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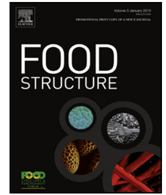

Destabilization of multilayered interfaces in digestive conditions limits their ability to prevent lipolysis in emulsions

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ABSTRACT

Delivery of lipid fractions in the lower small intestine can induce feelings of satiety, but is only possible when lipids escape the highly efficient lipolysis and adsorption in the upper gastrointestinal (GI) tract. Our objective was to gain insight in the stability of multilayered interfaces in simulated GI conditions, and their suitability for intestinal delivery of undigested lipids. Oil-in-water emulsions ($d_{32}\sim$5–30 μm; one-to five-layered interfaces) were produced by sequentially adsorbing biopolymers with opposite charges at pH 3.0: whey protein isolate (WPI) (cationic), pectin (anionic), chitosan (cationic). Corresponding multilayered structures were characterized using reflectometry. Influence of layer composition and thickness on its protectiveness against lipolysis of emulsions was studied in simulated GI conditions.

Multilayered WPI/pectin emulsions had an improved physical stability compared to WPI-stabilized emulsions, during both storage and *in vitro* gastric incubation, whereas chitosan-containing emulsions were physically unstable. Reflectometry and CLSM results showed that a greater number of layers increased the adsorbed amount, forming a mesoscopically heterogeneous structure. Under simulated intestinal conditions, however, outer layers instantaneously destabilized. Accordingly, similar initial lipolysis rates were recorded for all emulsions. Yet, compared to only WPI the final extent of lipolysis was lowered by addition of a second and a third layer under mild *in vitro* conditions. This moderate protective effect disappeared when harsher digestive conditions were applied.

From this work, it became clear why multilayered interfaces (initially built under acidic pH) can improve gastric stability of emulsions, but are prone to disintegration under intestinal conditions. This knowledge is important for designing food systems that control release of lipolytic products in targeted locations of the GI tract; the emulsions reported here are expected to be suitable for duodenal release.
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1. Introduction

A key strategy to control the worldwide expansion of obesity prevalence is by large-scale application of dedicated dietary interventions. These may target, for example, induction of feelings of satiety, thus leading to reduced food intake. Several gastrointestinal processes are involved in food intake regulation, such as gastric distension, and the sensing of nutrients and their digestion products. Gastric distension triggers mechanosensor-mediated signals and suppresses the release of the stomach hormone ghrelin. The presence of macronutrients in the small intestine induces the release of several gut peptides that are known to be associated with food intake, such as cholecystokinin (CCK), peptide YY (PYY), and glucagon-like peptide-1 (GLP-1) (van Avesaat, Troost, Ripken, Hendriks, & Masclee, 2015). Especially the presence of nutrients in more distal parts of the small intestine induces the release of gut peptide hormones that control feelings of satiety and hunger in humans, hence reducing food intake (Alleleyn, van Avesaat, Troost, & Masclee, 2016; Maljaars, Peters, Mela, & Masclee,

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2008). This mechanism is referred to as 'the ileal brake'. For instance, ileal infusion of safflower oil, rich in linoleic acid, has been found to reduce hunger efficiently (Maljaars, Romeyn, Haddeman, Peters, & Masclee, 2009).

In practice, it is difficult to control lipolysis because the human gastrointestinal tract has evolved towards efficient food digestion, through a range of processes that allow optimized lipolysis and absorption of digestion products (Bakala N'Goma, Amara, Dridi, Jannin, & Carrière, 2012). Lipase and bile salts play a major role in this respect. Lipolysis takes place at the surface of lipid droplets, where lipase encounters the lipid substrate (triacylglycerols). At this interface, bile salts play multiple important roles that promote lipolysis: stabilizing new interfacial area, displacing surface-active molecules from the oil-water interface *via* competitive adsorption, facilitating lipase adsorption at the interface (Maldonado-Valderrama, Wilde, Macierzanka, & Mackie, 2011). Moreover, unadsorbed bile salts play an even more important role in the aqueous phase, to solubilize digestion products and, hence, prevent enzyme inhibition (Sarkar, Ye, & Singh, 2016).

The availability of the lipid substrate for lipase is determined not only by the amount of oil-water interfacial area, but also by the interfacial structure. For example, galactolipids (Chu et al., 2009) and Pluronics (Wulff-pérez, Vicente, Martín-rodríguez, & Gálvez-ruiz, 2012) have been shown to provide a physical barrier against lipolysis in emulsions, *via* steric hindrance. Yet, this is an exception to the general trend that most emulsifiers that conventionally stabilize food emulsions (low molecular-weight emulsifiers and amphiphilic biopolymers) only have a minor influence on lipolysis (Golding et al., 2011; Hur, Decker, & McClements, 2009). Recently, specific Pickering particle-stabilized interfaces have been shown to delay lipolysis to a certain extent (Sarkar, Murray et al., 2016; Tzoumaki, Moschakis, Scholten, & Biliaderis, 2013), but to truly control lipolysis, more protective interfacial structures are required.

