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Oil-in-water Pickering emulsions via microfluidization with cellulose nanocrystals: 2. In vitro lipid digestion

Long Bai, Shanshan Lv, Wenchao Xiang, Siqi Huan, David Julian McClements, Orlando J. Rojas

Abstract

Bio-based engineered nanomaterials are being explored for their utilization within foods to improve quality and enhance functionality. In this study, we investigated the impact of a naturally-derived particle stabilizer, cellulose nanocrystals (CNC), on the gastrointestinal fate and digestion of corn oil-in-water Pickering emulsions. A static 3-stage gastrointestinal tract (GIT) model was used to simulate the mouth, stomach and small intestine. The digestion of the CNC-coated lipid droplets was monitored by measuring the release of free fatty acids (FFAs) in the small intestine stage over time. The final extent of FFAs released was reduced by ~40% by using emulsions containing 10 wt% of the dispersed phase, corn oil, stabilized with CNC (0.75 wt% of the aqueous phase). Three main mechanisms are proposed for this effect: (1) the irreversible adsorption of CNC to the lipid droplet surfaces inhibited bile salt and lipase adsorption; (2) coalescence and flocculation of the lipid droplets reduced the surface area available for the bile salts and lipase to bind; and (3) accumulation of FFAs at the surfaces of the lipid droplets inhibited lipolysis. Our findings suggest that CNC can be used as a food-grade particle stabilizer to modulate the digestion of Pickering emulsified lipids, which is useful for the development of given functional foods.

Keywords:
- Cellulose nanocrystals
- Pickering emulsion
- Simulated GIT model
- Lipid digestion
- Microfluidization

1. Introduction

Over the past thirty years or so, the global prevalence of obesity and overweight has risen by more than 27%, bringing the number of affected individuals to around 2.1 billion (Ng et al., 2014). In particular, obesity is seen as a major health concern because it is linked to increased risks of other chronic diseases, such as diabetes, cardiovascular disease, hypertension, cancer, and depression (Qasim et al., 2018). A good diet and plenty of physical activity can prevent obesity, but once someone has become obese it is difficult to reduce their weight owing to the physiological changes that increase appetite and encourage weight regain (Polidori, Sanghvi, Seeley, & Hall, 2016). More lasting weight loss can sometimes be achieved using drugs, such as lipase inhibitors (Bessesen & Van Gaal, 2018). However, pharmaceutical intake over extended periods is undesirable and so there is a need for innovative, effective, and long-lasting alternative treatments. Particularly, it is desirable to develop food-based approaches that can be integrated into an individual's regular diet.

Foods that are able to stimulate appetite suppression through the gut-brain axis have been examined for this purpose (Jose, Peter, & Gustavo, 2007). For instance, human studies have shown that infusion of undigested fats into the far end of the small intestine activates the ileal brake mechanism (Marciani et al., 2008). The fat arriving in the ileum induces the expression of hormones that slow down intestinal motility and reduce appetite (Maljaars, Peters, Mela, & Masclee, 2008). Conversely, more recent studies have shown that when fats are fed through the mouth, rather than directly infused into the small intestine, these effects are less pronounced (Poppitt et al., 2018). This may be because the ingested fats are broken down in the mouth and stomach, and then rapidly digested in the small intestine so that little undigested fat reaches the ileum. Consequently, there is an interest in designing foods that will slow down the digestion of fats within the small intestine so as to promote satiety.

The digestion of lipids within the human gastrointestinal tract (GIT) is an extremely dynamic and complex phenomenon involving many different endogenous and exogenous molecular species (McClements & Li, 2010; Sarkar, Ye, & Singh, 2016; Scheuble et al., 2018). Mucin may bind to the surfaces of the lipid droplets and alter their surface
properties and aggregation state. Bile salts and phospholipids may adsorb to the surfaces of the lipid droplets and displace any existing emulsifiers (Sadeghpour, Rappolt, Misra, & Kulkarni, 2018). Lipases may attach to the oil/water interfaces and digest the lipids inside the droplets (Ye, Cao, Liu, Cao, & Li, 2018). Bile salts may combine with lipid digestion products to form mixed micelles that solubilize and transport fatty acids and oil-soluble bioactives. Calcium ions may bind to free fatty acids to form insoluble calcium soaps (Devraj et al., 2013). Knowledge of these different physiochemical and physiological processes is required to develop food systems with delayed digestion characteristics within the human gut.

