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Lipase diffusion in oil-filled, alginate micro- and macrobeads

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ABSTRACT

Triglycerides, which are broken down in the lower part of the intestinal tract, give a stronger ileal brake feedback, resulting in a feeling of satiety and causing people to eat less. The digestion of triglycerides into fatty acids by lipase in the intestine can be delayed by encapsulating oil droplets. In this study the release of fatty acids and oil droplet breakdown in a simulated intestinal system was investigated, for oil droplets encapsulated in alginate micro- (10.7 μm) and macrobeads (1.77 mm). It was found that fatty acid release rate was greatly decreased by encapsulating the oil droplets into an alginate matrix compared to loose droplets. Microscopic imaging of the breakdown of the oil droplets showed a sharp front moving from the bead interface to the centre of the bead, and the change in position of the front scaled linear with time. The motion of the front is well described by combining the mass balance for lipase with a Maxwell-Cattaneo type equation, for the mass flux vector. The front in microbeads seemed to move slightly slower (0.15 (± 0.04) μm per minute) than for the macrobeads (0.20 (± 0.02) μm per minute). The release of free fatty acids in microbeads was faster than in macrobeads, despite the slower front movement, because of the larger amount of surface area available.

1. Introduction

Lipids which are digested in the ileum of the intestinal tract induce a negative feedback mechanism, which increases feelings of satiety, causing people to eat less (Alleleyn, van Avesaat, Troost, & Masclee, 2016; Maljaars et al., 2011; Spiller et al., 1984; Welch, Saunders, & Read, 1985). This effect is called the ileal brake, and is stronger the further along in the intestinal tract the lipids are digested (Welch et al., 1985). Forcing lipids to be digested further down the intestinal tract might have an application in the treatment or prevention of obesity. Lipase is an enzyme, which breaks down triglycerides into glycerol and free fatty acids (FFA), and is active at the oil-water interface. Therefore most systems intending to delay lipid digestion are based on decreasing the accessibility of lipase to the triglyceride interface. One method to slow the diffusion of lipase to the oil-water interface is by encapsulating oil droplets into a hydrogel bead, where the porous gel acts as a barrier between the lipase and the oil droplets (Corstens et al., 2017; Li, Hu, Du, Xiao, & McClements, 2011). Because the lipase first has to diffuse through the matrix before it reaches the oil droplet interface, fatty acid release can be slowed down until the ileum is reached.

Alginate is a polysaccharide that has often been used for medical purposes, including encapsulation of cells and drugs (Covarrubias, de-Bashan, Moreno, & Bashan, 2012; Lee & Heo, 2000; Lim & Sun, 1980; J.; Liu et al., 2010; Pasparakis & Bouropoulos, 2006; Sultana et al., 2000). It is a non-toxic and biodegradable polymer which gels in the presence of divalent cations. The method of gelation is very gentle, and thus appropriate for compounds sensitive to heat or strong chemicals. Paques et al. developed a water-in-oil emulsification method that is capable of creating beads of approximately 10 μm (Paques, van der Linden, Sagis, & van Rijn, 2012; van Leusden et al., 2017). Because particles smaller than 25 μm do not negatively affect sensorial aspects of food, these microbeads can be added without affecting sensorial perception (Tyle, 1993).

The factors that influence the rate of diffusion of components in gels include: gel type (X. C. Liu et al., 2002; Martinsen, Storrø, & Skjåk-Bræk, 1992) and density (Corstens et al., 2017; Martinsen et al., 1992)), the diffusing component's size (Stewart & Swaisgood, 1993), shape (Pluen, Netti, Jain, & Berk, 1999), and charge (Huguet & Dellacherie, 1996; Stewart & Swaisgood, 1993), and environmental conditions such as pH (Huguet & Dellacherie, 1996) and ionic strength (Huguet & Dellacherie, 1996; Stewart & Swaisgood, 1993; van Leusden et al., 2017). In diffusion in hydrogel beads, also the size of the beads is

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important (Corstens et al., 2017; Li et al., 2011).

When oil is encapsulated in alginate beads for the delayed release of fatty acids, one cannot change the component (lipase) or environment, because those are naturally present in the human biological system. We can however, control the gel properties. Li et al. showed that the digestion of oil droplets encapsulated in alginate beads decreased with increasing bead size (0.8–3.4 mm), and increased degree of cross-linking with calcium (Li et al., 2011). Corstens et al. also showed that digestion of oil droplets encapsulated in alginate beads decreased with increasing bead size (0.55–1.15 mm), and with increasing alginate concentration, which influences the mesh size of the alginate gel (Corstens et al., 2017).

Encapsulating oil droplets in an indigestible hydrogel matrix delays the release of fatty acids in the intestinal tract. The rate of the digestion is entirely dependent on the rate with which lipase is able to diffuse into the bead and reach the oil droplets. In this study we investigated the diffusion of lipase in gelled alginate beads. We investigated the influence of size in a wider range than previously reported: macrobeads of 1.77 mm vs. microbeads of 10.7 μm. We determined the rate of free fatty acid release, for both macro and microbeads, and used microscopic imaging to track the breakdown of oil droplets as a function of time. We then model this process by combining the mass balance for diffusion of lipase in the bead with a Maxwell-Cattaneo type equation for the mass flux vector.

