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Title:

Optimizing the gelation and digestibility of artificial casein micelles by varying their calcium phosphate content: an in vitro digestion study

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Abstract: (Your abstract must use **Normal style** and must fit in this box. Your abstract should be no longer than 300 words. The box will 'expand' over 2 pages as you add text into it.)

Preparation of Your Abstract

- 1. The title should be as brief as possible but long enough to indicate clearly the nature of the study. Capitalise the first letter of the first word ONLY (place names excluded). No full stop at the end.
- 2. Abstracts should state briefly and clearly the purpose, methods, results and conclusions of the work.

Introduction: Clearly state the purpose of the abstract

Methods: Describe your selection of observations or experimental subjects clearly

Results: Present your results in a logical sequence

Discussion: Emphasize new and important aspects of the study and conclusions that are drawn from them

Milk protein plays an integral role in human nutrition and exhibits a range of functional properties primarily attributed to casein micelles (CM), formed upon the sequestration of amorphous calcium phosphate (CaP) nanoclusters by casein proteins. Beyond nutritional properties, CM have a fundamental role in milk coagulation, gelation and stability. Under gastric conditions, CM form a semi-solid curd which is thought to delay gastric emptying and improve protein digestion and absorption. Understanding the influence of protein and mineral composition on micelle structure and digestibility is essential for the development of sustainable animal-free dairy alternatives.

Here, we investigate the impact of varying CaP content on the structure and digestibility of artificial casein micelles (ACMs). ACMs were prepared by controlled mixing of bovine sodium caseinate with three CaP to achieve α values 0.5, 0.7, 1.0; where alpha(α) represents the fraction of bound phosphocaseins. ACM formation was monitored by the change in solution turbidity along with pH and free calcium ion conductivity. The micelle size distribution was characterized by nanoparticle tracking analysis (NTA), while rheological measurements provided insights into the viscoelastic properties of gels formed under acidic conditions. Digestibility was evaluated using a modified INFOGEST 2.0 protocol, implemented on a semi-automated robotic platform for improved reproducibility. Protein hydrolysis and peptide release during simulated oral, gastric and intestinal phases were analysed using SDS-PAGE and OPA assay.

Results showed that ACM mineral composition influenced ACM size and modulated viscoelasticity during gelation. Variation in mineral content regulated the digestion rate and peptide release. Interestingly, ACMs with an intermediate mineral content approximating that of milk (α 0.7) formed a stronger curd that was more resistant to enzymatic digestion. OPA assay confirmed progressive peptide release increasing from oral to gastric, peaked in intestinal phase. These findings support the design of sustainable and functional dairy alternatives with tailored properties.

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