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DOCTORAL DISSERTATION ABSTRACT

Scientific discipline: chemical sciences

Title of the doctoral dissertation: Development of methodology for a comprehensive study of biocolloids and their application in dairy industry

Doctoral dissertation abstract:

In this work, a methodology was developed for the comprehensive analysis of simple dairy-derived colloidal systems, including milk proteins such as casein and lactoferrin, as well as more complex systems such as cream. The investigated systems were subjected to targeted modification in order to tailor their functional properties and broaden their application potential. This research direction is important because it helps address problems related to the limited functionality of natural milk components, the design of fortified foods, the improvement of protein-related analytical quality, and the development of new carrier and bioactive systems with application potential in the dairy industry and the biomedical field.

As part of this work, a method for the natural enrichment of cream with vitamin D3 using *Lactiplantibacillus plantarum* was developed. It was demonstrated that the efficiency of vitamin D3 biosynthesis depends on the process conditions, and the most favorable results were obtained when the lyophilized form of the bacteria was used under non-mixing conditions and light exposure. It was also found that the application of the bacteria affected the fatty acid profile of the cream, leading to a decrease in the proportion of saturated fatty acids (SFA) and an increase in the proportion of monounsaturated fatty acids (MUFA), which may contribute to improving the health-promoting properties of the product.

An important part of the study also involved the physicochemical characterization of bovine lactoferrin (bLF). It was shown that the stability of this protein system depends on pH and ionic strength, and that the presence of low-molecular-weight impurities, such as salts and peptides, may deteriorate the quality of the analyses. The application of ultrafiltration (UF) enabled effective purification of the sample without significantly affecting the molecular mass of the protein, while at the same time improving sample representativeness and the reliability of identification results obtained by MALDI-TOF/MS. These findings confirmed the importance of proper sample preparation for further studies on the physicochemical and biological properties of LF.

The modification of milk proteins with metal ions constitutes a promising platform for designing bioactive systems with improved or expanded functionality. In this work, casein (CN) was modified with silver ions Ag(I), and LF was functionalized with ruthenium ions Ru(III). Based on isothermal studies, it was shown that the sorption of Ag(I) by casein is heterogeneous and multi-step in nature, and that protein desalting increases its ability to bind silver. It was also demonstrated that Ag(I) binding leads to changes in the organization of casein micelles and in protein conformation.



Spectroscopic and microscopic results indicated that this process involves the initial binding of Ag(I) by functional groups of casein, followed by partial bio-reduction leading to the formation of silver nanoparticles and Ag-CN nanocomplexes. The resulting Ag-CN systems exhibited antibacterial activity together with lower cytotoxicity than free silver ions, indicating their potential for the design of biocompatible antiseptic formulations.

In turn, the results of isothermal and kinetic studies indicated that the formation of LF-Ru complexes proceeds spontaneously and within a short time, and that the Ru(III) binding mechanism may be described as saturation-like, involving the gradual occupation of a limited number of accessible binding sites. The results of spectroscopic studies, SAXS, and computational analyses confirmed that the presence of Ru(III) induces changes in the structural organization and physicochemical properties of LF, without causing significant disruption of its global stability. The obtained LF-Ru complexes were characterized by limited ruthenium release, whereas their rapid degradation under the action of pepsin indicated the need for additional encapsulation in order to preserve system integrity under physiological conditions. At the same time, it was demonstrated that Ru(III) binding affects the biological response of LF, decreasing the viability of cancer cells while being better tolerated by fibroblasts, which confirms the biomedical potential of these systems.

From an application-oriented perspective, this work demonstrates that milk and its fractions can be regarded as platform raw materials for modern high-value dairy processing. The proposed approach combines market-oriented analysis with an in-depth understanding of interaction chemistry and shows that casein, lactoferrin, and cream can perform roles that go beyond their classical nutritional functions, namely as an antiseptic carrier, an active coordination scaffold, and a matrix for natural biofortification, respectively. Thus, the dissertation reinforces the concept of dairy processing based on the selective recovery of fractions, the precise design of their properties, and their targeted use in functional, nutraceutical, and biomaterial applications.

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doctoral student's signature