Food Hydrocolloids 54 (2016) 255–265

Contents lists available at ScienceDirect

Food Hydrocolloids

journal homepage: www.elsevier.com/locate/foodhyd

# Impact of colloidal structure of gastric digesta on *in-vitro* intestinal digestion of whey protein emulsion gels

Qing Guo [a], Aiqian Ye [a, *], Mita Lad [a, 1], Douglas Dalgleish [b], Harjinder Singh [a]

[a] Riddet Institute, Massey University, Palmerston North, New Zealand
[b] Department of Food Science, University of Guelph, Guelph, Ontario, Canada

ARTICLE INFO

*Article history:*
Received 29 July 2015
Received in revised form
5 October 2015
Accepted 8 October 2015
Available online 17 October 2015

*Keywords:*
Emulsion gel
Food digestion
3D polymer network
Lipid regulation

ABSTRACT

Two whey protein emulsion gels containing 10 mM NaCl (soft gel) or 200 mM NaCl (hard gel) were formed and subjected to sequential digestion, including an *in vitro* gastric step followed by an *in vitro* intestinal step. The breakdown of gel particles, stability of oil droplets, microstructure and free fatty acid release during digestion were examined. The results showed that after 60 and 240 min gastric digestion the digesta of both gels had different physicochemical forms. The 60 min gastric digesta of both gels was mainly composed of gel particles ranging from ~1 to ~1000 μm. The 240 min gastric digesta of the soft gel mainly consisted of individual oil droplets and small gel particles (~10 μm) while that of the hard gel mainly consisted of small gel particles (~10 μm). During intestinal digestion, the breakup and coalescence of oil droplets occurred simultaneously for all gastric digesta. The remaining network structure of gel particles hindered their breakdown especially in the hard gel during intestinal digestion, which delayed lipid digestion significantly. Lipolytic products appeared to accumulate around oil droplet surfaces. Needle-shaped crystals that may be composed of free fatty acids were also found during intestinal digestion.



## 1. Introduction

The intestine is the major location for lipid digestion, although a lingual lipase (secreted by salivary glands) and gastric lipase in the stomach can digest as much as 30% of dietary triglyceride (Ganong & Barrett, 2005). The pancreatic lipase of the intestine prefers to hydrolyse the Sn1 and Sn3 positions of triglycerides to give monoglycerides and the corresponding free fatty acids (Carriere, Barrowman, Verger, & Laugier, 1993).

Lipid absorption occurs in the small intestine with the assistance of micelles and vesicles. These micellar phases are constituted of bile salts and biliary phospholipids which can capture lipolytic products from oil droplet surfaces and transport these products to the enterocyte cells that line the gut wall (Maldonado-Valderrama, Wilde, Macierzanka, & Mackie, 2011). The absorption of lipid is completed in the proximal 100 cm or more of the jejunum, and is faster than that of carbohydrate and protein. In general, the human body has an excess capacity for lipid digestion and absorption (Borgström, Dahlqvist, Lundh, & Sjövall, 1957). Overconsumption of lipid is thought as a leading contributor to obesity which relates to cardiovascular disease and diabetes. Controlling lipid digestion could lead to decrease energy intake by enhancing satiety or suppressing appetite responses.

One of main mechanisms that has been used to regulate lipid digestion is the manipulation of interfacial composition, which has been expected to alter the access of bile salts and digestive enzymes onto the emulsion surfaces (Golding & Wooster, 2010; Singh & Ye, 2013). However, this effect via using different surfactants (non-ionic or ionic), building multi-layers or increasing crosslinking in adsorbed layers is not significant (Day et al., 2014; Golding et al., 2011; Malaki Nik, Wright, & Corredig, 2011; Pinheiro et al., 2013; Qiu, Zhao, Decker, & McClements, 2015; Sandra, Decker, & McClements, 2008; Zeeb, Weiss, & McClements, 2015). In general, the stability of emulsions (coalescence, creaming and phase separation) within the gastrointestinal tract plays a more important role in determining the rate and extent of lipid digestion than the initial interfacial layer properties (Li, Ye, Lee, & Singh, 2012, 2013; Wooster et al., 2014).