The digestion of lipid droplets can be retarded by coating them with certain types of emulsifiers (Bellesi, Martinez, Ruiz-Henestrosa, & Pilosof, 2016; Sarkar, Zhang, Murray, Russell, & Boxal, 2017). For instance, many molecular-based emulsifiers commonly used in the food industry are not very effective at inhibiting lipid digestion, such as milk proteins (Winuprasith et al., 2018), polysaccharides (Yao et al., 2013) or small-molecule surfactants (Chang & McClements, 2016). The origin of this phenomenon is the ability of bile salts to displace these emulsifiers from the oil droplet surfaces (McClements & Li, 2010), which is probably a mechanism developed through evolution to ensure full digestion of lipids so that all the calories can be effectively utilized.

Recently, there has been an increased interest in the utilization of particle stabilizers to produce Pickering emulsions and modulate their digestion (Sarkar, Zhang, Holmes, & Ettelaie, 2018; Tzoumaki, Moschakis, Scholten, & Biliaderis, 2013). This type of stabilizer may be more effective for this purpose than traditional molecular-based emulsifiers because of their higher desorption energy and stronger resistance to droplet coalescence (Bai, Huan, et al., 2019; Bai, Xiang, Huan, & Rojas, 2018; Wu & Ma, 2016). The high desorption energy makes them more difficult to be displaced by bile salts from the oil droplet surfaces, thereby inhibiting the subsequent adsorption and activity of lipase (Tzoumaki et al., 2013). However, the nature of the particles used to formulate Pickering emulsions must be selected carefully to ensure that they have the required performance (Sarkar, Li, Cray, & Boxall, 2018). For instance, acid-sensitive particles may be degraded when exposed to the strongly acidic gastric fluids, thereby leading to exposure of the lipids to the lipase in the small intestine. Moreover, only few of the particles currently studied for use in Pickering emulsions are economically viable or suitable for commercial applications (Barkhordari & Fathi, 2018; Berton-Caron & Schröen, 2015; Serpa et al., 2016; Tzoumaki, Moschakis, Kossosoglou, & Biliaderis, 2011; Wei & Huang, 2019). Therefore, it is necessary to find cost-effective Pickering stabilizers that can also adjust the lipid digestion.

Cellulose nanocrystals (CNC) are one of the most promising nanoparticle-based stabilizers of Pickering emulsions because of their lower desorption energy and stronger resistance to droplet coalescence (Bai, Huan, et al., 2019; Bai, Xiang, Huan, & Rojas, 2018). They are resistant to digestion by digestive enzymes within the human gut (Nisor-Atindana et al., 2017; Torcillo-Gómez & Foster, 2016). The ability of CNC to form an indigestible physical barrier around the oil droplets in Pickering emulsions may make it particularly suitable for controlling lipid digestion under GIT conditions. Indeed, it was recently shown that depositing a layer of CNC around protein-coated lipid droplets decreased the rate and degree of lipid digestion (Sarkar, Li, et al., 2018; Sarkar et al., 2017). On the other hand, a recent report on lipid digestion with another type of nanocellulose, cellulose nanofibrils, indicates that the existence of dietary fiber in the system promotes the coalescence and binding of fat, which minimizes the total accessible area for bile salt and lipases, decreasing the lipid digestion (DeLoid et al., 2018). In contrast to cellulose nanofibrils, CNC particles adsorb at the oil/water interfaces; therefore, they can be considered for the formulation of emulsions that interfere with lipid digestion under similar or different mechanisms.

