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# Composite whey protein–cellulose nanocrystals at oil-water interface: Towards delaying lipid digestion

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ABSTRACT

Lipid digestion is an interfacial process that is largely controlled by the adsorption of lipase + colipase + bile salts onto the surface of the emulsified lipid droplets. Therefore, engineering oil-in-water (O/W) interfaces that prevent competitive displacement by bile salts and/or delay the transportation of lipase to the hydrophobic lipid core can be effective strategies to delay lipolysis. In this study, we present such an interface using composite protein-particle system, consisting of whey protein isolate (WPI) (1 wt %) + cellulose nanocrystals (CNCs) (1–3 wt%). Droplet size, microscopy at various length scales (confocal, electron microscopy), ζ-potential and kinetics of fatty acid release were used to assess how the presence of CNCs impacted the microstructural stability of the emulsions in *in vitro* duodenal conditions (pH 6.8, 37 °C). Adding CNCs at sufficiently high concentrations (3 wt%) significantly decreased the rate and degree of lipolysis as compared to that of protein-coated emulsion droplets. The principal cause of this altered lipolysis profile was the binding of bile salts by CNCs, which restricted both the interfacial displacement and solubilisation of lipid-digestion products by bile salts. The CNCs can be envisaged to be strongly bound to the protein-coated droplets by virtue of hydrogen bonding with the underlying protein. Furthermore, the ability of the CNCs in the continuous phase to bridge several protein-coated droplets reduced the overall surface area available for the lipolysis. Composite WPI + CNC interface holds promise in designing physiologically relevant emulsions to target satiety or delivery systems for sustained release of lipophilic components.



## 1. Introduction

Obesity is a growing global health crisis, which has more than doubled in extent in the last 25 years. As of 2014, more than 1.9 billion adults were overweight globally, and of those over 600 million people were obese (WHO, 2015). Among many strategies to achieve weight management, food scientists, psychologists and nutritionists are attempting to enhance the satiating and satiety-promoting properties of food in order to generate appetite suppression. In the case of lipids, satiety hormones can be triggered by the presence of undigested lipids in the ileum via the so-called "ileal brake" mechanism (Maljaars, Peters, Mela, & Masclee, 2008). Hence, a delayed lipid digestion in the intestine that will release the lipids in a more sustained manner and has potential to promote the feeling of satiety has become a target of research in the literature.

Nearly 70–90% of the lipid digestion takes place in the duodenal i.e. in the upper part of the small intestine in healthy human adults. Lipid digestion is essentially an interfacial process that involves complex adsorption phenomenon of lipase/colipase and bile salts onto the surface of the oil droplets (Sarkar, Ye, & Singh, 2016c; Singh & Sarkar, 2011). Bile salts are bio-surfactants that competitively push out the interfacial materials originally present at the surface, facilitating the adsorption of pancreatic lipase-colipase complex and subsequent lipolysis (Maldonado-Valderrama, Wilde, Macierzanka, & Mackie, 2011). In the last decade two key food-structuring approaches have been investigated to delay intestinal lipolysis, which would allow the detection of undigested lipids in the distal parts of the intestine. The first one involved modulating the interfacial parameters of emulsion (size, charge, interfacial composition *etc.*) in order to prevent competitive displacement of the original interfacial materials by bile salts and adsorption of lipase thus delaying the process of binding of lipase/colipase complexes to act on the bile-coated oil droplets (Corstens et al., 2017; Golding, 2014; Golding & Wooster, 2010; Sandra,

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Decker, & McClements, 2008; Sarkar, Horne, & Singh, 2010c; Sarkar et al., 2016a). The second approach included restricting the transport of lipase to the emulsified lipid droplets via encapsulation of the emulsion droplets within a gel system (Guo, Bellissimo, & Rousseau, 2017; Guo, Ye, Lad, Dalgleish, & Singh, 2014; Sarkar et al., 2015). It is now clearly recognized that both ionic surfactants and biopolymers are generally easily displaced from the emulsion droplet surface by bile salts during duodenal digestion (Mackie, Gunning, Wilde, & Morris, 2000; Maldonado-Valderrama et al., 2011; Sarkar, Horne, & Singh, 2010b; Sarkar et al., 2016c). However, lately, it has been demonstrated that particle-laden interfaces, such as those created by chitin nanocrystals (Tzoumaki, Moschakis, Scholten, & Biliaderis, 2013), intact or fused whey protein microgel particles (Sarkar et al., 2016a) were not displaced by bile salts, by virtue of high desorption energy of these particles from the interfaces.

One alternative strategy is to coat protein-stabilized interfaces with a layer of particles, which would provide a steric hindrance to the transport of lipase to hydrophobic lipid core. Recently, such protein-particles laden composite interfaces have shown promising effects on enhancing the gastric stability of oil-in-water (O/W) emulsions (Sarkar, Zhang, Murray, Russell, & Boxal, 2017). In this study, cellulose nanocrystal particles (CNCs) were used to create O/W emulsions with composite protein-CNC interfaces by carefully exploiting the electrostatic attraction between cationic whey protein and negatively charged CNCs at pH 3 (Sarkar et al., 2017). The presence of higher concentration of particles (3 wt%) increased the interfacial shear viscosity of the underlying protein film by almost 40 times and thus, increased the resistance of the interfacial protein film to subsequent rupture by pepsin in the gastric regime at pH 3 (60% intact interfacial protein remained after gastric digestion). The composite WPI-CNC interfacial layer inhibited droplet coalescence in the gastric phase. Such droplet coalescence would have occurred rather spontaneously in an emulsion stabilized by WPI alone, where almost no intact interfacial protein tends to remain after gastric digestion (Sarkar, Goh, & Singh, 2010a; Sarkar, Goh, Singh, & Singh, 2009b; Sarkar & Singh, 2016b; Sarkar et al., 2017; Singh and Sarkar, 2011). Besides formation of rigid composite interface, the network formation by the CNCs in the bulk (continuous) phase was also hypothesized to reduce the overall kinetics of interfacial proteolysis (Sarkar et al., 2017).