A more compact or thicker interfacial structure may be more protective against lipolysis, and can be achieved by, *e.g.*, combining more than one material. Multilayered interfaces can be produced through layer-by-layer adsorption of oppositely charged biopolymers and is based on electrostatic attraction, with the electrostatic charge alternating after each added layer due to charge overcompensation (Schönhoff, 2003). For example, whey proteins, casein, gum Arabic, modified starch, gelatin, carrageenan, pectin and chitosan have been used previously for that purpose (Dickinson, 2011; Guzey & McClements, 2006). Whey proteins and pectin have often been combined to create multilayered interfaces (Mao and Miao, 2013; Wackerbarth, Schön, & Bindrich, 2009; Zhang and Zhong, 2015), and layers close to the oil-water interface have been suggested to become strongly intermingled due to the attraction between oppositely charged molecules, causing a dense packing (Wackerbarth et al., 2009). In addition, a greater number of layers would increase the distance over which functional ingredients, such as fatty acids and vitamins, need to be transported across the interface, which may also lead to additional protection against digestion (Wackerbarth et al., 2009). A nice example of mechanically strong multilayered capsules can be found in the work of Rossier-Miranda and co-workers (Rossier-Miranda, Schroën, & Boom, 2010), who combined pectin with whey protein fibrils to fortify the interfacial shell. These capsules were physically stable at low pH (pH 2.0–3.5), while they disintegrated more rapidly at high pH (pH 7.0), because of the loss of charge interactions.

Multilayered emulsions have already been studied in relation to lipolysis, but most studies found only a minor influence of additional layers on *in vitro* lipolysis (Corstens et al., in press). It is difficult to compare the results published so far, notably because of the broad range of experimental conditions used. Some studies found that a chitosan outer layer delayed lipolysis of emulsions (Klinkesorn and McClements, 2010; Mun, Decker, Park, Weiss, & McClements, 2006), and this was attributed to the formation of a thick cationic layer restricting lipase adsorption, although this was not systematically confirmed (Li and McClements, 2014). In some studies, adsorbing a second layer facilitated lipolysis (Lesmes, Baudot, & McClements, 2010; Tokle, Mao, & McClements, 2013), which was attributed to enhanced removal of additional layers from the surface while passing through different stages of the gastrointestinal tract, compared with single layer material. Conversely, other studies found that a second layer formed a more protective interface structure compared with primary emulsions, resulting in delayed lipolysis (Hu, Li, Decker, Xiao, & McClements, 2011; Klinkesorn and McClements, 2010; Li et al., 2010; Mun et al., 2006). Hence, it was not possible to draw consistent conclusions from available studies on a potential protective effect of multilayered emulsions with respect to lipolysis.

To elucidate the underlying mechanisms, we systematically investigated the structure of multilayered interfaces in emulsions and on model surfaces, and the *in vitro* gastric resistance and intestinal stability against lipolysis of emulsified dietary lipids. Oil-in-water (O/W) emulsions were produced with a different layer composition and thickness, using whey proteins in combination with pectin, and in combination with chitosan. We performed a detailed characterisation of the interfacial structure using, amongst others, confocal fluorescence microscopy and reflectometry under digestion-relevant conditions. *In vitro* lipolysis studies were performed under various conditions and we were able to relate these results to the interfacial composition and thickness.

2. Materials and methods

2.1. Materials

Safflower oil was purchased from De Wit Specialty oils (19200 Safflower Oil High Linoleic Refined, The Netherlands). Three biopolymers were used: whey protein isolate (WPI) (BiPro, Davisco Food International, Eden Prairie, Minnesota, USA; purity 97.5%), pectin from citrus peel (≥74% galacturonic acid, ≥6.7% methoxy groups, Sigma Aldrich, St. Louis, MO, USA) and low molecular weight chitosan (75–85% deacetylated, Sigma Aldrich, St. Louis, MO, USA). From Sigma Aldrich (St. Louis, MO, USA) we also purchased: citric acid, calcium chloride, sodium citrate dihydrate, sodium hydroxide, sodium chloride, sodium phosphate dibasic, sodium phosphate monobasic, fluorescein isothiocynate isomer I (FITC), 4-morpholineethanesulfonic acid monohydrate, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC), *N*-hydroxysulfosuccinimide sodium salt (Sulfo-NHS), hexamethyldisilazane (HMDS), toluene, hexane, 2-propanol, pepsin from porcine gastric mucosa, lipase from porcine pancreas, pancreatin from porcine pancreas (8× USP specification; including trypsin, amylase, lipase, ribonuclease, protease), porcine bile extract (crude extract, purity estimated to be 30–60%, containing glycine and taurine conjugates of hyodeoxycholic acid and other bile salts according to the supplier) and phenolphthalein reagent. Enzyme activities were measured according to Minekus et al. (2014), and found to be 48 U/mg for lipase from porcine pancreas, 41 U/mg for pancreatin from porcine pancreas, and >400 U/mg for the used pepsin. Ethanol (absolute, for analysis) was purchased from Merck (Amsterdam, The Netherlands). All materials were used directly without further purification. Ultrapure water obtained from a Millipore Milli-Q system (Darmstadt, Germany) was used throughout the study.

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