Food Hydrocolloids 52 (2016) 47–56

Contents lists available at ScienceDirect

Food Hydrocolloids

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

Comparative behavior of protein or polysaccharide stabilized emulsion under in vitro gastrointestinal conditions

Fernando A. Bellesi [a, b], María J. Martinez [a, b], Víctor M. Pizones Ruiz-Henestrosa [a, b], Ana M.R. Pilosof [a, b, *]

[a] Departamento de Industrias, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria (1428), Buenos Aires, Argentina
[b] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina

ARTICLE INFO

Article history:
Received 23 January 2015
Received in revised form
28 April 2015
Accepted 5 June 2015
Available online 23 June 2015

Keywords:
Lipid digestion
Emulsion
Interfacial film

ABSTRACT

In the present work, an in vitro model digestion was used to compare the behavior of emulsions stabilized by proteins or polysaccharide upon digestion and to analyze its relationship with the kinetics and extent of lipid digestion. Oil/water emulsions were prepared using different emulsifiers (β-lactoglobulin, soy protein isolate and hydroxypropylmethylcellulose (HPMC)). The emulsion digestion was carried out in two continuous stages at 37 °C: 1) under simulated gastric conditions (1 h) using pepsin and phosphatidylcholine (simulated gastric fluid: pH 2.5, NaCl, NaH_2PO_4, KCl and $CaCl_2$) and 2) under simulated intestinal conditions (1 h) with bile salts, pancreatic lipase, trypsin and chymotrypsin (simulated intestinal fluid: pH 7.0, K_2HPO_4, NaCl and $CaCl_2$). The changes in the particle size distributions, the interfacial area and their microstructures were analyzed as a function of the digestion time. The free fatty acid release during the simulated intestinal stage was also determined and an empirical model was fitted to estimate different kinetic parameters. Irrespective of the composition/structure of emulsions, the initial surface area was found to determine the initial rate of lipolysis. Soy protein was the protein that forms the most resistant emulsion to digestion, showing a degree of free fatty acid release similar to HPMC, which is a non digestible emulsifier. The results are discussed on the basis of the role of bile salts and its effect on oil/water interfaces.

1. Introduction

The capacity to control the lipid digestion within the gastrointestinal human tract could facilitate the development of functional foods that could result in decreasing the risk of suffering some diseases associated with the high lipid absorption such as obesity and cardiovascular diseases (Golding et al., 2011; Li, Hu, & McClements, 2011; Li & McClements, 2011; Lowe, 1994; Singh, Ye, & Horne, 2009; Wooster et al., 2014). Moreover, this knowledge is important to develop lipid based delivery systems that would facilitate the incorporation of bioactive substances that could have healthy benefits (McClements, Decker, Park, & Weiss, 2008; Salvia-Trujillo, Qian, Martín-Belloso, & McClements, 2013).

An important part of the lipids in processed foods are consumed in the form of oil–water (o/w) emulsions, in which they are embedded in form of droplets in an aqueous medium. The surface of these droplets is coated by a layer of interfacial active molecules, such as proteins or polysaccharides. These molecules adsorb at fluid interfaces playing an important role not only in the formation and stability of emulsions but also in the rate of the digestion process (Bouyer et al., 2011; Qian & McClements, 2011; Wan et al., 2014). It has been reported in recent works that the characteristics of the interfacial layers surrounding the fat droplets could play a significant role in the extent of lipid digestion, as well as the release rate of any entrapped lipophilic components (Bellesi, Pizones Ruiz-Henestrosa, & Pilosof, 2014; Malaki Nik, Wright, & Corredig, 2011; Ye, Cui, Zhu, & Singh, 2013).

