Colloids and Surfaces B: Biointerfaces 161 (2018) 547–554

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Full Length Article

Comparative interfacial *in vitro* digestion of protein and polysaccharide oil/water films

Fernando A. Bellesi[a,b], Víctor M. Pizones Ruiz-Henestrosa[a,b], Julia Maldonado-Valderrama[c], Teresa Del Castillo Santaella[c], 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
[c] Grupo de física de Fluidos, Departamento de Física Aplicada, Universidad de Granada, Av. Fuentenueva S/N, 18071, Granada, España

ARTICLE INFO

Article history:
Received 28 June 2017
Received in revised form 2 November 2017
Accepted 8 November 2017
Available online 10 November 2017

Keywords:
Interface
Digestion
Lipolysis
Bile salts

ABSTRACT

The behaviour of proteins (β-lactoglobulin (βlg) and soy protein isolate (SPI)) and a surface active polysaccharide (hydroxypropylmethylcellulose, HPMC) o/w interfacial films under simulated gastrointestinal conditions using the interfacial tensiometer Octopus were compared and related to the performance of the emulsions (using the same emulsifiers) under *in vitro* digestion.

The evolution of interfacial tension (γ) was used to investigate the effect of gastrointestinal fluids on o/w interfacial films. Clear differences were observed among these emulsifiers. During the gastric phase, HPMC showed the lowest change in γ values as compared to protein films. The most important changes occurred during the intestinal stage where it was observed an important decrease of γ associated with the rapid penetration of BS, followed by a lower rate of decrease attributable to the accumulation of FFA at the interface. In the last stage, the subphase was exchanged by buffer alone, to remove the reversibly adsorbed digestion products. SPI formed the most resistant interface to the remotion of digestion products, followed by HPMC and finally by βlg. The results agree with the degree of lipolysis reported for the emulsions stabilized by these emulsifiers, which suggest that lipid digestion could be modulated by the ability of emulsifiers to prevent the BS activity (to adsorb at the O/W interface or remove the inhibitory digestion products from the interface). Thus, emulsifiers-BS interactions appears as a key factor in controlling the lipolysis.

1. Introduction

Lipids are widely used as food ingredients in many processed foods where they provide texture and flavour producing an important impact on the physicochemical properties [1,2]. Moreover, dietary lipids have an essential function in the body, for example they are a source of energy and provide fat-soluble vitamins, essential fatty acids as well as carotenoids or phytosterols [3]. However, the high lipid absorption is associated with serious health problems such as cardiovascular diseases, obesity, diabetes and hyperlipidaemia [4–6]. In fact, the World Health Organization reported a significant increment in the prevalence of the overweight and obesity in the last decade attributed to changes in dietary patterns and lifestyles [7–9]. For this reason, in the recent years, an important number of works may be found on the control of lipid digestion within the gastrointestinal human tract to facilitate the development of functional foods to reduce the risk of suffering these problems [10–13]. Thus, it would be of importance to develop long-term strategies to reach a reduction of the consumption of dietary fats producing healthy foods with a lower fat content but with the same sensory properties [14]. In this context, previous studies have shown that emulsion-based delivery systems can be designed to control the rate and extent of lipid digestion within the gastrointestinal tract [13,15] enabling the design of functional foods to control lipid digestion or retaining, protecting, and releasing hydrophilic bioactives within the gastrointestinal tract [16,17].

Dietary fats are consumed in different forms such as o/w emulsions where the oil droplets are contained in an aqueous medium and the surface of these oil droplets is coated by a layer of interfacial active molecules [18,19].

After passing through the mouth and oesophagus, the food is mixed in the stomach with the gastric juice. Although the pH

* Corresponding author at: 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).

https://doi.org/10.1016/j.colsurfb.2017.11.027
0927-7765/

remains in between 1 and 3, it could appreciably increase after food digestion [20]. As described in the literature, the meal could remain here between 1 and 3 h before passing to the small intestine. The protein hydrolysis starts in the stomach through the action of pepsin, affecting the structure of proteins used as emulsifiers [20]. The effects of pepsin on protein structure depend on the nature and protein conformation [13,21]. Singh and Sarkar [22] reported that β-lactoglobulin (βlg) in solution resists the hydrolysis carried out by pepsin but the protein is hydrolysed when unfolded at the o/w interface.

