Potential impact of interfacial composition of proteins and polysaccharides stabilized emulsions on the modulation of lipolysis. The role of bile salts

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ABSTRACT

Important insights into the role of interfacial composition and structure in controlling the digestion of oil-water emulsions have been gained in the last decade. The driving interest relies on: i) the necessity of controlling the digestion of lipids to decrease or delay fat intake to address the obesity crisis existing worldwide and ii) assuring the bioaccessibility of bioactive emulsified lipids or hydrophobic bioactive compounds.

This article mainly reviews the relationship between the composition and structure of protein and polysaccharides stabilized emulsions and their susceptibility to in vitro lipolysis. The analysis concentrates on emulsions where (1) proteins or (2) polysaccharides are used as single emulsifiers, (3) emulsions stabilized by protein-polysaccharide conjugates, (4) protein-polysaccharide multilayer emulsions where the primary emulsion is formed by a protein, (5) protein-polysaccharide emulsions where proteins are the main emulsifiers and the polysaccharides perform as stabilizers.

The mechanisms involved in the control of the rate and extent of lipolysis are discussed with special attention given to the interactions between emulsions components and bile salts as a critical point for controlling lipids digestion.

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1. Introduction

During the last decade, important insights into the role of interfacial composition and structure in controlling the digestion of oil-water emulsions have been gained. The driving interest relies on: **i)** the necessity of controlling the digestion of lipids to decrease or delay fat intake to address the obesity crisis existing worldwide and the implications for long-term chronic diseases, **ii)** assuring the bioaccessibility of bioactive emulsified lipids (e.g omega-3 fatty acids) or the delivery of hydrophobic bioactive compounds included in the core lipid.

A good overview of the biochemistry of human lipid digestion is given in previous reviews (Golding & Wooster, 2010; Singh, Ye, & Horne, 2009). In brief, lipids digestion starts in the stomach where about 20% of the lipolysis takes place by acid-stable gastric lipase. When the partially digested food moves from the stomach into the small intestine, it is mixed with bile salts (BS) and pancreatic secretions in the duodenum forming an emulsion stabilised by bio-surfactants. One of the key roles of BS is to prepare the surface of the fat to improve the access of lipolytic enzymes to the lipid substrates (Maldonado-Valderrama, Wilde, Macierzanka, & Mackie, 2011). Therefore, the lipids digestion occurs essentially in the small intestine where about 80% of the lipolysis takes place at the oil-water interface mediated by the pancreatic lipase-colipase complex, releasing the sn-2-monoacylglycerol and two free fatty acids (FFA) from triacylglycerols (Golding & Wooster, 2010). Lipolysis products are then incorporated into BS micelles to be transported in the aqueous medium and absorbed to the mucosa of the small intestine.

In vitro digestion studies are widely used with the aim of predicting the lipolysis of food emulsions in the digestive tract, because animal and human studies are costly and lengthy; moreover they are limited due to ethical considerations. Most of these studies are performed in static models where gastric and small intestinal digestion (GI) is mimicked in two consecutive steps (Minekus et al., 2014). In vitro models enable the prediction of emulsions changes during oral and GI digestion as well as the

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release of FFA. They allow the screening of comparatively large numbers of samples and/or conditions, studying the separate and combined effects of each stage of digestion (oral, gastric, small intestinal, large intestinal) on the release of FFA. Nevertheless, the diversity of existing models may hinder the comparison of results across studies. The employed models differ in the inclusion of one or more of the stages of digestion, digestion times, pH, the nature and concentration of digestive enzymes, concentrations of electrolytes and biosurfactants (bile acids, phospholipids). Finally, while most of the models are operated in static mode (with pre-fixed concentrations and volumes of digested materials, enzymes salts, etc), there are also a limited number of dynamic models that mimic the continuous changes of the physicochemical conditions to better simulate the human digestive tract (Alminger et al., 2014).

Despite the key role of interfaces in determining the behaviour of emulsions on digestion, there are few studies dealing with the effects of digestion conditions on interfacial structures. The research group from the University of Granada is pioneer in developing a specific device (the OCTOPUS) based on the subphase exchange technique, that allows to apply a customized interfacial in vitro digestion process in which the interfaces are subjected subsequently to conditions mimicking the passage through the gut (Maldonado-Valderrama, Holgado-Terriza, Torcello-Gomez, & Cabrerizo-Vilchez, 2013). It allows to measure in situ the evolution of the interfacial tension throughout the whole simulated GI transit and the mechanical properties of the interfacial layer (interfacial dilatational modulus) after each digestion stage (mouth, stomach and small intestines).

Several mechanisms can be involved in the effect that the composition and structure of emulsions and interfacial films surrounding the oil droplets have on lipids digestion (Fig. 1). The main mechanisms are summarized as follows:

- Flocculation and coalescence of oil droplets under gastroduodenal conditions that, by decreasing the interfacial area available for lipase/colipase adsorption, may retard lipolysis.