During the digestion process, the emulsion is exposed to physical and biochemical changes that result in flocculation, coalescence, aggregation, droplet disruption, etc. (McClements & Li, 2010; Singh et al., 2009). In humans, the lipid digestion process takes place firstly in the stomach but it is more important in the small intestine through the action of pancreatic lipase (70–90% of the fat

* Corresponding author. Departamento de Industrias, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria (1428), Buenos Aires, Argentina.
E-mail address: apilosof@di.fcen.uba.ar (A.M.R. Pilosof).

http://dx.doi.org/10.1016/j.foodhyd.2015.06.007
0268-005X/

digestion) (Bauer, Jakob, & Mosenthin, 2005; Maldonado-Valderrama et al., 2008; Mun, Decker, Park, Weiss, & McClements, 2006).

The emulsion is mixed in the stomach with acidic digestive juices. These juices contain gastric components such as pepsin and surface active components such as phosphatidylcholine (PC) that alter the interfacial composition of oil droplets. It has been reported that the pH of the human stomach is between 1 and 3 and the meal could remain for 1–3 h, depending on the ingested food (composition, initial pH, buffering capacity, quantity, etc.). The partially digested food in the stomach, called chyme (Ekmekcioglu, 2002; Kalantzi et al., 2006; Lindahl, Ungell, Knutson, & Lennernäs, 1997), is mixed in the duodenum with sodium bicarbonate, bile salts (BS), phospholipids, and enzymes secreted by the liver, pancreas and gall bladder. The sodium bicarbonate secreted in the small intestine causes the pH increment from 1 to 3 (gastric conditions) to 6–7 where the pancreatic enzymes present maximum activity (Bauer et al., 2005). Trypsin and chymotrypsin are the main proteases in the intestinal juice and catalyze the breaking of specific peptides bonds (Ma, Tang, & Lai, 2005; McClements et al., 2008).

The lipid hydrolysis is an interfacial phenomenon that requires the adsorption of pancreatic lipase at the lipid droplet surface. This adsorption is facilitated by the presence of BS, released from the gall bladder through the bile duct, and colipase secreted by the pancreas (Fillery-Travis, Foster, & Robins, 1995; Mun et al., 2006). The adsorption of BS facilitates the emulsification of the lipids, as they affect the interfacial layer of emulsion droplets and prepare them for enzymatic hydrolysis (Bauer et al., 2005; Bellesi et al., 2014; Torcello-Gómez, Maldonado-Valderrama, Jódar-Reyes, Cabrerizo-Vílchez, & Martín-Rodríguez, 2014). Therefore, the nature of the interfacial layer surrounding the lipid droplets should have an important role in the lipid digestion. Previous works have shown that the characteristic of interfacial films could affect the BS adsorption under simulated intestinal conditions, which could alter the lipid digestion (Bellesi et al., 2014; Maldonado-Valderrama et al., 2008; Mun, Decker, & McClements, 2007; Singh & Sarkar, 2011; Singh et al., 2009; Torcello-Gómez & Foster, 2014). When the lipase is adsorbed at the o/w interface, the triglycerides are hydrolyzed into free fatty acid (FFA), monoglycerides and diglycerides. These products are then incorporated into the BS micelles and transported to be absorbed by the epithelium layer. Moreover, the intestinal proteases (trypsin and chymotrypsin) carry out the hydrolysis of the protein interfacial films (Di Maio & Carrier, 2011; Martigne, Julien, & Sarda, 1987).

It has been recently described the behavior of lipid emulsions under gastrointestinal conditions focusing in different aspects (Golding et al., 2011; Singh & Ye, 2013; Wooster et al., 2014; Ye et al., 2013). Malaki Nik et al. (2011) have reported the effect of the emulsifier type in the physicochemical behavior of o/w emulsions during the in vitro digestion. They observed that soy protein isolate (SPI) emulsions were more digested than whey protein isolate (WPI) emulsions. Torcello-Gómez, A., et al. (2014) have analyzed the behavior of different non-ionic surfactants (pluronics) under simulated duodenal conditions from an interfacial point of view using olive oil as the oil phase. These authors concluded that the surfactants could resist the interfacial displacement of BS, retarding or limiting the lipase activity. Ye et al. (2013) have focused on the effect of calcium in the kinetics of FFA release in emulsions stabilized by WPI, Tween 20, sodium caseinate and lecithin under duodenal conditions and they showed that the addition of calcium promote the rate and extent of FFA release (through the removal of FFA from the surface area). Moreover they concluded that the increase of FFA was dependent on the emulsifying agent. The proteins, capable to interact with calcium, reduced the availability of calcium and consequently decreased the lipase activity more than low molecular weight emulsifiers (lowest capacity to interact with calcium). It has also been demonstrated that emulsions stabilized whit gum arabic were more digested than emulsions stabilized by proteins (WPI), as the former showed the higher FFA release. The protein molecules adsorbed at the interface could hinder the arrival of the lipase molecules to the interface (Helbig, Silletti, Timmerman, Hamer, & Gruppen, 2012).