This partially digested food (chyme) enters in the small intestine where it is modified by different enzymes (trypsin, chymotrypsin and lipase), coenzymes, such as colipase and surface active components (bile salts (BS) and phospholipids) that have an important effect in the interfacial film coating the oil droplets [23]. Sodium bicarbonate released from the pancreas aids in maintaining the pH close to 7. Ionic strength is due to the presence of Ca^{2+}, Na^{+}, Cl^{-}, $PO_4{}^{-3}$, etc. contained in the duodenal juices secreted by the liver, pancreas and gallbladder [20,24]. These are the conditions that allow the maximum activity of the intestinal enzymes [25]. In the adults human tract most of the lipid digestion occurs in the duodenum (~70%), although the lipolysis starts in the stomach [12,26,27]. The duodenal lipolysis is carried out by the pancreatic lipase, which adsorbs onto the oil droplets surface to hydrolyse the triglycerides into 2-monoglycerides (MG) and free fatty acid (FFA). This process is facilitated by the presence of the BS (secreted by the gallbladder) which adsorb at the oil droplet surface increasing the available surface area [28].

At the end of the gastrointestinal digestion the surface of the remaining oil droplets will be formed by a mixture of different substances with interfacial activity such as: peptides, the lipolysis products (FFA, MG and diglycerides (DG)), BS and enzymes [26].

In the last decade it has been demonstrated that the nature of the emulsifier and therefore the characteristics of the interfacial films they form upon adsorption, play a crucial role in the behaviour of the emulsions during the digestion process [11,13,23,29–34] and specifically in the lipolysis kinetics. In a previous work, the performance of protein stabilized emulsions (β-lactoglobulin (βlg) and soy protein isolate (SPI)) and a surface active polysaccharide stabilized emulsion (hydroxypropylmethylcellulose, HPMC), under *in vitro* gastrointestinal digestion, were compared [13]. Under similar digestion conditions the emulsions stabilized by SPI and HPMC showed a lower degree of lipolysis than that stabilized by βlg. The lower extent of lipids digestion of SPI was attributed to the behaviour of the soy protein interfaces that could resist the displacement carried out by the BS under duodenal conditions [23], retarding the adsorption of lipase as well as to the desorption of the lipolysis products, also mediated by the BS. Regarding HPMC emulsions, the lower extent of lipolysis was attributed to its resistance to enzymatic hydrolysis and abilities to adsorb and "sequester" BS in the bulk, as well as at the interface [35].

Despite the key role attributed to the interfaces in determining the behaviour of emulsions during digestion, there are few studies dealing with the effects of digestion conditions on interfacial films, mainly because of technical difficulties in mimicking the effect of the successive digestion conditions throughout the whole gastrointestinal transit of interfaces. New technologies such as the OCTOPUS (interfacial tensiometer with a subphase multi-exchange device) allow to study the effect of the successive digestion stages (mouth, gastric and duodenal stages) in the properties of the interfacial films [33]. It allows to measure *in situ* the evolution of the interfacial tension throughout the whole simulated gastrointestinal transit and the mechanical properties of the interfacial layer after each digestion stage. In these stages, the interfacial film is subjected to different experimental conditions by mimicking the passage to the stomach and the duodenum in the presence of the salts, biosurfactants and the main enzymes present in the physiological medium. Maldonado-Valderrama, et al. [32] studied the *in vitro* digestion of interfacial protein structures using the interfacial tensiometer Octopus. They reported different susceptibilities of interfacial layers of βlg and β-casein upon gastric digestion, where pepsin partially hydrolysed the interfacial film formed by βlg, lowering both the interfacial coverage and the interfacial elasticity of the network. However, pepsinolysis of the interfacial adsorbed β-casein showed a reduction of 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 a subsequent work using the Octopus device, del Castillo-Santaella, et al. [36] reported that the modification of βlg conformation (pulsed light (PL) treatment) induced the formation of a more susceptible interfacial film than that formed by native βlg during pepsin and trypsin digestion. Analysis of the lipolysis and desorption phases also indicated a slightly lower resistance to lipolysis offered by the PL-βlg layer, as deduced from the highest desorption degree observed for PL-βlg.