- Steric factors inhibiting the interfacial anchoring of lipase/colipase (big head groups protruding in the aqueous phase, thick interfacial films, rigidity of interfaces).
- Resistance of interfacial films to adsorption/displacement by BS.
- Accumulation at the interface of inhibitory lipolysis products (i.e, FFA, monoacylglycerols) due to a decrease in available BS and phospholipids that can be bound by adsorbed or unadsorbed emulsion components.
- Accumulation at the interface of inhibitory lipolysis products (i.e, fatty acids, monoacylglycerols) due to their binding to adsorbed emulsifiers.
- Inhibition of fatty acids uptake due to their binding to unadsorbed components.
- Decrease in available calcium by its binding to adsorbed or unadsorbed components. Calcium plays a critical role in the dynamics of fat digestion (Golding & Wooster, 2010).

This review will be focused mainly on the analysis of the relationship between the composition and structure of emulsions and the degree of lipolysis and less on the physico-chemical behaviour of emulsions during in vitro digestion. The analysis will concentrate on emulsions where (1) proteins or (2) polysaccharides are used as single emulsifiers, (3) emulsions stabilized by protein-polysaccharide conjugates, (4) protein-polysaccharide multilayer emulsions where the primary emulsion is formed by a protein, (5) protein-polysaccharide emulsions where proteins are the main emulsifiers and the polysaccharides perform as stabilizers.

The mechanisms involved in the control of the rate and extent of lipolysis of the different emulsions will be discussed. Special attention will be given to the interactions between emulsions components and BS as a critical point for controlling lipids digestion.

2. Protein stabilized emulsions

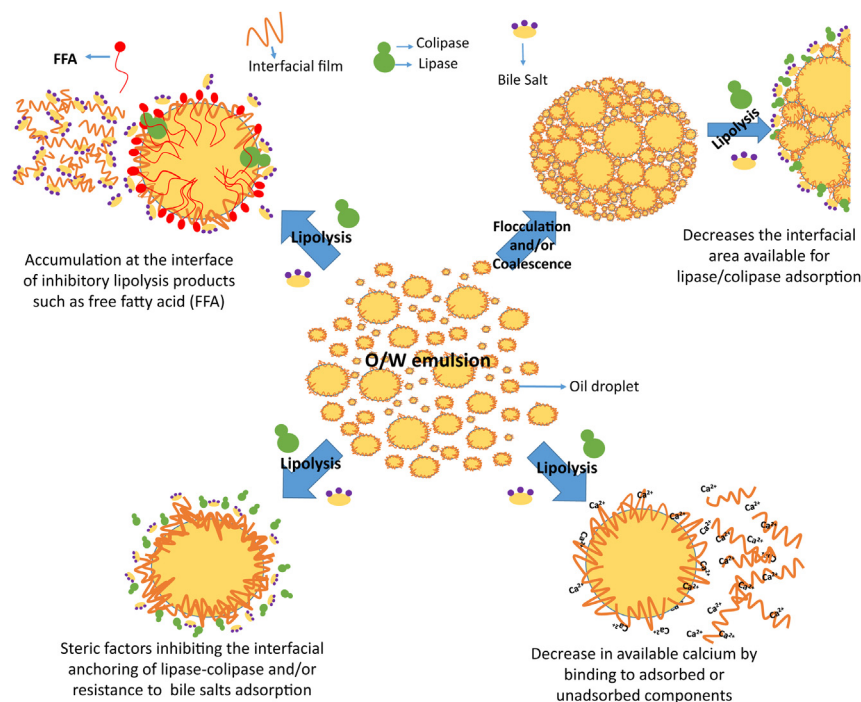Proteins are known specifically for their surface activity, which

Fig. 1. Mechanisms involved in the modulation of lipolysis of emulsions.

allows them to play a major role in the formation and stabilization of emulsions by a combination of electrostatic and steric mechanisms (Dickinson, 1992). Proteins are not equally surface active, even though all are amphiphilic.

The most commonly used proteins in food emulsions are derived from milk, soybean and eggs. The proteins derived from milk have been extensively studied for their formation and stabilization of emulsions as they exhibit good surface-active properties and form interfacial layers with desirable rheological properties (Rodríguez Patino & Pilosof, 2011; Singh & Ye, 2013). The main milk proteins are caseins, which have rather flexible structures and whey proteins which are typical globular proteins. The major globulins of soy protein are conglycinin and glycinin. Structural modifications by physical or chemical methods improve their surface behaviour and functionality (Morales, Martínez, Pizones Ruiz-Henestrosa & Pilosof, 2015). Egg white contains as many as 40 different proteins, among them ovalbumin is the main constituent responsible for the egg white functionality (Powrie & Nakai, 1986).