In this study, we have focused on duodenal lipolysis of those emulsions stabilized by composite WPI-CNC interfacial layer, latter created by interfacial electrostatic complexation of mutually complimentarily charged species at pH 3. We hypothesize that the presence of such unmodified CNCs at the WPI-stabilized O/W interface could prevent the competitive displacement by bile salts via steric and/or electrostatic effects and thus might contribute to delaying the lipid digestion. To our knowledge, this is the first study that reports the impact of composite protein-particle laden O/W interface on kinetics of lipid digestion in simulated duodenal conditions.

## 2. Materials and methods

### 2.1. Materials

Whey protein isolate (WPI) powder containing 96.3 wt% protein was kindly gifted by Fonterra Limited (Auckland, New Zealand). Cellulose nanocrystal powder (CNC, sulphated) was purchased from CelluForce™, Canada. Sunflower oil was purchased from a local supermarket (Morrisons, UK). Porcine bile extract B8631, porcine pancreatin (P7545, 8 × USP) and sodium azide were purchased from Sigma-Aldrich Company Ltd, Dorset, UK. All other chemicals used were of analytical grade and were obtained from Sigma-Aldrich Chemical Company unless otherwise specified. Milli-Q water having an ionic purity of 18.2 MΩ·cm at 25 °C (water purified by treatment with a Milli-Q apparatus, Millipore Corp., USA) was used for all the experiments.

### 2.2. Preparation of emulsions

Appropriate quantities of WPI were dispersed in 10 mM citrate buffer solution at pH 3 (adjusted using 0.1 M HCl) for 2 h to ensure complete dissolution. Oil-in-water emulsions (20 wt% oil) stabilized by WPI (1 wt%), hereafter cited as W1 were prepared by homogenizing 20.0 wt% sunflower oil and 80.0 wt% WPI solution using two passes through a two-stage valve homogenizer (Panda Plus 2000, GEA Niro Soavi Homogeneizador Parma, Italy) operating at first/second stage pressures of 250/50 bars, respectively at 25 °C. The maximum temperature reached by the emulsions during the homogenization step was 37 °C. For preparing the protein-particle-stabilized emulsions (schematic diagram shown in Fig. 1), the primary emulsions (40 wt% oil, 2 wt% WPI) were dispersed in CNC dispersions (2–6 wt% in citrate buffer at pH 3) (1:1 w/w) to achieve final concentration of 20 wt% oil, 1 wt% WPI and 1 or 3 wt% CNCs, hereafter reported as W1C1 or W1C3, respectively. All the three emulsion samples were prepared in triplicates. Sodium azide (0.02 wt%) was used as a preservative for the emulsions during refrigerated storage at 4 °C.

### 2.3. Particle size analysis of emulsions

The droplet size distribution of each of the three emulsions before and after duodenal digestion was measured using static light scattering (Malvern MasterSizer 3000, Malvern Instruments Ltd, Malvern, Worcestershire, UK). The absorbance of the emulsion droplets was set to 0.001. Refractive indices of 1.456 and 1.33 were selected for the sunflower oil and the continuous phase, respectively. Mean droplet size was reported as Sauter-average diameter ($d_{32}$) and volume-average diameter ($d_{43}$) calculated on five measurements on triplicate samples.

### 2.4. Zeta-potential

The ζ-potential of each of the emulsions before and after duodenal digestion (180 min) with or without the addition of bile salts and/or pancreatin was measured using a Zetasizer Nano ZS (Malvern Instruments Ltd, Malvern, Worcestershire, UK). Emulsions were diluted to 0.005 wt% droplet concentration in citrate buffer before digestion at pH 3 and simulated duodenal fluid (SDF) buffer after digestion at pH 6.8 The samples were equilibrated in respective buffers for 1 h and then were carefully transferred into DTS1070 folded capillary cells to measure the electrophoretic

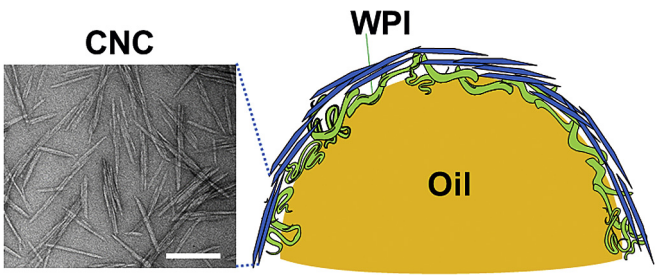


**Fig. 1.** Schematic representation of the emulsion droplets (yellow) stabilized by WPI (green) and CNC (blue) with negative-stained TEM micrograph of CNC (left). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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