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# Behaviour of Milk Protein Ingredients and Emulsions Stabilised by Milk Protein Ingredients In the Simulated Gastrointestinal Tract

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# Abstract

Milk clotting behaviours in the stomach impact the digestion rates of protein and fat. A variety of milk protein products are applied as functional ingredients in many foods. This research was conducted to investigate the digestion behaviours of various commercial dairy ingredients and lipids in emulsions stabilised by these ingredients using a dynamic *in vitro* digestion model, i.e., a human gastric simulator (HGS), with a focus on the effect of different structures of clots formed in dairy ingredients during gastric digestion on hydrolysis of proteins and/or lipids.

Skim milk powder (SMP), milk protein concentrate (MPC) 4851, MPC 4861, sodium caseinate, whey protein isolate (WPI) and heated (90°C, 20 min) WPI were used in the present study. Results showed that SMP and MPC 4851, which contained casein micelles, formed ball-like clots with a relatively dense network after 10 min of gastric digestion. These clots did not disintegrate after 220 min of digestion. MPC 4861 and sodium caseinate generated clots at around 40 min, and a loose, fragmented structure was observed at the end of the gastric digestion due to a lacking micellar structure of caseins. No clot was observed in WPI or heated WPI after 220 min gastric digestion, although aggregation occurred at around 40 min in heated WPI. These differences in coagulation behaviours apparently affected the rate of gastric emptying and protein hydrolysis by pepsin in the gastric system. In SMP and MPC 4851, the gastric emptying and hydrolysis of caseins was much slower than that observed in MPC 4861 and sodium caseinate. The most rapid gastric emptying of proteins was observed in the WPI samples both with and without heating. This is attributed to the formation of varied structured clots at different times under the gastric conditions.

The effect of protein concentration on the gastric behaviour of these dairy ingredients in solution was then examined, with a particular emphasis on the structure of clots. SMP and MPC 4851 have been selected as model protein ingredients. Their gastric behaviours were investigated over a protein concentration range of 0.5-5.0% (w/w). The results showed that the digestion behaviour of SMP and MPC 4851 followed a similar pattern. The rate of pH changes in the emptied digesta during digestion was protein concentration dependent. With an increase in protein concentration, the decrease in pH slowed. The protein concentration had no apparent impact on the casein clotting time.

Clots were formed in the first 10 min of digestion in all samples. However, in both SMP and MPC 4851, when protein concentration was lower than 2.0% (w/w) the clots consisted of small protein pieces with a loose, porous and open structure after a 220 min digestion. Whereas a cheese ball-like clot with a denser network was observed at the end of gastric digestion when the protein concentration varied from 2.0% to 5.0% (w/w). Such a difference in the structure apparently affected the rate of protein hydrolysis. A more rapid hydrolysis ($P < 0.05$) of the clotted protein was observed when protein concentration was lower than 2.0% (w/w) compared to the samples containing a higher proportion of protein (2.0%-5.0%, w/w).

To study the effect of different coagulation behaviours on the digestion of oil droplets in oil-in-water emulsions, these dairy ingredients (with the exception of SMP) were used to prepare an oil-in-water emulsion (20.0% soy oil and 4.0% protein, w/w). They were digested under the dynamic gastric conditions using the HGS. The gastric digesta was emptied at 20 min intervals. Then all digesta were mixed to investigate the lipid digestion under the small intestinal conditions. Changes in physicochemical properties of emulsions, involving the particle size, the microstructure, the oil content of the emptied gastric digesta and the amount of free fatty acids (FFAs) released during the small intestine stage, were determined using an *in vitro* small intestinal digestion model.

Aggregation of MPC 4851-stabilised emulsion took place after 5 min of digestion in the HGS with the largest size. The aggregates remained in the stomach and did not disappear during the whole gastric digestion. The hydrolysis of the aggregated network by pepsin was largely slowed by the reduced ability of the simulated gastric fluid (SGF, containing pepsin) to diffuse into the larger sized aggregates. MPC 4851-stabilised emulsion thus resulted in the slowest release of oil droplets into the small intestine. In comparison, MPC 4861 and sodium caseinate-stabilised emulsions aggregated in the stomach at approximately 40 min, forming smaller sized aggregates. These aggregates disintegrated at the mid and late-stages of digestion in these two emulsions. Therefore, MPC 4861 and sodium caseinate-stabilised emulsions had a more rapid delivery of oil droplets into the small intestine. In relation to the WPI-stabilised emulsions both with and without heating, the aggregations formed at a similar time to that which was observed in MPC 4861 and sodium caseinate-stabilised-emulsions; i.e., at approximately 40 min. However, they had the smallest sized aggregates amongst all samples and they

disintegrated quickly with further digestion. WPI-stabilised emulsions both with and without heating had the fastest gastric emptying and hydrolysis by pepsin in the early and mid-stages of the gastric digestion process. Thus, the highest level of oil content contained in the emptied gastric digesta was produced from both WPI-stabilised emulsions. In the mixed gastric digesta, which were subjected to the small intestinal digestion, the oil contents contained in the different emulsion samples varied. This difference impacted the extent of lipid digestion by pancreatic lipase. The sample with a higher oil content released a greater amount of FFAs compared to the sample with a lower oil content. The extent of lipid digestion of different emulsion samples adhered to the following pattern: MPC 4851-stabilised emulsion < MPC 4861-stabilised emulsion < sodium caseinate-stabilised emulsion, WPI-stabilised emulsions both with and without heating.

Overall, the gastric behaviours of dairy ingredients either in solutions or emulsions were affected by the formation of structured clots/aggregates. The differences in clotting/aggregation times and their structures were greatly dependent on the component and structure of protein, the processing prior to digestion and the susceptibility to proteases. These differences in protein coagulation/aggregation behaviour impacted the rates of protein hydrolysis and gastric emptying. The oil content and protein composition of the gastric digesta transferred into small intestine and the extent of lipid digestion in small intestine were also affected. These results are important in an application perspective. They provide useful information for the design and development of healthier food products by allowing greater control over the manipulation of protein bioavailability, which subsequently provides greater control over lipid metabolism.

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# List of Abbreviations

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|---|---|
| α-La: | α-lactalbumin |
| β-Lg: | β-lactoglobulin |
| BSA: | Bovine serum albumin |
| CCP: | Colloidal calcium phosphate |
| HGS: | Human gastric simulator |
| MPC: | Milk protein concentrate |
| WPI: | Whey protein isolate |
| WPNI: | Whey protein nitrogen index |
| SDS-PAGE: | Sodium dodecyl sulfate-poly acrylamide electrophoresis |
| SGF: | Simulated gastric fluid |
| SIF: | Simulated intestinal fluid |
| $d_{4,3}$: | Average volume-weighted diameter |
| $d_{3,2}$: | Average surface-weighted diameter |
| PI: | Isoelectric point |
| w/w | Weight/weight |
| w/v | Weight/volume |
| v/v | Volume/volume |