An excellent review on the factors that affect the digestibility of protein-based lipid emulsions that discusses the behaviour of emulsions in the oral, gastric and intestinal environment has been reported by Singh and Ye (2013). Generally, the very low pH in the stomach causes flocculation or coalescence in the gastric environment. Moreover protein interfaces are prone to proteolysis in the gastric and duodenal environment that further affects their stability. Nevertheless flocs may be further re-dispersed under intestinal environment by increased pH and presence of bio-surfactants (Bellesi, Martinez, Pizones Ruiz-Henestrosa, & Pilosof, 2016).

Mun, Decker, and McClements (2007) studied the comparative behaviour of sodium caseinate and whey protein isolate (WPI) corn oil-water emulsions and FFA release under in vitro intestinal model carried out at pH 7, with pancreatic lipase. The FFA release from sodium caseinate emulsion was slightly higher than that from WPI emulsion. When WPI and caseinate fish oil-water emulsions were passed through a simulated GI tract that included mouth, stomach, and small intestine phases different results were obtained (Chang & McClements, 2016). An almost complete digestion of the fish oil was observed for both emulsions. Nevertheless, the initial digestion rates for the caseinate-coated lipid droplets was slower than for the WPI droplets, which was attributed to the severe flocculation and coalescence of the casein coated lipid droplets that occurred before the addition of lipase.

Li, Ye, Lee, and Singh (2012) further examined the influence of gastric digestive reaction on subsequent in vitro intestinal digestion of sodium caseinate-stabilized emulsions. Digestion in simulated gastric fluid containing pepsin accelerated coalescence of the emulsion droplets during subsequent digestion in simulated intestinal fluid containing pancreatic lipase. However, the changes in the size, the microstructure and the proteolysis of the interfacial proteins of the emulsions under gastric conditions did not influence the rate and the extent of lipid digestion in the subsequent intestinal environment.

Kenmogne-Domguia, Meynier, Viau, Llamas, and Genot (2012) also studied the consequences of the gastric phase at pH 2.5 or 4.0 (with or without pepsin) on intestinal lipolysis of BSA stabilized rapeseed oil-water emulsions. The pH had a limited impact but pepsin favoured flocculation and coalescence of the droplets, modulating the early stage of lipolysis but not its final extent.

Singh and Sarkar (2011) compared the behaviour of milk protein based emulsions (cationic-lactoferrin or anionic-β-lactoglobulin) during their passage through the GI tract. Emulsions at pH 7.0 had ζ-potentials of ~ +52 and −55 mV respectively and were uniformly dispersed, with droplets being under 5 μm in size. The amount of FFA released after the gastric digestion step was similar for both emulsions. The results from the sequential processing suggested

that not only the charge but the nature of the protein are not particularly important in the final digestion of lipids. Also, the physical stability of emulsions was similar in this sequential study.

More recently Zhang, Zhang, Zhang, Decker, and McClements (2015) compared the influence of milk protein emulsifier type (cationic-lactoferrin or anionic-caseinate) on the GI fate of emulsified lipids using a simulated gastrointestinal tract: mouth, stomach, small intestine. The initial rate of lipid digestion depended strongly on emulsifier type, being 18.6 and 6.4%FFA.min^{-1} for lactoferrin and caseinate, respectively. The slower initial rate of lipid digestion in the caseinate-stabilized emulsions was attributed to extensive droplet flocculation in the gastric phase, which would restrict the access of lipase to lipid droplet surfaces. However, complete lipid digestion occurred for all these emulsions at the end of the small intestine phase.

Malaki Nik, Wright & Corredig (2011) used various in vitro digestion models to investigate the lipolysis of emulsions prepared with WPI or soy protein isolate (SPI). Both emulsions showed monomodal particle size distributions, with $D_{3,2}$ values of 0.14 and 0.23 μm, respectively. SPI emulsions consistently showed a higher degree of lipolysis (and initial rate) compared to the WPI-emulsions regardless of the in vitro digestion model used. WPI-emulsion was more stable than SPI-emulsion during in vitro gastric digestion but further proteolysis of WPI peptides by trypsin and chymotrypsin, combined with interfacial displacement by BS, resulted in a loss of stability due to droplet coalescence. As shown by ζ-potential measurements, both proteins were displaced from the interface by BS; however, the displacement was much faster for the WPI-emulsions. Whey proteins despite being faster displaced from the interface and having smaller initial droplets size, exhibited the lowest degree of lipolysis. This unexpected behaviour was not addressed.

Bellesi et al. (2016) studied the behaviour of SPI stabilized oil-water emulsions under simulated GI digestion in comparison with β-lg emulsions. The lower extent of lipid digestion of SPI emulsion, as measured from the in vitro FFA release, could be related to the behaviour of the SPI interfaces that could resist the displacement carried out by the BS. Bellesi, Pizones Ruiz-Henestrosa & Pilosof (2014) have observed in dynamic co-adsorption and sequential experiments that SPI film was more resistant than β-lg to BS displacement and that SPI could compete with the BS for the interface. As a result, the adsorption of lipase would be less facilitated as well as the desorption of the lipolysis products that is BS-dependent.