In a previous report, we developed a microfluidization method to produce fluid-like CNC-stabilized Pickering emulsions containing relatively small and monomodal oil droplets (Bai, Lv, et al., 2019). We also showed that these emulsions had good physical stability over a wide range of pH values, ionic strength, and temperature. These Pickering emulsions may therefore be suitable for applications in commercial food and beverage products. At present, however, there is little understanding of how these emulsions will behave under gastrointestinal conditions. Moreover, the effectiveness and regulatory pathways of CNC-based Pickering emulsification on impacting lipid digestion has also not been fully unveiled. If they are digested more slowly than conventional emulsions, they may be useful for creating products that enhance satiety. Conversely, if they are digested at the same rate as conventional emulsions, they may be more useful for delivering hydrophobic bioactive components. The purpose of the current study is therefore to use a simulated GIT model to investigate the impact of the CNC-coating on the gastrointestinal fate and digestibility of the lipid droplets in Pickering emulsions.

2. Experimental

2.1. Materials

Cellulose nanocrystals (CNC) produced by acid hydrolysis of wood fibers were obtained as an aqueous suspension (12.1 wt%, pH ∼7.0) from the USDA’s Forest Products Laboratory (FPL, Madison, WI) and acquired through the Process Development Center (University of Maine, Maine). The pH of the stock CNC suspension was 7. Sodium chloride (NaCl), Calciumflour white stain, and Nile red were purchased from the Sigma-Aldrich Co. (St. Louis, MO). Gastrointestinal components, including mucin (from porcine stomach), peptic (from porcine gastric mucosa, activity 800–2500 units/mg), lipase (from porcine pancreas, Type II, activity 2.0 UPS units/mg), and bile extract (porcine) were also purchased from the Sigma-Aldrich Co. The bile extract contained 49 w/w% bile salt (10–15 w/w% glycodeoxycholic acid, 3–9 w/w% taurodeoxycholic acid, 0.5–7.0 w/w% deoxycholic acid, 1.0–5.0 w/w% hydrodeoxycholic acid, and 0.5–2.0 w/w% cholic acid) and 5.0 w/w% phosphatidyl choline, with a mole ratio of bile salt to phosphatidyl choline of ca. 15:1. Corn oil was purchased from a local supermarket. Double distilled water (Milli-Q) was used throughout the experiments to prepare solutions and emulsions.

2.2. Nanocellulose morphology

The morphology of pristine CNC was determined using atomic force microscopy (AFM) equipped with a NanoScope V controller (Dimension Icon, Bruker Corporation, Billerica, MA), operating in tapping mode. The scanning area was 5 μm × 5 μm. The image correction applied was flattening during image analysis (NanoScope 8.15 software, Bruker). Several drops of diluted CNC suspension were dripped onto freshly pre-cleaned mica plate and then the sample was dried and stored at ambient temperature before imaging.

2.3. CNC-stabilized Pickering emulsions

CNC-stabilized oil-in-water Pickering emulsions were prepared by homogenizing the oil (10 wt%) and aqueous (90 wt%) phases, as described previously (Bai, Lv, et al., 2019). Particularly, the stock CNC suspension was diluted with electrolyte-free Milli-Q water and, after dilution, the final pH of the aqueous phase for emulsion preparation was 6.5. Together with the role of ionic strength and ionic species, which may be relevant factors, no buffer solutions were used except for Milli-Q water. The oil phase consisted of corn oil while the aqueous...
phase consisted of CNC (0.1–1.0 wt%) and NaCl (0.06 wt%) in Milli-Q water. A coarse emulsion was formed using a blender and then a fine Pickering emulsion was formed using a microfluidizer (3 passes, 13 kpsi). The pH value for all the emulsions was approximately 6.5. All the Pickering emulsions obtained were stored at 4 °C before use.