In a previous work (Bellesi et al., 2014) it was shown from the analysis of the competitive and sequential adsorption of three proteins and BS that soy protein film was particularly more resistant to BS displacement than β-lg or egg white film, which could impact in further action of lipase and thus on lipid digestion. Therefore, the objective of the present work was to study the behavior of soy protein stabilized o/w emulsions under simulated gastrointestinal digestion in comparison with β-lg, which has been extensively used to stabilized o/w emulsions (Sarkar, Horne, & Singh, 2010a, 2010b; Singh et al., 2009), and a non-ionic polysaccharide (hydroxypropylmethylcellulose (HPMC)), that has been selected because of its known interfacial activity (Camino, Sanchez, Rodríguez Patino, & Pilosof, 2012) and resistance to enzymes action. Furthermore, the rate of lipid digestion was determined and related to the performance of emulsions upon in vitro digestion.

2. Materials and methods

2.1. Materials

BioPURE β-lactoglobulin (β-lg) was obtained from DAVISCO Foods International, Inc. (Le Sueur, Minnesota) with a protein composition (dry basis) of 97.8%, being β-lactoglobulin 93.6% of total proteins. Denatured soy protein isolate (thermally procedure), being 98% water soluble, was obtained from defatted soybean flour (Sambra S.A., Brazil) as indicated by Carp, Bartholomai, and Pilosof (1997).

Methocell (food grade) HPMC from the Dow Chemical company was kindly supplied by Colorcon (Argentina) and used without purification. The characteristics of this HPMC was indicated previously by Camino and Pilosof (2011).

2.2. Methods

2.2.1. Emulsion preparation

Oil–Water emulsions were prepared by mixing a commercial sunflower oil and emulsifiers solutions (2% w/w) at a 10:90 ratio using an ultrasonic processor Vibra Cell, model VCX 750 (Sonics & Materials, Inc., Newton, CT, USA) at a frequency of 20 kHz and an amplitude of 20% for 15 min. The glass tube with the sample was introduced in a glycerine-jacketed at 0.5 °C to dissipate the heat produced during the sonication keeping the sample temperature below 25 °C (Camino & Pilosof, 2011; McClements, 2004).

2.2.2. In vitro digestion model

15 ml of o/w emulsion were previously incubated at 37 °C. The in vitro digestion begins with the addition of 15 ml of a simulated gastric fluid (SGF) (pH 2.5, 100 mM NaCl, 3 mM $CaCl_2$, 5 mM NaH_2PO_4, and 22 mM KCl) under continuous and moderate agitation. Pepsin from porcine gastric mucosa (P700, powder ≥ 250 units/mg solid) and L-α-phosphatidylcholine from egg yolk (type XVI-E, P3556) both purchased from Sigma–Aldrich were dissolved in SGF. The different aliquots were withdrawn at different times of the gastric stage (t_g): 0 (t_{g0}), 10 (t_{g10}), 30 (t_{g30}) and 60 (t_{g60}) min. The aliquot t_{g0} (at the beginning of the gastric stage) was immediately taken after the incorporation of pepsin and PC and it is associated with the initial effect of this biomolecules. Finally, the proteolysis was stopped by increasing the pH to 7 (1 M $NaHCO_3$).