Few works have been carried out to prove if crosslinking the adsorbed proteins could increase the resistance of protein films to lipolysis. Sandra, Decker, and McClements (2008) investigated the influence of β-lg interfacial heat cross-linking on the in vitro digestibility of emulsified lipids by pancreatic lipase. The rate and extent of lipid digestion did not differ greatly between unheated or heated β-lg stabilized emulsions.

A new approach to enhance the stability of protein nano-emulsions and to control the lipolysis through spontaneous cross-linking of the interfacial casein layer with genipin (a functional ingredient isolated from the fruit of Gardenia jasminoides E.) has recently been reported (Hu et al., 2015). Cross-linking casein-emulsified nanoemulsions enhanced their stability under gastric environment and prevented flocculation. The intestinal digestibility of lipid droplets was delayed very significantly after cross-linking the interfacial casein layer with genipin.

The hypothesis that lipid digestion can be controlled by strengthening the interfacial network that would resist displacement by BS has also been proven by Sarkar, Murray et al. (2016). They tested if fused (heat-treated) microgel stabilized interfaces (pickering emulsions) should be able to protect the lipids against

the action of lipase more significantly as compared to the non-heat treated whey protein microgel particles and thus contribute to delaying lipid digestion. When simulating the overall gastrointestinal digestion whey protein microgels were broken down by proteases irrespective of whether further heat treatment was applied or not, as evidenced by SDS-PAGE, surface charge measurements and confocal microscopy. Such protease-responsive nature of the whey protein microgels particles enhanced the lipolysis kinetics of pickering emulsions significantly, due to the interfacial presence of remnants of particles/peptides as compared to intact microgel particles during in vitro intestinal digestion.

Further, Maldonado-Valderrama et al. (2013) studied the behaviour of two interfacial protein structures (β-lg and β-casein) upon in vitro digestion by means of a new apparatus, the OCTOPUS, which enables to mimic the sequential in vitro digestion process in a single droplet. The two protein interfacial layers showed different performance upon gastric digestion. Pepsin partially hydrolysed the interfacial adsorbed β-lg molecules under gastric conditions, lowering both the interfacial coverage and the interfacial elasticity of the network. Conversely, pepsinolysis of the interfacial adsorbed β-casein lowered the interfacial coverage but affected the interfacial packing of the resulting network increasing the interfacial dilatational modulus. Nevertheless, the extent of lipid hydrolysis was found to be similar and comparable to that in the absence of coverage (pure oil-water interface) indicating that proteins do not comprise a barrier to lipolysis.

In conclusion, the protein itself, where crosslinked or forming pickering particles, does not seem to be an effective barrier to lipolysis, even if some delay in lipolysis may occur. This behaviour may be attributed to their low resistance to proteases action or to penetration/displacement by BS that facilitates the anchoring of lipase/colipase complex as well as the removal of inhibitory products. Nevertheless, the type and structure of protein clearly affects the physico-chemical behaviour of protein-stabilized emulsions during in vitro digestion. The importance of droplet size on lipase kinetics has been highlighted (Giang et al., 2015, 2016). They supported the idea that droplet coalescence during the intestinal phase was the main reason for the marked slowdown of the kinetics of lipid digestion of whey protein stabilized emulsion by causing a sharp reduction of the interfacial area available for the adsorption of pancreatic lipase-colipase.

3. Polysaccharides stabilized emulsions

Most high-molecular-weight polysaccharides, being hydrophilic, do not have much of a tendency to adsorb at fluid interfaces. Most common polysaccharides used in the formulation of food emulsions are pectin, xanthan, carrageenan, arabic gum, guar gum and alginate.

Although several reports show that polysaccharides exhibit surface/interfacial activity, it has been attributed to the presence of protein impurities (2–4%) associated to the gums (Dickinson, 2003). Therefore we will consider them as non-surface active polysaccharides. Nevertheless they are mostly used as stabilizers in the preparation of food emulsions.

Some hydrophobically modified polysaccharides can be used as main emulsifiers: cellulose derivatives (methylcellulose, MC; carboxymethylcellulose, CMC; hydroxypropylcellulose, HPC and hydroxypropylmethylcellulose, HPMC) or the propylene glycol esters of alginic acid. Even, ethylcellulose and hydroxypropylmethylcellulose appear to be more surface active than milk proteins (Rodríguez Patino & Pilosof, 2011).

