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Engineering artificial casein micelles for future food

Background: Milk and dairy products supply roughly a third of the required dietary energy, lipids, and protein in the Western diet (Fox and Kelly, 2004). However, a growing number of consumers are adopting a dairy-free diet for a variety of reasons, such as lifestyle choices (e.g. vegan diet). Consequently, the demand for dairy alternatives has grown in recent years, but the production of plant-based cheese analogues remains a challenge for food manufacturers, as the functionality (e.g. melting behaviour, elasticity), organoleptic properties, and nutritional quality of these products are often inferior to their dairy counterparts. Therefore, recombinant production of milk proteins, and especially caseins, by microorganisms is receiving increasing interest. To use these novel proteins to produce animal-free cheese, it is required to simulate the assemblies in which caseins occur naturally (i.e. casein micelles), since these structures are essential for the rennet-induced coagulation during cheesemaking and the texture of cheese (Hettinga & Bijl, 2022). In order to do so, first the assembly of natural bovine caseins into so-called artificial casein micelles needs to be understood, after which this knowledge can be employed in the design of casein micelles from recombinant caseins. Therefore, our research focuses on engineering artificial casein micelles under various conditions relevant for future food applications.

Methods: Artificial casein micelles were prepared according to Schmidt et al. (1977) from solutions of bovine caseinate and salts by simulating the mineral concentrations in milk. The process conditions (e.g. temperature, pH, rate) were varied to assess the tunability of the process and to study how this influences the properties and coagulation of the micelles. Next, the assembly of mixtures of individual caseins, as well as dephosphorylated caseins (resembling future recombinant caseins without post-translational modifications), into casein micelles was studied. The following properties were then investigated: micelle size using dynamic light scattering, calcium phosphate nanocluster size by small-angle X-ray scattering, protein and mineral partitioning between the micellar and serum phase, micelle hydration, and the coagulation behaviour and melting behaviour of the curds with rheometry.

Results and discussion: Multiple properties of the artificial casein micelles could be tuned by tailoring the preparation process and manipulating the caseins. Firstly, the amount of caseins that constituted the micellar phase differed greatly at different process conditions and especially decreased upon dephosphorylation. Consequently, the size of the micelles could be controlled between 100 and 300 nm in diameter. Secondly, the degree of casein mineralisation could be tuned, while the size of the calcium phosphate nanoclusters remained unaffected. In turn, this influenced the hydration of the micelles. Lastly, the prepared artificial casein micelles could be coagulated and the resulting curds showed similar melting behaviour as curds from natural bovine casein micelles. This demonstrates the ability to tailor the preparation process and engineer artificial casein micelles with finetuned properties based on the target functionality. Therefore, our research paves the way for the future production of animal-free cheese from recombinant casein.

References

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