2.4. In vitro digestion

The CNC-stabilized Pickering emulsions were passed through a 3-stage simulated GIT model consisting of mouth, stomach, and small intestine phases. The details of this simulation method have been described in a previous publication (Winuprasith et al., 2018). The controls used in these experiments were CNC suspensions analyzed under the same conditions but that did not contain oil. The results for the controls were subtracted from those of the samples. As a reference, a corn oil-in-water emulsion stabilized by a typically used polysaccharides-based emulsifier, gum arabic (GA), was prepared using the same microfluidization procedure and then used in the same digestion model. The GA concentration used was 0.75 wt%, water-to-oil ratio of the GA-stabilized emulsion was 90:10, and the mean droplet diameter was 1.3 μm, which was similar to the CNC-stabilized emulsions.

2.4.1. Initial system

The initial emulsion (20 mL) containing 2 wt% oil was placed into a 100-mL glass beaker and incubated in an incubator shaker (Innova Shaker, Model 4080, New Brunswick Scientific, New Jersey, USA) at 37 °C for 2 min.

2.4.2. Mouth stage

The initial emulsion was mixed with 20 mL of simulated saliva fluid (SSF) containing 0.03 g/mL mucin. The SSF was preheated to 37 °C for 2 min before adding to the initial sample. After mixing, the pH of the samples was adjusted to pH 6.8 and then the system was stirred continuously at 100 rpm in the incubator (37 °C for 5 min) to mimic agitation condition in the mouth.

2.4.3. Stomach stage

Simulated gastric fluid (SGF) was prepared by dissolving 2 g of sodium chloride (NaCl) and 7 mL of hydrochloric in Milli-Q water. The total volume of SGF was then adjusted to 1 L. The sample from the mouth stage (20 mL) was mixed with 20 mL of SGF containing 0.0032 g/mL pepsin in a 100 mL glass beaker. The mixture was adjusted to pH 2.5 and then stirred continuously at 100 rpm in the incubator (37 °C for 2 h) to mimic the conditions in the stomach.

2.4.4. Small intestine stage

The sample from the stomach phase (20 mL) was transferred into a 100 mL clean glass beaker and then placed into a water bath at 37 °C connected to an automatic titration unit used as a pH-stat (Metrohm, USA Inc., Riverview, FL, USA). The sample was adjusted to pH 7.00 using NaOH solution. Simulated intestinal fluids (1.5 mL), containing 0.25M CaCl2 and 3.75M NaCl, were added into the reaction vessel, followed by 3.5 mL of bile salt solution (5 mg/mL) with continuous stirring. The pH of the system was adjusted back to pH 7 using NaOH solution. Freshly prepared 2.5 mL lipase solution (1.6 mg/mL) was added to the reaction vessel, and then the automatic titration unit was started. The titration unit was used to record and maintain the pH at 7 by titrating 0.15 M NaOH solution into the reaction vessel for 2 h. The temperature was controlled at 37 °C throughout the experiment. The digestion of the lipids in the small intestine was monitored by measuring free fatty acid (FFA) release over time (Lv et al., 2018).

It should be noted that various factors can possibly alter the titration with the pH-stat method during lipid digestion, which in turn would affect the measured FFA releasing profiles. However, studies involving similar determinations (DeLoid et al., 2018) support the reliability of pH-stat for such purposes.

2.5. Droplet size, charge, and microstructure

The size and charge of oil droplets were measured using the same methods described previously (Bai, Lv, et al., 2019). Briefly, laser diffraction was used to measure the particle size distribution and mean particle diameter (D32), and the particle electrophoresis was used to measure the ζ-potential.

The microstructure of Pickering emulsions was characterized using confocal scanning fluorescence microscopy and fluorescent microscopy with oil sensitive (Nile Red) and CNC sensitive (Calcofluor white stain) fluorescence dyes as described previously (Bai, Lv, et al., 2019).

2.6. Statistical analysis

Means and standard deviations were calculated from measurements made on at least two newly prepared samples with three repeated measurements per sample. Statistical analyses were performed via analysis of variance (ANOVA) using Duncan’s new multiple range test (Minitab 16.2.4, Minitab Inc., State College, PA). A p-value of < 0.05 was considered statistically significant.