3.1. Polysaccharides as main emulsifiers

Few studies on digestion of emulsions, where a polysaccharide is the main emulsifier, may be found in the literature. Recently, Bellesi et al. (2016) showed that HPMC stabilized emulsion underwent small changes under GI environment because of their non-ionic nature and undigestibility by gastric or duodenal proteases. Thus they were more resistant under gastric conditions than protein emulsions (β-lg or SPI). This lower degree of destabilization under the gastric condition presents a special interest, since it has been associated with the rate of gastric emptying delay, alterations in the release of the hormones involved in human digestion and consequently with the satiating effect (Malaki Nik, Wright, & Corredig, 2010; Marciani et al., 2009).

A low extent of lipolysis was observed for HPMC emulsions as compared to β-lg that makes this polysaccharide of interest to delay lipid digestion.

HPMC as single emulsifier, has also been shown to delay lipolysis of olive oil-water emulsions in relation to the conventional food surfactant Tween 20 (Torcello-Gómez & Foster, 2016). This ability was ascribed to the lower emulsification capacity of cellulose ethers, giving rise to larger droplets and hence smaller initial interfacial area available for the lipolysis reaction. Moreover, cellulose ethers seemed to resist complete desorption from the oil-water interface by the BS, which may make difficult the access of lipase to the interface. Similar lipolysis curves were found independent of either molecular weight, substitution pattern or initial concentration of HPMC.

Pizones Ruiz-Henestrosa, Bellesi, Camino & Pilosof (2017) studied the FFA release of HPMC stabilized emulsions as affected by the molecular weight or hydrophobicity of HPMC. The emulsion formed with the less hydrophobic HPMC was more susceptible to lipolysis. This behaviour could not be attributed to differences in the size/surface area available to the action of lipase/colipase nor to differences in the interfacial film properties but could rely on molecular events occurring at the interface upon BS adsorption. Besides both HPMC have shown similar abilities to adsorb and "sequester" BS in the bulk as well as at the interface, some special features arose from their different methyl/hydroxypropyl ratio. The self-assembly of the less hydrophobic HPMC in the bulk was more hindered by BS (Torcello-Gómez & Foster, 2014). Thus in parallelism, they proposed that BS adsorbed onto the interface formed by the less hydrophobic HPMC, would provoke a higher disentanglement of the molecules allowing more sites available for lipase adsorption, resulting in a higher rate and extent of lipolysis.

3.2. Protein-polysaccharide conjugates stabilized emulsions

Maillard-type conjugates produced by the dry-heating of a mixture of these two kinds of biopolymers can improve the poor protein solubility, colloidal stability and interfacial functionality of proteins under certain conditions (Kato, 2002).

Lesmes and McClements (2012) examined how β-lg-dextran conjugates affect the behavior of emulsions under conditions simulating the stomach and small intestine. Conjugation affected emulsion characteristics and responsiveness to pH, gastric pepsin, CaCl₂ and BS. These effects were ascribed to the dextran moieties. The final amount of FFA released from conjugate-stabilized emulsions appeared to be decreased compared to the β-lg stabilized control emulsion. Contrarily, Xu et al. (2014) showed that the release of FFA did not differ greatly between the unconjugated and conjugated whey protein isolate–beet pectin stabilized emulsions.

3.3. Polysaccharides in multilayer emulsions

Multilayer emulsions are formed by depositing charged biopolymers onto the surfaces of oppositely charged droplets through electrostatic attraction. Nanolaminated coatings are formed by

carrying out this process a number of times using two or more oppositely charged biopolymers (McClements, 2010).

The lipolysis of these multilayer emulsions has been extensively examined to determine the effect of the multilayer on the rate and extent of FFA release. It is supposed they may have better resistance against the stresses during digestion than primary emulsions due to a higher resistance against mechanical disruption. In addition, thick and robust interfacial membranes might sterically hinder lipase to access the emulsified lipids and, therefore, prevent or retard the release of FFA (Zeeb, Lopez-Pena, Weiss & McClements, 2015).

Li, Hu, Du, and McClements (2011) used an intestinal digestion model to test in vitro digestibility by pancreatic lipase of emulsions containing lipid droplets coated by: β-lg; β-lg/alginate; β-lg/alginate/chitosan. Biopolymer layers had little impact on lipid digestion at 5 mM calcium suggesting that they became detached from the droplet surfaces, but they slowed down digestion considerably at 20 mM calcium, suggesting the formation of a calcium alginate gel that restricted lipases access to emulsified lipids. Nevertheless as the emulsions were not subjected to gastric digestion it may be expected a different result in a gastrointestinal environment.

