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# **Behaviour of milk protein–stabilized oil-in-water emulsions in simulated physiological fluids**

A thesis presented in partial fulfilment  
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*Dedicated to*  
*My Beloved Parents*



## Abstract

Emulsions form a major part of processed food formulations, either being the end products in themselves or as parts of a more complex food system. For the past few decades, colloid scientists have focussed mainly on the effects of processing conditions (*e.g.* heat, high pressure, and shear) on the physicochemical properties of emulsions (*e.g.* viscosity, droplet size distribution and phase stability). However, the information about the behaviour of food structures post consumption is very limited. Fundamental knowledge of how the food structures behave in the mouth is critical, as these oral interactions of food components influence the common sensorial perceptions (*e.g.* creaminess, smoothness) and the release of fat-soluble flavours. Initial studies also suggest that the breakdown of emulsions in the gastrointestinal tract and the generated interfacial structures impact lipid digestion, which can consequently influence post-prandial metabolic responses. This area of research needs to be intensively investigated before the knowledge can be applied to rational design of healthier food structures that could modulate the rate of lipid metabolism, bioavailability of nutrients, and also help in providing targeted delivery of flavour molecules and/or bioactive components.

Hence, the objective of this research was to gain understanding of how emulsions behave during their passage through the gastrointestinal tract. *In vitro* digestion models that mimic the physicochemical processes and biological conditions in the mouth and gastrointestinal tract were successfully employed. Behaviour of model protein-stabilized emulsions (both positively charged (lactoferrin) as well as negatively charged [ $\beta$ -lactoglobulin ( $\beta$ -lg)] oil-in-water emulsions) at each step of simulated physiological processing (using model oral, gastric and duodenal fluids individually) were investigated.

In simulated mouth conditions, oil-in-water emulsions stabilized by lactoferrin or  $\beta$ -lg at the interfacial layers were mixed with artificial saliva at neutral pH that contained a range of mucin concentrations and salts. The  $\beta$ -lg emulsions did not interact with the artificial saliva due to the dominant repulsion between mutually

opposite charges of anionic mucin and anionic  $\beta$ -lg interfacial layer at neutral pH. However,  $\beta$ -lg emulsions underwent some depletion flocculation on addition of higher concentrations of mucin due to the presence of unadsorbed mucin molecules in the continuous phase. In contrast, positively charged lactoferrin emulsions showed considerable salt-induced aggregation in the presence of salts (from the saliva) alone. Furthermore, lactoferrin emulsions underwent bridging flocculation because of electrostatic binding of anionic mucin to the positively charged lactoferrin-stabilized emulsion droplets.

In acidic pH conditions (pH 1.2) of the simulated gastric fluid (SGF), both protein-stabilized emulsions were positively charged. Addition of pepsin resulted in extensive droplet flocculation in both emulsions with a greater extent of droplet instability in lactoferrin emulsions. Coalescence of the droplets was observed as a result of peptic hydrolysis of the interfacial protein layers. Conditions such as ionic strength, pH and exposure to mucin were shown to significantly influence the rate of hydrolysis of  $\beta$ -lg-stabilized emulsion by pepsin.

Addition of simulated intestinal fluid (SIF) containing physiological concentrations of bile salts to the emulsions showed competitive interfacial displacement of  $\beta$ -lg by bile salts. In the case of lactoferrin-stabilized emulsion droplets, there was considerable aggregation in the presence of intestinal electrolytes alone (without added bile salts) at pH 7.5. Binding of anionic bile salts to cationic interfacial lactoferrin layer resulted in re-stabilization of salt-aggregated lactoferrin emulsions. On mixing with physiological concentrations of pancreatin (mixture of pancreatic lipase, amylase and protease), significant degree of coalescence and fatty acid release occurred for both the emulsions. This was attributed to the interfacial proteolysis by trypsin (proteolytic fractions of pancreatin) resulting in interfacial film rupturing. Exchange of initial interfacial materials by bile salts and trypsin-induced film breakage enhanced the potential for lipolytic fractions of pancreatin to act on the hydrophobic lipid core. The lipid digestion products (free fatty acids and mono-

and/or diglycerides) generated at the droplet surface further removed the residual intact protein layers from the interface by competitive displacement mechanisms.

The sequential treatment of the cationic and anionic emulsions with artificial saliva, SGF and SIF, respectively, was determined to understand the impact of initial protein type during complete physiological processing from mouth to intestine. Broadly, both the protein-stabilized emulsions underwent charge reversals, extensive droplet flocculation, and significant coalescence as they passed through various stages of the *in vitro* digestion conditions. Except in the simulated mouth environment, the initial charge of the emulsifiers had relatively limited influence on droplet behaviour during the simulated digestion.

The results contribute to the knowledge of how structure and charge of the emulsified lipid droplets impact digestion at various stages of physiology. This information might have important consequences for developing suitable microstructures that allow controlled breakdown of droplets in the mouth and predictable release of lipids in the gastrointestinal tract.



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1. Goh, K. K. T., **Sarkar, A.**, & Singh, H. (2008). Milk protein–polysaccharide interactions. In: A. Thompson, M. Boland, & H. Singh, *Milk Proteins: From Expression to Food* (pp. 347–376). New York: Academic Press.
2. **Sarkar, A.**, Goh, K. K. T., & Singh, H. (2009). Colloidal stability and interactions of milk protein–stabilized emulsions in an artificial saliva. *Food Hydrocolloids*, 23(5), 1270–1278.
3. **Sarkar, A.**, Goh, K. K. T., Singh, R. P., & Singh, H. (2009). Behaviour of an oil-in-water emulsion stabilized by  $\beta$ -lactoglobulin in an *in vitro* gastric model. *Food Hydrocolloids*, 23(6), 1563–1569.
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6. **Sarkar, A.**, Horne, D. S., & Singh, H. (In Press). Pancreatin–induced coalescence of oil-in-water emulsions in an *in vitro* duodenal model. *International Dairy Journal*, [doi:10.1016/j.idairyj.2009.12.007](https://doi.org/10.1016/j.idairyj.2009.12.007).