3. Results and discussion

3.1. CNC structure

Initially, the morphology of pristine CNC was characterized using AFM (Fig. 1). The AFM images showed that CNCs were present as thin nanorods with a width in the range of 5–20 nm and a length in the range of 150–200 nm (Fig. 1a), which is in line with previous reports.
Bearing in mind that CNC metrology depends on the method applied (AFM, SEM, TEM, and others), we report here an AFM height dimension of 6 ± 2 nm. On the other hand, no interconnected fibrous network structures were observed in the sample, even after they had been dried for AFM testing, which is different from other types of nanocelluloses, such as cellulose nanofibrils or nano-brilliated cellulose fibers (Huan, et al., 2017; Winuprasith et al., 2018). Thus, it is expected that the assembly behavior of CNC nanorods in aqueous phase is quite different compared to the other nanocelluloses. It should be noted that the limited resolution of the AFM images, due to the small scan sizes and the high particle density on the surface, do not allow precise measurements. However, considering the difference between methods and the inherent variation in CNC dimensions, the values are expected to be appropriate for our purposes.

### 3.2. Influence of CNC concentration on gastrointestinal fate of lipid droplets

To assess the impact of the CNC-coatings on the gastrointestinal fate and digestion of the lipid droplets, Pickering emulsions prepared using different levels of CNC were passed through a 3-stage *in vitro* GIT model. The properties of the initial emulsions have been described in detail in our previous companion study (Bai, Lv., et al., 2019). In summary, the initial Pickering emulsions produced via microfluidizer contained relatively small oil droplets ($D_{32} = 1$ to $3 \mu m$) that were evenly distributed throughout the samples without any evidence of flocculation during storage (14 days). The size of the CNC-coated droplets in these emulsions decreased with increasing CNC concentration, which can be attributed to the greater surface area that can be covered. For the sake of comparison, GA-stabilized emulsions were prepared as a reference. These emulsions had a similar initial mean droplet diameter as the CNC-stabilized systems, which facilitated comparison of the results.

It should be noted that the evaluation of the droplet size and ζ-potential during the three-stage GIT needs to factor the changes in pH, ionic strength, interfacial and bulk composition as well as the complex associations in the surrounding environment. They are likely to affect the properties of CNC adsorbed on the surface of the droplets and, hence, limit the measurements at the given GIT stage, an unavoidable and challenging fact. Therefore, the reported data should be taken as approximations and calls for the need of new experimental approaches.

#### 3.2.1. Mouth stage

After exposure to the simulated mouth, all the Pickering emulsions stabilized by CNC had well-dispersed oil droplets with similar dimensions as those in the original emulsions (Figs. 2, 4 and 5), suggesting that the CNC-coated droplets were relatively stable to oral conditions. In our previous study, we found that this type of Pickering emulsions was stable across a wide range of pH values, temperature and ionic strength (Bai, Lv., et al., 2019), which is attributed to the strong electrostatic and steric repulsions between the droplets. The fact that we did not see any flocculation in these emulsions under oral conditions suggests that they were also resistant to the effects of mucin in the simulated saliva. The magnitude of the ζ-potential in all the emulsions slightly decreased after the mouth stage (Fig. 3), which is probably a result of electrostatic screening by ions in the simulated saliva (Singh & Sarkar, 2011). GA-coated droplets also appeared to be stable to droplet aggregation under oral conditions, which can be attributed to strong inter-droplet electrostatic repulsion (Bai, Huan, Gu, & McClements, 2016; Bai, Huan, Li, & McClements, 2017; McClements, Bai, & Chung, 2017; Ozturk, Argin, Ozilgen, & McClements, 2015).