The focus of the current work is to compare the behaviour of protein (βlg and SPI) and a surface active polysaccharide (hydroxypropylmethylcellulose, HPMC) o/w interfacial films under simulated gastrointestinal conditions using the interfacial tensiometer Octopus and to relate their behaviour to the performance of the emulsions (using the same emulsifiers) under *in vitro* digestion as previously reported [13]. As far as we know, such a comparison between gastrointestinal *in vitro* interfacial digestion and emulsion digestion using different macromolecular emulsifiers has not been reported.

The results of the present work will provide a deeper understanding of the interfacial aspects that underlie the behaviour of o/w emulsions. In this way we provide additional route to manipulate interfaces to control lipid digestion.

2. Materials and method

2.1. Materials

2.1.1. Emulsifiers

BioPURE βlg was obtained from DAVISCO Foods International, Inc. (Le Sueur, Minnesota) with a protein composition (dry basis) of 97.8%, being βlg 93.6% of total proteins. Denatured soy protein isolated (SPI, thermally procedure), being 98% water soluble, was obtained from defatted soybean flour (Sambra S.A., Brazil) as indicated by Carp, et al. [37].

Methocell HPMC (food grade) from the Dow Chemical company was kindly supplied by Colorcon (Argentina) and used without purification [38].

Commercial sunflower oil was purified according to Bahtz, et al. [39] by mixing it with Florisil 60–100 Mesh, from Fluka, in order to minimize the surface active impurities present in the oil. Moreover, the glass material used in the present work was also cleaned properly to avoid some possible contamination with surface-active components [23].

2.1.2. Simulated gastrointestinal fluids

The simulated gastric fluid (SGF) was prepared using NaCl (100 mM), $CaCl_2$ (3 mM), NaH_2PO_4 (5 mM) and KCl (22 mM). The pepsin from porcine gastric mucosa (P700, powder ≥ 250 units/mg solid, Sigma Aldrich) was dissolved in the SGF and the pH was adjusted at 2.5 using HCl 1 M. The pepsin was dissolved immediately prior its use to avoid loss of proteolytic activity.

It was utilized the protocol reported by Sarkar, et al. [40] to prepare the duodenal juice (SIF), that consists of a mixture formed by K_2HPO_4 (39 mM), NaCl (150 mM) and $CaCl_2$ (30 mM) at pH 7.0. The intestinal proteases (trypsin: type I, T8003 and chymotrypsin: type II, C4129, both provided by Sigma Aldrich) were used at physiological concentrations (40 U/mg emulsifier and 0.45 U/mg emulsifier, respectively) [41]. It was carried out the lipolysis with lipase from porcine pancreas (L3126, type II, at a final concentration of 54.4 mg/ml) in the presence of bovine BS (B3883, 5 mg/ml), both from Sigma Aldrich.

2.2. Methods

2.2.1. Interfacial properties

The interfacial tension, as well as the dilatational rheology measurements of the o/w interfaces, have been done using the pendant drop method in an interfacial tensiometer (OCTOPUS), equipped with a subphase multi-exchange device, developed at the University of Granada [33]. The OCTOPUS consists of an arrangement with two coaxial capillaries connected to one of the channels of a specific micro injector, which can operate independently [32,42].

A droplet solution was formed at the tip of double PTFE (constant volume: 10 μl) immersed in a glass cuvette filled with the purified oil. This cuvette is covered by a thermostated compartment (37.0 ± 0.1 °C). The whole set-up is computer controlled with a software (DINATEN) which fits the experimental drop profile to the Young-Laplace equation by using a drop profile analysis tensiometry to accurately determine (±0.1 mN/m) the interfacial tension (γ), drop volume (V) and the interfacial area (A).

The interfacial tension of the pure o/w interface was checked daily in order to corroborate the absence of surface-active contaminants and values of 24.0 ± 0.4 mN/m were obtained at 37 °C. The reproducibility of the results was assured from the standard deviation of at least three independent measurements. A one-way ANOVA analysis ($P < 0.05$ is significant) was performed.