* Corresponding author. Riddet Institute, Massey University, Private Bag 11 222, Palmerston North, 4442, New Zealand.
*E-mail address:* A.M.Ye@massey.ac.nz (A. Ye).
[1] Current address: Fonterra Research and Development Center, Palmerston North, New Zealand.

http://dx.doi.org/10.1016/j.foodhyd.2015.10.006
0268-005X/

Recently, the digestion of oil droplets incorporated in a semi-solid or solid food matrix has received some attention. Wooster et al. (2014) designed a series of emulsion-filled gels by incorporating caseinate/monoglyceride (CasMag) stabilized emulsions into a thermally reversible gelatine network (which melts in the stomach), a colloidal casein network (yoghurt) and a concentrated starch particulate dispersion. The impact of the different biopolymer networks on lipid digestion was investigated. The authors found that yoghurt and concentrated starch dispersions effectively protected oil droplets against coalescence during *in vitro* gastric digestion. However, these networks did not slow down lipid digestion compared to the reference emulsion during *in vitro* intestinal digestion. The rate of lipid digestion was lower for the CasMag emulsion and the emulsion-filled gelatine gel mainly because of coalescence of oil droplets during gastric digestion. A similar effect was observed *in vivo* by measuring changes in triglyceride concentration. Lamothe, Corbeil, Turgeon, and Britten (2012) investigated the kinetics of matrix degradation and fatty acid release for different cheeses (mild Cheddar, aged Cheddar, light Cheddar and Mozzarella) in the simulated gastrointestinal tract. They found that Mozzarella was the most rapidly digested and underwent complete digestion within 180 min. This was attributed to a more porous protein matrix and lower firmness and cohesiveness than the other cheeses. The extent of matrix disintegration of mild Cheddar was significantly lower than that of other cheeses because of its elastic, cohesive and firm structure. This also led to a lower extent of lipid hydrolysis. Therefore, tuning the structural characteristics of food matrices provides a potential way to control lipid digestion.

In our previous studies, we designed a series of whey protein emulsion gels with different structures and hardness. The breakdown properties of gels in the human mouth and mechanical/biochemical breakdown of gel boluses in the human gastric simulator were investigated (Guo, Ye, Lad, Dalgleish, & Singh, 2013, 2014). We found that the fragmentation degree of gels increased linearly with gel hardness, with the soft gel reaching a larger threshold size for swallowing than the hard gel. Furthermore, the hard gel that appeared to be formed by crosslinking of thick protein-coated oil droplets could prevent oil droplets release from the gel network during *in vitro* gastric digestion. By contrast, the soft gel with oil droplets incorporated in a fine-stranded protein matrix released large quantities of oil droplets. The breakdown experienced in the mouth and stomach permanently change the physical and chemical form of gels, and will largely influence their digestion in the intestine.

In the present study, *in vitro* oral processing and gastric digestion of whey protein emulsion gels were carried out first for preparing the samples for simulated intestinal digestion. The behaviour of whey protein emulsion gel in an *in vitro* intestinal model was investigated, with a focus on the effect of colloidal structures of gastric digesta (or chyme) on lipid digestion.