#### 3.2.2. Stomach stage

At the end of the stomach stage, the mean droplet diameter ($D_{32}$) of all CNC-stabilized Pickering emulsions was slightly higher than that of the initial emulsions (Fig. 2), but still relatively small, which was also confirmed by confocal microscopy (Fig. 4). The size range of droplet diameter was between 1.9 and 3.7 μm; we note that the percentage increased compared to the initial emulsions was limited, e.g., less than 30% for all samples. Furthermore, the structure of CNC was maintained at highly acidic gastric condition, as indicated by the clear blue contour around oil droplets observed in the fluorescent micrographs (Fig. 5). This result suggests that the CNC-coatings provided good protection against droplet aggregation under simulated gastric conditions. Even so, the ζ-potential analysis showed that the surface potential changed from strongly negative in the mouth to slightly positive in the stomach (Fig. 3), which has also been reported by other researchers (Lv et al., 2018). This is probably the result of the low pH, high ionic strength and the complex composition of the gastric environment, which modulates the electrostatic interactions of the CNC-coated oil droplets. In
particular, the CNC loses some of its charge at low pH values and the magnitude of the surface potential may be reduced by the presence of any cationic proteins or multivalent counter-ions in the gastric fluids. The GA-coated droplets were also relatively stable to droplet aggregation under simulated stomach conditions (Figs. 2 and 4), presumably because of the strong steric repulsion (Ozturk et al., 2015). In summary, our results suggest that both kinds of polysaccharide-coated lipid droplets were relatively stable to aggregation under gastric conditions, which is quite different to lipid droplets coated by food-grade proteins (de Figueiredo Furtado, Silva, de Andrade, & Cunha, 2018; Winuprasith et al., 2018), which tend to flocculate.

3.2.3. Small intestinal stage

Compared to the stomach stage, there were major changes in the size, charge, and microstructures of the CNC-stabilized Pickering emulsions after the small intestinal stage (Figs. 2–4). An increase in the mean droplet size was noted, especially for the emulsions prepared at low CNC concentrations (Fig. 2), which suggests that aggregation occurred after digestion. It should be noted that compared to that with 0.1 wt% CNC, a larger increase in droplet size occurred for the samples with 0.2 wt% CNC. This is likely caused by the more limited surface area when entering the small intestinal stage, which leads to a stronger interaction/reaction with digesting components that alter the droplet morphology. The \( \zeta \)-potential of the oil droplets in the emulsions became strongly negative again (Fig. 3), which besides the presence of CNC, is due to various anionic substances, including free fatty acids and bile acids. Meanwhile, the small intestine contains a variety of anionic species, which dominate the overall \( \zeta \)-potential of the system, thereby promoting negative electrostatic charges. The confocal images suggested that the emulsions contained relatively large lipid-rich droplets (Fig. 4), which may be due to the fusion of undigested oil droplets, the formation of large, aggregated vesicles, the role of CNC and micelles as well as calcium soaps and associated structures. Interestingly, the size of these lipid-rich particles appeared to be smaller in the emulsions containing the highest CNC levels (Figs. 2 and 4). The CNC-stabilized emulsions appeared to contain more spherical lipid-rich droplets after digestion, which was also confirmed by fluorescent microscopy (Fig. 5), whereas the GA-stabilized ones contained more diffuse, irregularly-shaped particles (Fig. 4). These spherical particles may have been undigested oil droplets whereas the irregular particles may have been mixed micelles or calcium soaps, indicating a high potential of CNC-stabilized Pickering emulsions to interfere lipid digestion.

3.3. Lipid digestibility in Pickering emulsions

The hydrolysis of lipids by pancreatic lipase generates free fatty acids, monoacylglycerols, and hydrogen ions (H\(^+\)). Consequently, the extent of lipid digestion can be followed by recording the volume of NaOH required to maintain the pH at neutral values during digestion (DeLoid et al., 2018). The lipid digestion profiles of various Pickering emulsions measured using the pH-stat method are shown in Fig. 6, and the initial rate and final extent of FFAs released are presented in Fig. 7. The initial rate of FFAs released was estimated by fitting a linear profile to the data during the first 20 min of digestion, while the final digestion extent was calculated from the average of the last 5 min of digestion.
For all emulsions, the lipid digestion profiles were fairly similar: rapid generation of FFAs throughout the first 20 min followed by a slower evolution at later times (Figs. 6 and 7a). Nevertheless, there were differences between the digestion profiles depending on stabilizer type and level. The emulsions were nearly fully digested in the GA-stabilized emulsions, which was attributed that the polysaccharide molecules were completely displaced from the lipid droplet surfaces by the bile salts, thereby allowing the lipase to directly adsorb and hydrolyze the triglycerides (Fig. 4). This result demonstrates the poor performance of conventional food-grade emulsifiers (even for high-molecular biopolymer) at inhibiting lipid digestion.