2. Materials and methods

2.1. Materials

$CaCl_2.2H_2O$, ethanol absolute, NaOH, NaCl (Merck millipore, Darmstadt, Germany), Tween 60, Lipase from porcine pancreas (Sigma, Steinheim, Germany), Polyglycerol polyricinoleate (PGPR) 90 Kosher (Danisco, Kopenhagen, Denmark), Medium Chain Triglycerides (MCT) (Miglyol 812 N, Sasol, Germany), and Sodium alginate extracted from brown algae (Algin, Texturas, Barcelona, Spain), were all used as received. Solutions were made in demineralized water.

2.2. Production of calcium crystals

Calcium crystals were made according to the method of Paques et al. (Paques, van der Linden, van Rijn, & Sagis, 2012). In short: A dispersion was made of 6% PGPR in MCT oil and allowed to mix for 2 h. A volume of 5% of a 0.1 molal $CaCl_2.2H_2O$ solution in ethanol was added to this MCT solution and emulsified (Sonicator S-250A sonicator, Branson Ultrasonics, USA) for 2 min. The resulting mixture was heated and stirred overnight at 60 °C without cover to allow the ethanol to evaporate, producing a dispersion of calcium crystals in oil.

2.3. Production of the microbeads

Oil filled alginate microbeads were made based on the method described by Van Leusden et al. (van Leusden et al., 2017). The inner oil droplets were made by mixing 5.0% (w/w) MCT with demi-water containing 0.3% (w/w) Tween 60 with an Ultra-Turrax (Ultra-Turrax T 25, IKA Werke, Germany) at 8000 rpm for 2 min. The emulsion was further homogenised (Delta instruments, Drachten, The Netherlands) at 180 bar for 3 passes. To the emulsion 2.0% (w/w) alginate was added and allowed to dissolve for 2 h. While mixing with the Ultra-Turrax at 8800 rpm, 10% (v/v) of the previous emulsion was slowly added to MCT oil containing 4% (w/w) PGPR. After full addition the double emulsion was mixed for a further 3 min. After mixing, 5 mL of calcium crystal dispersion was added per mL of primary emulsion. This mixture was gently stirred for at least 18 h to allow gelation of the beads.

The beads were removed from the oil phase by successive centrifugation and redispersion steps. The beads were centrifuged for 2 h at 3500 g. The pellet was redispersed in 25 mM $CaCl_2$ solution, homogenised at 100 bar for 3 passes and then once again centrifuged at 1000 g for 1 h. The sediment was redispersed in demi-water, after which the centrifugation was repeated. The highly concentrated beads were stored in demi-water and diluted before use.

2.4. Production of the macrobeads

Oil filled alginate macrobeads were made based on the method described by (van Leusden et al., 2017). The inner oil droplets with alginate were made as described in the section of the production of microbeads. The emulsion was put in a syringe and expressed through a needle of 0.3 mm (BD Microlance 0.3 × 13 mm) into a 25 mM $CaCl_2$ water bath, where the droplets were formed approximately 5 cm above water level. The beads were stirred for 1 h and then stored in the $CaCl_2$ solution, at 4 °C, for a further 24 h. The beads were taken from the $CaCl_2$ solution, the excess $CaCl_2$ solution was absorbed with filter paper, and the beads were stored at high concentrations in demi-water.

2.5. Size determination

The size of the beads was measured by light microscopy (Axioskop 50) equipped with a camera (AxioCam HRc) (both from Zeiss, Germany). Images were analysed with ImageJ.

2.6. Lipase accessibility

The lipase accessibility to the oil droplets was investigated in: 1) a 2.0% (v/v) dispersion of microbeads, 2) a 2.0% (v/v) dispersion of macrobeads, 3) an equivalent amount of non-encapsulated emulsion, as made for the production of the alginate beads, but before addition of the alginate. The volume fraction was estimated by drying samples of the beads overnight in an oven at 105 °C. The dry weight of the beads was used to calculate the volume fraction of the beads, assuming no loss of oil occurred. The accessibility was measured in a diluted, simulated intestinal system based on the system described by (Minekus et al., 2014). This consisted of 150 mM of NaCl and 1 mg/mL pancreatic lipase at pH 7.0 and 37 °C. The conversion of MCT to the acidic fatty acids was followed by the amount of NaOH that needed to be added to keep the pH at 7.0. This was done with the pH-STAT (Metrohm, Herisau, Switzerland). The reaction was followed for 2.5 h and duplicate experiments were performed. The free fatty acid release was calculated with the following equation:

$$\text{FFA } \% = \frac{V_{NaOH} M_{NaOH} M_{w,lipid}}{3\omega_{lipid}}$$

where V_{NaOH} is the volume of NaOH titrated into the solution to keep it at pH 7.0, M_{NaOH} the molarity of the NaOH solution, $M_{w,lipid}$ the average molecular weight of the MCT oil, which was calculated to be 508 g/mol (based on the composition specified by the manufacturer), and ω_{lipid} the fraction of lipid present in the beginning of the experiment. The factor 3 is present because every triglyceride contains 3 fatty acids which can be released.

The oil droplet breakdown was also followed with light microscopy. The same method as for the pH-STAT was used, and at regular time intervals a small sample of the solution was taken. The sample was immediately heated to 80 °C for 10 min to inactivate the lipase. For every sample at least 25 microbeads were investigated in the range of 9.0–12 μm. The macrobeads were cut in half and viewed in a microscope slide with a dip, to prevent the pressure of the cover slide to influence the measurements. For every sample at least four macrobeads were investigated, and the location of the breakdown front was determined at different points along the circumference of the bead.