The computer controlled dosing system also allows to induce area deformations and periodic automatically controlled sinusoidal interfacial compressions and expansions were performed during the formation of the adsorbed layer by decreasing and increasing the drop volume at the desired amplitude and angular frequency. Oscillations were performed at a frequency of 0.1 Hz and each perturbation consisted of 10 oscillations cycles applied when the interfacial tension reach a plateau. The amplitude of the oscillation was 10% of the initial drop volume in order to guarantee that the rheological parameters are independent of the amplitude. The response obtained from the variations of the interfacial tension with the area deformation is used to determine the dilatational properties [43].

The dilatational modulus (E) is composed by the storage part (E'), that is called dilatational elasticity, representing the real part of the term, and a loss component (E"), describing the imaginary part of E (Eq. (1)):

$$E(i\omega) = E'(\omega) + iE''(\omega) \quad (1)$$

where $E''/\omega = \eta$ is the interfacial viscosity ($\omega = 2\pi f$, which is the angular frequency of the generated area variations) [44].

2.2.2. Interfacial in vitro digestion protocol

A coaxial double capillary system was used to make the subphase exchange experiments without disturbing the interfacial layer. As indicated previously, the OCTOPUS allows to perform the analysis of the sequential adsorption of different types of molecules at the same interface.

It was designed an *in vitro* gastrointestinal model that mimics the principal events that occur during the digestion process. The gastric and the duodenal stages used in the present work are summarized in Table 1. Firstly, the corresponding emulsifier interfacial film was formed at the o/w interface (control). Once the interfacial tension reaches a plateau, the drop subphase was exchanged by the SGF to mimic the passage to the stomach. An hour later, the bulk solution was exchanged by the SIF to simulate the duodenal stage. At the end of the *in vitro* digestion process the subphase was also exchanged by the SIF (with no enzymes and BS) in order to remove the reversibly adsorbed molecules (BS, FFA, enzymes, etc.).

Table 1
Stage and composition of the digestion process.

Stage	Composition
Control	Emulsifier solution (1% w/w)
SGF	NaCl, $CaCl_2$, NaH_2PO_4, KCl pH 2.5 + Pepsin
SIF 1	NaCl, $CaCl_2$, NaH_2PO_4 pH 7.0 + Trypsin + Chymotrypsin
SIF 2	NaCl, $CaCl_2$, K_2HPO_4 pH 7.0 + Pancreatic Lipase + BS
DES	NaCl, $CaCl_2$, K_2HPO_4 pH 7.0

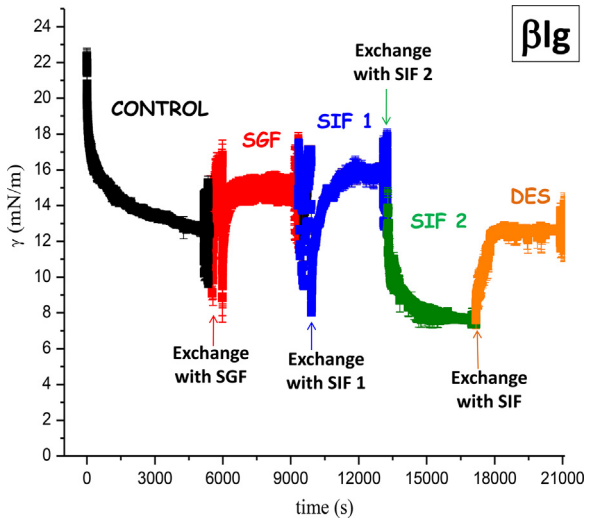

Fig. 1. Evolution of the interfacial tension (γ) during the in vitro digestion of the βlg adsorbed layer at the O/W interface. The conditions of each digestion stages are summarized in Table 1. Error bars mean SD, n = 3.

3. Results and discussion

3.1. In vitro *digestion of o/w interfacial films*

As a first step for the analysis of the interfacial behaviour of the emulsifiers (proteins and HPMC) during the *in vitro* digestion, their irreversible adsorption at the o/w interface was corroborated by exchanging the subphase with clean buffer. No changes were observed in the interfacial parameters (data not shown), which indicates that these emulsifiers exhibit a strong desorption barrier.

Figs. 1–3, show the time evolution of the interfacial tension (γ) for βlg (Fig. 1), SPI (Fig. 2) and HPMC (Fig. 3) adsorbed layers under gastrointestinal conditions. Table 1 reflects the composition of each digestive medium used in the different stages corresponding to the simulated digestion process. The values of γ and E obtained at the end of each one of these stages are summarized in Table 2.