In contrast, for the CNC-stabilized Pickering emulsions (Fig. 6), the initial rate of lipid digestion was slower than that for the GA-stabilized emulsions, especially at the lowest and highest CNC levels used (Fig. 7a). Moreover, the final level of FFAs generated by the end of the small intestinal phase was also smallest at the lowest and highest CNC concentrations (Fig. 7b). It can be speculated that the relatively slow rate of lipid digestion at the lowest CNC level was caused by the relatively large size (low specific surface area) of the oil droplets in these emulsions. Moreover, the increase of the droplet size after digestion was the oil droplets (Bai, Lv, et al., 2019), thereby restricting access of the lipase molecules to the lipids. We should warn on the limitations of relying on droplet sizing to explain the results. Keeping this in mind, however, our results related to lipid digestion compare to those of other food-grade nanocrystals, e.g., chitin nanocrystals (Tzoumaki et al., 2011; Tzoumaki et al., 2013) and starch crystals (Jo, Ban, Goh, & Choi, 2018).

Several other physicochemical phenomena may account for the relatively slow rate of lipid digestion in Pickering emulsions containing high levels of CNC (Fig. 8). The irreversibly strong adsorption of CNCs to the oil droplet surfaces may have inhibited the adsorption of bile salts and lipases. Moreover, at sufficiently high levels, CNC formed a densely packed coating that restricted the ability of lipases to penetrate through and access the lipids. This hypothesis is supported by the fluorescent microscopy images, which show a thick layer of CNC around the oil droplets (see the blue contour and domains surrounding the oil droplets, Fig. 5c). This result also demonstrated that the indigestible CNC could sustain the attack from enzymes in the small intestine (Qin, Yang, Gao, Yao, & McClements, 2017; Sarkar, Li, et al., 2018; Sarkar et al., 2017), avoiding the direct rupture of lipid droplets. Furthermore, since all the emulsions remained fairly the same when entering into small intestinal stage, the flocculation and coalescence of the oil droplets in the small intestine (Fig. 4) may also have reduced the surface area of lipids available for the lipases to act on (Fig. 8), which further suppressed their lipolysis function. Previous studies have shown that there exists uncovered areas in CNC-coated interfaces due to the non-aligned interfacial adsorption of these thin nanorods (Fig. 8a) (Kalashnikova, Bizot, Bertoncini, Cathala, & Capron, 2013; Kalashnikova, Bizot, Cathala, & Capron, 2011). These unprotected areas are usually large enough to allow the access of nanometer-sized bile salts and lipase molecules to reach the lipid surface (Ruiz-Rodriguez, Meshulam, & Lesmes, 2014). This phenomenon would account for the rapid onset of lipid digestion observed immediately after the Pickering emulsions were exposed to the small intestinal environment (Figs. 6 and 7a). Increasing CNC concentrations may decrease the dimensions and surface area occupied of these gaps (Bai, Ly, et al., 2019), which thereby resulted in the slower digestion rate observed at higher CNC loadings (Figs. 6 and 7). It seems, however, that under the conditions used in this study it was not possible to completely block the gaps in the CNC coatings, since some lipid digestion did occur in all systems.

The presence of the interfacial gaps might also have partially inhibited the removal of the FFAs generated at the oil droplet surfaces during lipid digestion, particularly with less assistance from bile salts, owing to the accumulation effect of FFAs at limited surface area (Ruiz-Rodriguez et al., 2014). Furthermore, CNC may interact with the bile acids through hydrogen bonding and other interactions, which reduce the transport of bile salts that otherwise remove FFAs. Removal of FFAs by incorporation into either bile salts or calcium soaps is known to be important for ensuring that the lipid digestion reaction proceeds.

