1. PHD PROJECT DESCRIPTION (4000 characters max., including the aims and work plan)

Project title: Innovative Strategies in the Isolation and Functionalization of Lactoferrin various forms

1.1. Project goals.

The study aims to enhance the extraction and functional utilization of Lactoferrin, focusing on its potential health benefits and its role in the dairy industry. It seeks to develop refined isolation methods for Lactoferrin from various dairy sources while considering the impact of seasonal variations, cow breeds, and diets. The project will examine the stability of raw materials under different conditions to define necessary storage and transportation parameters. The research includes analyzing microbiological purity, acidity, protein content, and Lactoferrin glycosylation, alongside employing advanced methods like SDS-PAGE, MALDI-TOF-MS, and HPLC to quantify and characterize Lactoferrin. The study will also synthesize magnetic ferrite particles modified with polysaccharides to enhance Lactoferrin binding efficiency. Additionally, the project will investigate Lactoferrin's biologically active properties, its potential in promoting wound healing, and its efficacy against specific pathogens in plant models. The goal is to leverage this research to improve microbial management strategies in clinical, environmental, and food safety contexts.

1.2. Outline

Lactoferrin, a globular glycoprotein prominently found in cow's milk and secreted fluids such as saliva, tears, and nasal secretions, exhibits a broad spectrum of biological activities. These include strong antimicrobial, antiviral, and immunomodulatory effects, which are critical in both innate and adaptive immune responses. The protein also supports wound healing processes by promoting cell proliferation and tissue regeneration, and it has been shown to stimulate hair growth, likely through its regulatory effects on cell function and immune modulation. Variability in lactoferrin (LTF) levels within the human body can significantly impact short-term immune function, making the maintenance of stable LTF sources crucial for health. For instance, low LTF levels are often associated with increased susceptibility to infections and slower recovery rates, which highlights its role in disease prevention and management [1-3]. Despite the recognized health benefits of bovine lactoferrin and its commercial production for about three decades, current production methods still encounter significant challenges. These include protein contamination and the loss of biological activity during extraction and processing, which can drastically reduce its effectiveness. Traditional isolation techniques often involve harsh conditions or extensive processing times that can denature the protein, stripping it of its beneficial properties.

1.3. Work plan will be realized according to main research task:

1. Develop and optimize isolation methods for Lactoferrin (LTF) from various dairy sources. Consider the impact of seasonal variations, cow breeds, and diets on Lactoferrin content and other components.

2. Examine the stability of raw materials under varying conditions, including the impact of temperature over different time periods. Stability studies will help define parameters that must be maintained during storage and transportation of the raw material.

3. Analyze basic parameters such as microbiological purity, acidity, total protein content, Lactoferrin content, Lactoferrin glycosylation degree, the fraction of biologically active Lactoferrin, lactose content, lactic acid, free amino acids, total fat content including specific fatty acids, ash, and dry mass content. Additionally, assess the dispersion stability of the raw material.

4. Conduct microbiological research using serial dilution and solid media culture methods to determine the total number of bacteria and fungi, estimating colony-forming unit counts, and identify microorganisms.

5. Employ spectroscopic methods to study total protein content and use separation methods like SDS-PAGE combined with MALDI-TOF-MS to determine Lactoferrin glycosylation levels. High-performance liquid chromatography (HPLC-ESI-3Q-MS and/or HPLC-DAD) and ELISA tests will be utilized to quantify Lactoferrin.

6. Synthesize magnetic ferrite particles using three different methods: co-precipitation in an alkaline environment, thermal decomposition, and hydrothermal methods. Different iron precursors will be used for the synthesis, and the possibility of doping the particles with other metals, such as cobalt, will be explored.

7. Modify the synthesized ferrite particles by coating them with polysaccharides such as modified agarose (sepharose), heparin, and chitosan to enhance their efficiency in binding Lactoferrin.

8. Determine the biologically active properties of Lactoferrin, including its ability to bind iron, degree of denatured/degraded protein, and its antibacterial, antifungal, antiviral properties, as well as its ability to promote the growth of eukaryotic cells and enhance host resistance to viral infections.

9. Investigate the application of Lactoferrin in promoting wound healing and assess its uptake by eukaryotic cells using flow cytometry and electron microscopy with gold-labeled antibodies.

10. Conduct tests on plant models to assess the antiviral properties of Lactoferrin against specific pathogens like Potato virus Y (PVY) and Potato leafroll virus (PLRV), examining the impact of different concentrations and frequency of application on plant resistance compared to controls.

1.4. Literature

1. Dyrda-Terniuk, Tetiana, Paweł Pomastowski. 2023. „The Multifaceted Roles of Bovine Lactoferrin Molecular Structure, Isolation Methods, Analytical Characteristics, and Biological Properties". *Journal of Agricultural and Food Chemistry*, 71: 20500–20531. https://doi.org/10.1021/acs.jafc.3c06887.
2. Walczak-Skierska, Justyna, Fernanda Ferreira Silva Souza Monedeiro, Joanna Rudnicka, Paweł Pomastowski. 2024. „Optimizing Milk Quality and Shelf Life: Investigating Refrigeration Effects on Fatty Acid and Protein Profiles". *ACS Food Science & Technology* 4: 382–391https://doi.org/10.1021/acsfoodscitech.3c00468.
3. Monedeiro-Milanowski, Maciej, Fernanda Monedeiro, Paweł Pomastowski. 2023. „Silver Lactoferrin as Antimicrobials : Mechanisms of Action and Resistance Assessed by Bacterial Molecular Profiles". *ACS Omega* 8: 46236–46251. https://doi.org/10.1021/acsomega.3c07562.I

1.5. Required initial knowledge and skills of the PhD candidate

PhD candidate should be skilful and intellectually manipulative, familiar with the separation (SDS-PAGE, LC-MS, GC-MS, LDI) and instrumental techniques (FTIR, SERS, UV-VIS). Candidate should have also expiration with microorganisms isolation, culturing and identification as well as preparing different culture media. Experienced with the sample preparation and their further analysis using LDI TOF MS as well as skills in the mass spectra recoding. Knowledge in field of protein biochemistry will be highly honoured. Knowledge about work in programs used to identify mass profiles of microorganisms like FlexAnalysis, FlexControl, and MALDI Biotyper will be favoured.

1.6. Expected development of the PhD candidate's knowledge and skills

PhD candidate will gain knowledge and skills in field of microbiology, analytical chemistry and dairy science. Candidate will get specialized knowledge in microbial identification, metal-protein complex synthesis and their physicochemical characterization by separation and instrumental.

Moreover, the interpretation of analytical and statistical data will be developed during PhD study. During the study student will be able to present obtained data in form of high-impact factor publication and posters and oral presentations at domestic and international conferences. As part of the PhD project, it is planned to develop new technological solutions with a high level of invention, legally protected by a patent.