Proteins are building blocks of cells that directly provide potential gene function through enzymatic catalysis, molecular signaling and physical interactions. Research related to this area concerns proteomics. With the advent of the entire genome sequencing, large-scale proteomics began to dominate rapidly in post-genomic age.

One of the first analytical techniques used in proteomics was two-dimensional gel electrophoresis (2D-GE), in which the migration of electrically charged particles in an electric field and protein separation takes place. However, at present, proteomic research involves a wide variety of analytical methods such as Fourier Transformed Infrared Spectroscopy (FT-IR), Raman Spectroscopy, Ultraviolet Spectroscopy (UV-VIS), Nuclear Magnetic Resonance (NMR) or fluorescence methods. However, the most powerful tool in this area of research is mass spectrometry, which includes protein identification/characteristics and their posttranslational modification (PTM). PTMs are chemical changes of amino acid residues that affect the physicochemical properties of proteins. The introduction of new chemical groups into specific protein positions can lead to many changes in the function of a protein whose different isoforms can participate in different biological processes. The presence of PTM in a protein leads to changes in interactions with other proteins or affects the tertiary structure of proteins. The importance of PTM is widely used in biochemical, clinical and food technology processes. Although the degree of modification is usually low, functional effects can be severe when affecting functional domains. Therefore, the main objective of the project is studies aimed at developing PTM analysis methods based on milk proteins and whey. For this purpose, state-of-the-art research instruments will be used, such as matrix-assisted laser desorption/ionization time of flight (MALDI-TOF MS) and nanostructure-assisted (NALDI). In order to obtain biologically active proteins, cow's milk and whey, which for a long time was seen only as waste, will be used. During the project implementation, physicochemical characterization of isolated proteins will be carried out by means of gel electrophoresis and zeta potential measurements will be carried out to determine their isoelectric points. In addition, a classical method of digestion in solution and using capillary enzyme reactors (CER) will be developed, as well as an attempt to combine CER with a fractionation robot. An in-depth review and testing of different PTMs play an important role in paving the way for the identification and targeting of new biomarkers to facilitate the development of effective diagnostics and treatments. In addition, a better understanding of the PTMs present in milk during processing and storage can help modulate the effect of heat treatment on nutritional and technological properties. Knowing where modified proteins are modified gives a new insight into how each place can have a positive or negative effect on a particular milk property.

The undoubted advantage of mass spectrometry is its very high sensitivity, selectivity, wide range of mass analysis, which in combination with other analytical techniques such as liquid chromatography (LC-MS) or capillary electrophoresis (e.g. capillary zone electrophoresis (CZE-MS) and two-dimensional gel electrophoresis (2D-GE)) gives a powerful tool for the analysis of very complex mixtures such as peptides and proteins. The sequencing of profiled peptides by MALDI-TOF MS and a variation of this approach NALDI-TOF MS provide an additional aspect of the information content of proteomic profiles, which is crucial in detecting new biomarkers. The importance of mass spectrometry in this context was underlined by the 2002 Nobel Prize in Chemistry. Protein digestion is usually carried out with trypsin. Proteolytic peptides are washed out and prepared on a MALDI plate. MALDI-TOF MS provides good compatibility of high throughput as well as good sensitivity for low femtomol values. Protein identification depends on the mass peptide fingerprint, which is used to search the protein sequence database. In MS/MS studies, the first stage is to select the precursor ion and break it down into pieces. Whereas the second stage is a mass analysis of the resulting fragments. Ions of fragments created as a result of peptide skeleton cleavage are the most beneficial for protein identification, because they allow for specific identification and quick computer search of protein sequence databases.