**Fig. 6.** Percent of total available free fatty acids (FFAs) released as a function of time during simulated small intestinal digestion of CNC-stabilized corn oil-in-water Pickering emulsions. The emulsions were stabilized by different levels of CNC or by 0.75 wt% gum arabic (reference).

**Fig. 7.** (a) Initial and (b) total percentage of FFAs released of CNC-stabilized corn oil-in-water Pickering emulsions in the simulated small intestinal digestion. The emulsions were stabilized by different levels of CNC or by 0.75 wt% gum arabic (reference). The different lowercase letters (a to d) indicate significant differences (p < 0.05) in the percentage of FFAs released.
The negatively-charged CNC may have bound free calcium ions, especially at higher CNC levels (Fig. 5c), which reduced their ability to remove the FFAs from the oil droplet surfaces, thereby inhibiting further digestion (Winuprasith et al., 2018).

In contrast to the long, flexible cellulose nanofibrils (CNF), a three-dimensional gel network in the continuous phase is not expected for CNC, owing to the limited entanglement between the free particles in aqueous phase (Bai, Huan, et al., 2018). This conclusion has been proved in the shear viscosity measurements made on the initial Pickering emulsions at various CNC concentrations (Bai, Lv, et al., 2019), showing that the presence of even high levels of CNC did not cause a large increase in viscosity. On the other hand, during the lipid digestion, the CNC-stabilized emulsions were highly diluted compared to the original samples, which significantly lowered the apparent viscosity of the samples. Furthermore, the fact that rapid creaming occurred in the Pickering emulsions after exposure to the stomach phase indicated that they were still fluid-like in the small intestinal stage (Fig. 9). Taken together, these results suggest that the ability of CNC to enhance the rheological properties of Pickering emulsions that otherwise restrict the contact of lipase molecules to the oil droplet surfaces was not an important factor. This effect is quite different from that reported in recent studies that have shown the inhibition of lipid digestion by CNF network formed in continuous phase (DeLoid et al., 2018; Winuprasith et al., 2018). Thus, the ability of CNC to inhibit lipid digestion in our study is primarily due to the formation of a less permeable coating around the oil droplets, which demonstrated the function of Pickering stabilization. Such effects are important in addressing human health, including obesity but also need to be considered when the bioavailability of lipophilic compounds (vitamins or fat-soluble bioactives) is a factor.

4. Conclusions

We have shown that Pickering emulsions containing CNC-coated lipid droplets can be produced using a microfluidizer. The rate and extent of lipid digestion in these emulsions could be modulated by the CNC loading in the emulsion formulation. At low CNC levels, the rate of lipid digestion was slow because relatively large lipid droplets were produced during homogenization. Conversely, at high CNC levels, the lipid digestion rate was slow because of the formation of a thick, less permeable CNC barrier at the oil droplet surfaces. The degree of lipid digestion after the small intestine phase could be reduced by as much as 40% at relatively high CNC levels. Three main mechanisms are proposed for this effect: (1) the irreversible adsorption of CNC to the oil droplet surfaces limited bile salt displacement and lipase adsorption; (2) the coalescence and flocculation of the oil droplets in the small intestine reduced the surface area of lipids available for bile salt and lipase binding; and (3) the accumulation of free fatty acids at the oil droplet surfaces inhibited lipase activity. Nevertheless, lipid digestion could occur in all of the emulsions, which suggested that the adsorbed CNC layer was somewhat permeable to lipase molecules to access the emulsified oil.

Our results suggest that CNC can be used as a particle stabilizer to coat oil droplets in food-grade Pickering emulsions and inhibit their digestion in GIT. These Pickering emulsions may be useful for developing functional foods that promote satiety, thereby inhibiting overeating. Alternatively, they could also be used to prolong the release of bioactive agents or to release them further down the GIT. The long-term goal of our research is to use nanocellulose to create value-added foods.
that can assist in weight management and promote human health under the concept of “wood-for-food”.

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