In a related work (Hu, Li, Decker, Xiao, and McClements (2011) caseinate (Ca) was used as to form a primary emulsion, pectin(P) was used as an anionic polyelectrolyte, and chitosan (C) was used as a cationic polyelectrolyte. The electrostatic layer-by-layer deposition approach was used to prepare multilayer emulsions containing lipid droplets coated by: (1) the same coating composition but different layer order (Ca−P−C and Ca−C−P); (2) the same outer layer but different coating compositions (e.g., Ca−P, Ca−P−C−P, and Ca−C−P). An intestinal digestion model was used to test the ability of lipase to release FFA from emulsions. Coating lipid droplets with dietary fiber layers (such as chitosan and pectin), did not have a major impact on the lipid digestibility. Nevertheless, there were some differences between multilayer emulsions with different biopolymer coatings. The Ca−C multilayer emulsion digested at an appreciably lower rate than the Ca emulsion which may have been due to the ability of the chitosan to form a cationic coating around the lipid droplets that at least partly prevented lipase from accessing the emulsified lipids. Alternatively, the chitosan may have been able to bind BS and thereby reduce the ability of the system to solubilize digestion products.

McClements (2010) examined the impact of the number of layers on lipid digestion, and found that the rate of lipid digestion decreases as the number of layers around the lipid droplets increases.

The crosslinking of coating polysaccharides has also been tested as it could increase the resistance of multilayers emulsions to lipolysis (Zeeb, Lopez-Pena et al., 2015; Zeeb, Weiss, & McClements, 2015). Through an in vitro GI model that included mouth, gastric, and intestinal phases, the impact of a polysaccharide crosslinking enzyme (laccase) on the lipolysis of multilayered fish gelatin-pectin oil-water emulsions was studied. The rate and extent of lipid digestion did not greatly differ between crosslinked and non crosslinked multilayered emulsions, even modulated by salt. FFA profiles showed that the stability of emulsified oil droplets plays a major role in the rate and extent of lipid digestion, rather than the initial layer properties.

In a recent work (Pinheiro, Coimbra, & Vicente, 2016) a dynamic gastrointestinal system was used to evaluate the behaviour of curcumin nanoemulsions stabilized by lactoferrin and lactoferrin/ alginate multilayer structure. This model simulates the main events that occur during digestion and consists of four compartments simulating the stomach, duodenum, jejunum and ileum. The overall extent of lipid digestion was fairly similar for both lactoferrin and multilayer nanoemulsion, which suggests that alginate coating did not prevent lipid digestion. However, there were some differences in the percentage of FFA released in the different stages and fractions of small intestine digestion.

All these results suggest that polysaccharides coatings are not very effective barriers to avoid lipase action, possibly because the interfacial multilayers are disintegrated when the emulsions are in the gastrointestinal environment (e.g. pH, ionic strength). However, although the structure of the initial surface layers may not have a very significant impact on lipid digestion, the polysaccharides involved in the interfacial layers may modulate available interfacial area for lipolysis as well as interact with BS or calcium, thus affecting lipolysis rate or extent.

Therefore it could not be necessary to attach polysaccharides to protein primary interfacial films as a strategy to control lipolysis, but simply including them as emulsion stabilizers.

3.4. Polysaccharides as stabilizers in emulsions

Some works have been done in this regard to prove the impact of unadsorbed polysaccharides (pH 7) in the release of FFA. Espinal-Ruiz, Parada-Alfonso, Restrepo-Sánchez, Narváez-Cuenca, and McClements (2014) examined the influence of polysaccharide type, chitosan (cationic), methylcellulose (nonionic), and pectin (anionic) and initial concentration (0.4−3.6% w/w) in corn oil-water emulsions stabilized by Tween-80. The rate and extent of lipid digestion decreased with increasing pectin, methylcellulose, and chitosan concentrations. The FFA released after 120 min of lipase digestion were 46, 63, and 81% for methylcellulose, pectin, and chitosan, respectively (3.6% initial polysaccharide), indicating that methylcellulose had the highest capacity to inhibit lipid digestion, followed by pectin, and then chitosan. The impact of the polysaccharides on lipid digestion was attributed to their ability to induce droplet flocculation, and/or to their interactions with molecular species involved in lipid hydrolysis, such as BS, fatty acids, and calcium.

Nevertheless Zhang et al. (2015) found that pectin addition increased the rate of lipid digestion in caseinate-stabilized emulsions (e.g., by 100% for 0.025% pectin), which was attributed to its ability to suppress droplet flocculation. Conversely, high levels of pectin in the lactoferrin-stabilized emulsions decreased the initial rate of lipid digestion (e.g., by >35% for 0.5% pectin), possibly due to calcium binding or gel forming effects.

Chang & McClements, 2016 compared the performance of fucoidan in WPI or caseinate fish oil-water emulsions through a simulated GI tract that included mouth, stomach, and small intestine phases. The presence of fucoidan increased the initial digestion rate of caseinate and WPI-stabilized emulsions due to its ability to modulate lipid droplet aggregation. The fucoidan appeared to suppress isoelectric aggregation of the droplets, which increased the surface area of lipids available for the lipase. On the other hand, the presence of fucoidan had little impact on the digestion of emulsions stabilized by lecithin or Tween, since it did not strongly impacted the lipid droplet aggregation.