3.1.1. Control stage

The control conditions consisted in the adsorption of each emulsifier onto the oil/water interface to form an interfacial film, which is then subjected to conditions of each compartment of the gastrointestinal tract thought the subphase exchange.

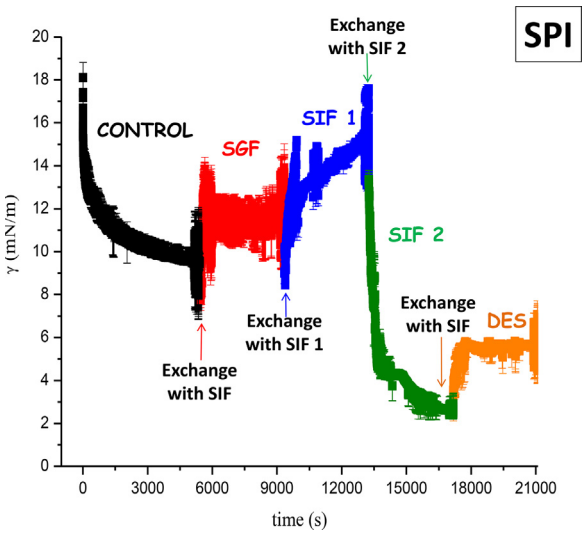

Fig. 2. Evolution of the interfacial tension (γ) during the in vitro digestion of the SPI adsorbed layer at the O/W interface. The conditions of each digestion stages are summarized in Table 1. Error bars mean SD, n = 3.

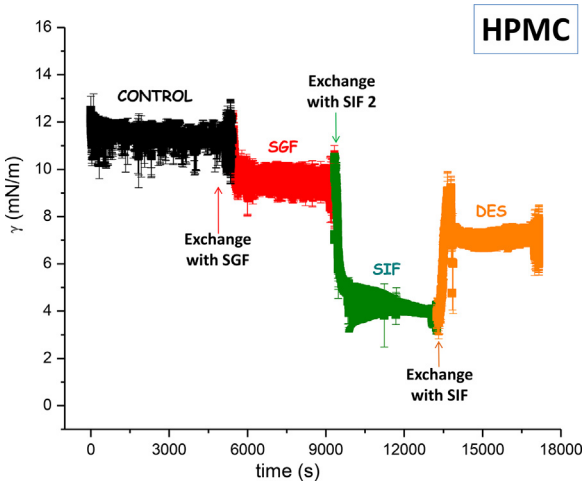

Fig. 3. Evolution of the interfacial tension (γ) during the in vitro digestion of the HPMC adsorbed layer at the O/W interface. The conditions of each digestion stages are summarized in Table 1. Error bars mean SD, n = 3.

The observed decrease of γ with time during this stage evidences the interfacial activity reported in the literature for these emulsifiers [23,45,46]. When comparing the interfacial behaviour of the proteins (βlg (Fig. 1) and SPI (Fig. 2)), similar dynamic curves were observed, being their adsorption kinetic lower than that for HPMC (Fig. 3), or with the behaviour reported for low molecular weight emulsifiers such as BS, tween 20 or lecithin [23]. As it was reported by MacRitchie [47] the protein adsorption at the o/w interface takes place in three different steps (diffusion to the interface; adsorption

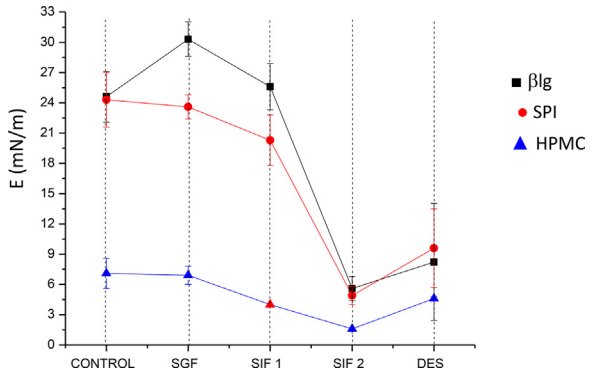

Fig. 4. Evolution of the Elastic modulus (E) during the in vitro digestion of the interfacial film formed by βlg, SPI and HPMC. Error bars mean SD, n = 3.

and unfolding of the protein at the interface) and the behaviour observed could be attributed to the complex and globular structure of these proteins which require more time to accommodate at the o/w interface. In contrast to the protein behaviour, HPMC reached very fast a steady-state value of γ associated with its lower molecular weight and structural complexity [45]. Although a decrease of γ is observed at the beginning of the measurement, all systems achieved an almost constant value of γ after 1800seconds of adsorption (Table 2).