## 2. Materials and methods

### 2.1. Materials

Whey protein isolate with a protein content of >90% was purchased from Fonterra Co-operative Group Limited, Auckland, New Zealand. Soybean oil was purchased from the Davis Trading Company, Palmerston North, New Zealand. The activity of the pepsin (Merck, Darmstadt, Germany) was 0.7 FIP units/mg. Bile extract porcine (complex mixtures of bile acids and bile acid conjugates), pancreatin (1 USP) and amano Lipase A from *Aspergillus niger* (≥12 000 U/g) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Milli-Q water (Millipore Corporation, Bedford, MA, USA) was used for all experiments. HCl (37%) was purchased from EMD Millipore Corporation (Billerica, MA, USA). All other chemicals were analytical grade. Artificial saliva and simulated gastric fluid (SGF) (pH 1.5, 3 g/L pepsin, 0.5 g/L amano lipase A and 150 mM/L NaCl) were prepared, respectively (Guo, Ye, Lad, Dalgleish, & Singh, 2014). Simulated intestinal fluid (SIF) containing 150 mM/L NaCl, 8 mM/L KCl, 10 mM/L $CaCl_2$, 5 mg/L bile extract and 6.4 mg/L pancreatin was prepared according to Sarkar, Horne, and Singh (2010b) and Ye, Cui, and Singh (2010) with some modifications.

### 2.2. Emulsion preparation

Pre-emulsions containing 10 wt% WPI and 20 wt% soybean oil were prepared using a high-speed mechanical mixer (L5M, Silverson, Massachusetts, USA) at 9000 rpm for 3 min. These pre-emulsions were then homogenized using 4 passes through a two-stage valve homogenizer (APV 2000, Albertslund, Denmark) operated at 300 and 25 bars for the first-stage and the second-stage, respectively (Ye & Taylor, 2009). Particle size of emulsions was ~0.45 μm. The stock emulsions were stored at 4 °C until further use.

### 2.3. Emulsion gel preparation

The required quantities of solid NaCl were added to the emulsion solutions to give final concentrations of 10 and 200 mM. The gels containing 10 and 200 mM NaCl were called the soft and hard gels, respectively, according to gel hardness (Guo et al., 2013). The solutions were gently stirred to allow the NaCl to completely dissolve. The emulsions were then put in sealed plastic cylindrical containers (inner diameter: 25 mm) and were heated in a water bath from 30 to 90 °C at a rate of 3 °C/min and held at 90 °C for 30 min. After heating, the gels were cooled at 4 °C until further use.

### 2.4. Consecutive digestion in the simulated gastrointestinal tract

The consecutive digestion steps in the upper gastrointestinal tract were simulated (Fig. 1). The gel bolus and gastric digesta at different digestion times were put into the next digestion step after *in vitro* oral processing and *in vitro* gastric digestion, respectively.

#### 2.4.1. In vitro oral and gastric processing

*In vitro* oral processing followed the procedures described by Guo et al. (2013). A mechanical grinder (MultiGrinder II EM0405, Sunbeam, Australia) was used to simulate oral breakdown and to create simulated boluses with similar size distributions to human gel boluses (~4.0 and 0.9 mm for the soft and hard gels respectively) (Guo et al., 2013). Simulated boluses of the two types of gel were prepared by mixing 200 g of ground gel and 40 mL of artificial saliva and warming at 37 °C for 2 min. *In vitro* gastric digestion of whey protein emulsion gel was carried out in a dynamic gastric model-human gastric simulator (HGS). The apparatus of the HGS and digestion procedures have been described in a previous paper (Guo et al., 2014). Briefly, the simulated gel bolus (200 g mixed with 40 mL of artificial saliva) was fed to the HGS. Before digestion, 70 mL of SGF containing pepsin was loaded to mimic the fasting condition of the human stomach. Then digestion was started immediately. The inflow rate of the SGF was 2.5 mL/min. Because of the biphasic nature of gastric emptying of solid food, the digesta began to be emptied out from 30 min (Collins, Horowitz, Cook, Harding, & Shearman, 1983; Siegel et al., 1988). For accurate control of the gastric emptying, the digesta were removed from the bottom of the HGS at 45 mL/15 min, equalling the gastric emptying rate of 3.0 mL/min. The contraction frequency was 3 times/min, simulating the actual stomach peristalsis. The temperature of the HGS was maintained at 37 °C by a heater and thermostat. The