The above apparent contradictory results may arise from the multiple roles performed in the emulsions by unadsorbed polysaccharides. They can stabilize or destabilize emulsions, depending on the primary emulsifier, thus changing the available surface area for lipase action. In addition they may bind biosurfactants and calcium that are necessary for the lipolysis reaction. All these roles modulated by their bulk concentration.

4. Role of bile salts

Many of the above discussed studies have been done under the hypothesis that strengthening the barrier properties (mostly mechanical or sterical) of interfacial films to lipase action could control

the rate and extent of lipolysis but many results have shown that this kind of barriers cannot significantly inhibit the action of lipase. However, increasing amount of works suggest that there exist an indirect impact of components of the interfacial film (or even unadsorbed components) on lipolysis, that rely on their ability to interact with biosurfactants and calcium.

According to Golding and Wooster (2010) the interfacial process of lipolysis involves essentially three key steps: (1) binding of the BS - lipase/colipase complex to the oil-water interface, (2) hydrolysis of the emulsified lipid to 2-monoacylglycerols and two FFAs and (3) desorption of these inhibitory lipolytic products by solubilisation in mixed BS and phospholipids micelles. Diffusion of micelles then delivers solubilized components across the unstirred water layer covering the luminal side of the enterocytes, thus facilitating uptake of lipophilic components by the enterocytes. Once this role is fulfilled, the BS micelles transit the remainder of the small and large intestine where they are progressively reabsorbed. However, if this transport mechanism fails or is slow, the accumulation of lipolytic products at emulsion interfaces will result in self-regulation of fat digestion.

As shown above, the first key step that regulates fat digestion is the surfactant action of BS. Due to its strong surface activity they rapidly adsorb at the oil-water interface, improving lipids emulsification by the increase of surface area and competing with emulsifiers previously adsorbed in the films, thus allowing (1) binding of the BS-lipase/colipase complex to the oil-water interface.

Knowing the type of displacement that takes place opens up a whole new area of research were strategies designed to control BS adsorption are hypothesized to control lipase adsorption and hence lipolysis (Maldonado-Valderrama et al., 2008). They investigated the competitive displacement of a model protein (β-lg) by BS from air-water and oil-water interfaces under in vitro duodenal digestion. The behavior of the films dilatational modulus suggested that the BS penetrate into, weaken, and break up the interfacial β-lg networks. AFM images suggested that almost total displacement occurred via an orogenic mechanism.

The results obtained by (Sarkar, Horne, & Singh, 2010) with milk protein emulsified systems interacting with BS (changes in droplet size, ζ-potential and confocal microstructures) in simulated intestinal conditions, also suggested that BS are adsorbed at the emulsion droplet surface pushing off protein from the interface.

Bellesi et al. (2014) studied the competitive and sequential adsorption of β-lg, soy proteins and egg white proteins and BS using a pendant drop tensiometer. Among all the proteins studied, soy protein films were more resistant to BS displacement. In addition soy proteins were the only protein that could compete with BS for the interface. This cooperative performance between soy protein and BS suggested the existence of favorable molecular interactions that in a further work (Bellesi et al., 2016) could account for by a decrease in the extent of lipolysis of soy protein stabilized emulsions as compared to β-lg emulsion.

The susceptibility of protein interfacial films to the displacement by BS would depend on the molecular feature of each protein as shown above as well as on the interfacial protein coverage. When the interface is poorly covered by protein, as in previous results cited in the literature (Maldonado-Valderrama et al., 2008) protein could be probably desorbed from the interface by BS adsorption. Nevertheless, if protein interfaces are saturated, BS could adsorb into defects of the protein network, and partially hinder protein interactions that account for the elastic behavior of films but would not totally displace the protein (Bellesi et al., 2014).

The other key step of lipolysis in which BS are involved is (3) desorption and transport of the inhibitory lipolytic products by solubilisation in BS micelles. Knowing the potential binding of BS by components forming an emulsion opens up another new area of

research to understand the importance of this mechanism in controlling/delaying lipolysis.

Soy protein has been shown to lower plasma total and LDL cholesterol in hyper-cholesterolemic humans and laboratory animals (Anderson, Johnstone, & Cook-Newell, 1995). The proposed mechanisms of action include a decrease in the intestinal absorption of bile acids (Potter, 1998).

Kahlon and Woodruff (2002) evaluated in vitro bile acid binding by soy protein, pinto beans, blackbeans and wheat gluten with a bile acid mixture under duodenal physiological pH of 6.3, relative to cholestyramine (a bile acid binding and cholesterol lowering drug). Assigning a bile acid binding value of 100% to cholestyramine, the relative bile acid binding for soy protein was 15%. Bile acid binding by soy protein was related to its potential influence on cholesterol lowering.

