) is helpful for the tracking of the proteins' post-translation modifications. Moreover, the combination of mass spectrometry with other analytical techniques such as high resolution liquid chromatography (LC-MS) or/and gel electrophoresis gives a powerful tool for the analysis of very complex mixtures such as peptides and proteins.