Recently Sarkar, Ye & Singh (2016) demonstrated quantitatively the role that BS play in the desorption of the inhibitory lipolytic products under simulated intestinal conditions. They showed that the presence of unadsorbed BS markedly enhanced the rate and the extent of lipid digestion. This could be attributed to considerable removal of lipolysis products (FFA, mono- and/or di-acylglycerols) in mixed micelles, which are known to inhibit lipid digestion, by the unadsorbed BS.

Very recent works highlighted the key role of the interaction of BS with surface active or non-surface active polysaccharides that can decrease unadsorbed BS content and hence potentially delay or inhibit the lipolysis. Most studies of BS interactions concentrate on interactions with soluble dietary fibres (SDF) that are non-starch plant polysaccharides resistant to digestion and absorption in the human GIT (Gunness, Flanagan, Shelat, Gilbert, & Gidley, 2012).

The principal mechanism by which SDFs are thought to reduce blood cholesterol is by preventing the re-absorption of BS from the small intestine into the enterohepatic circulation. One of the potential mechanisms that have been proposed to explain how SDF might interact with BS micelles in the small intestine, preventing their re-absorption, is the association/complexation of SDF and BS at a molecular level (Gunness & Gidley, 2010). Chitosan is particularly relevant for its ability to bind BS and in consequence its effect on the reduction of cholesterol blood levels (Chiappisi & Gradzielski, 2015).

From C NMR study, it was deduced that soluble dietary fibres such as glucan and arabinoxylan can interact with BS micelles either by forming dynamic complexes with the micelles or by trapping BS micelles in aggregate structures (Gunness, Flanagan, Mata, Gilbert, & Gidley, 2016).

A strong evidence exists on the interactions between BS and HPMCs. In the aqueous phase, BS are bound or "sequestered" by HPMC, mainly by interactions with the hydrophobic core of HPMC, thus being partially screened their charge. The binding of BS onto cellulose ethers has recently been assessed by differential scanning calorimetry (DSC) and linear mechanical spectroscopy (Torcello-Gómez & Foster, 2014, 2016). Hydrophobic interactions were postulated to take place between cellulose ethers and the BS, which were reflected in the inhibition of the thermal structuring of cellulose ethers. Recently the binding of BS by two HPMCs with different molecular structures was proven in the aqueous phase by particle size distribution analysis, cloud point temperature and electrical conductivity (Pizones Ruiz-Henestrosa et al., 2017).

5. Conclusions

A strong evidence exists from in vitro GI studies that it is not possible to totally inhibit free fatty acids release from protein/polysaccharide stabilized emulsions by modification of emulsion or interfacial composition and structure. However the rate or the

extent of lipolysis may be decreased. The rate at which FA are absorbed into the blood (i.e. postprandialtriglyceride levels) is now considered to be important for human health; high postprandial triglyceride levels are associated with the activation of various inflammatory pathways and are recognized as risk factors for cardiovascular disease and diabetes (Singh & Ye, 2013).

To this end, non-starch plant polysaccharides resistant to digestion and absorption in the human gastrointestinal tract proved to be more efficient than most proteins. However their action seems to be more related to their ability to interact with bile salts than to their role as a mechanical/sterical interfacial barrier to lipase/colipase action.

A better understanding of the mechanisms involved in the modulation of the lipolysis is needed as it will help to rationally select the emulsifiers or stabilizers to formulate emulsions with a reduced FA uptake. In this regard the following aspects should be deeply investigated: (a) the role of decrease in available calcium by binding to adsorbed or non-adsorbed components; (b) the resistance of interfacial films components to adsorption/displacement by BS; (c) the binding of BS and phospholipids to adsorbed or unadsorbed emulsion components as it may decrease the available biosurfactants for removal of lipolysis products (d) the binding of FA to adsorbed emulsifiers as it may difficult their removal from the interface (e) the binding of FA to unadsorbed components that may decrease their uptake.

The mechanisms (d) and (e) have not generally been considered as modulators of lipolysis. Nevertheless FA could be bound by interfacial or unadsorbed components that may inhibit or delay their absorption by the small intestine. In fact proteins − fatty acids interactions may occur in emulsions (Le Meste, Tainturier, & Gelin, 1997).

Chitosan may strongly interact with FA (Chiappisi & Gradzielski, 2015) and is known for its hypolipidemic properties (Zhang, Zhang, Mamadouba, & Xia, 2012). In vivo studies showed that it selectively reduced fat absorption in comparison to digestion-resistant maltodextrin (Santas, Espadaler, Mancebo, & Rafecas, 2012). The excretion of lauric, myristic and palmitic fatty acids of animals fed with chitosan was more than 10-, 5- and 2-fold higher, respectively, than in the cellulose group, whereas stearic acid excretion was not significantly altered. Oleic, linoleic and α-linolenic acid excretion were also significantly higher. Bile acid excretion was also increased by chitosan.

Finally, in vivo studies are needed to support conclusions obtained from in vitro models that cannot simulate the huge complexity of the physiological processes occurring in the human digestive tract.

Acknowledgements

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