Fig. 4 shows that both proteins formed a cohesive network with higher E values (Table 2) [48,49], while the HPMC exhibited the lowest E value, indicating that weaker intermolecular interactions between the adsorbed molecules took place, forming a more flexible structure [45].

3.1.2. Gastric stage: SGF

After the control stage, the emulsifier's solution was exchanged by SGF. During the gastric stage, significant changes in γ values were observed for proteins interfacial films (Figs. 1 and 2) as they increased to 14.5 mN/m (βlg) and 11.4 mN/m (soy protein) at the end of the gastric stage (Table 2), thus reflecting the effects of pepsinolysis in the protein interfacial films [32,36,49]. Macierzanka, et al. [50] observed a rapid increment in γ when introducing the pepsin, related to a rapid degradation of the protein interfacial films (βlg and β-casein) at the o/w interface. del Castillo-Santaella, et al. [36] showed that the pepsin hydrolysis dilutes the interfacial layer and, consequently, the interfacial tension increases (a reduction of the emulsifier concentration at the interface occurred). The exposure of pepsin susceptible sites owing the protein adsorption and interfacial unfolding of proteins at the o/w interface facilitates the action of pepsin contrarily to βlg in solution where it was reported their resistant to pepsinolysis [51]. The results corroborated the fact that the unfolding of βlg at the interface increment the susceptibility to pepsin hydrolysis through the exposition of new sensible sites to pepsin activity [52,53], demonstrating the importance of emulsification in protein digestibility studies.

On the other hand, the effect of the gastric stage on HPMC interfacial film was less notable than that observed for the pro-

Table 2
Interfacial tension (γ) and elastic modulus (E) obtained at the end of each digestion stages (mN/m.). Error bars mean SD, n = 3. Different letters indicate significant differences (P < 0.05).

Stage	βlg		SPI		E5LV	
	γ	E	γ	E	γ	E
Control	$12.6^{b} \pm 0.3$	$24.6^{b} \pm 2.5$	$9.5^{c} \pm 0.4$	$24.3^{b} \pm 2.7$	$11.28^{c} \pm 0.7$	$7.1^{c} \pm 0.1$
SGF	$14.5^{c} \pm 1,1$	$30.3^{c} \pm 1.7$	$11.4^{d} \pm 0.8$	$23.6^{b} \pm 1.2$	$10.05^{c} \pm 0.6$	$6.9^{c} \pm 0.5$
SIF 1	$15.5^{c} \pm 0.3$	$25.6^{bc} \pm 2.3$	$15.7^{e} \pm 0.2$	$20.3^{b} \pm 2.5$		
SIF 2	$7.5^{a} \pm 0.1$	$5.6^{a} \pm 1.2$	$2.6^{a} \pm 0.1$	$4.9^{a} \pm 0.9$	$3.5^{a} \pm 0.2$	$1.6^{a} \pm 0.7$
DES	$12.8^{b} \pm 0.2$	$8.23^{a} \pm 1.2$	$5.6^{b} \pm 0.2$	$9.6^{a} \pm 2.9$	$7.3^{b} \pm 0.3$	$4.6^{b} \pm 0.2$

tein films, as the polysaccharides are not hydrolysed by pepsin [54,55]. Fig. 3 shows a rapid decrease in γ values for HPMC films when the subphase was exchanged by SGF. After that, γ remained almost constant (Table 2). This fact agrees with the lower degree of destabilization reported for HPMC emulsions during *in vitro* gastric digestion [13]. The slight decrease in γ observed at the beginning of the gastric stage (Fig. 3) could be caused by the presence of components from the gastric fluid (pepsin and salts) that possibly increase the interfacial coverage. This behaviour supports previous studies reporting that surfactants can adsorb into the interface via defects within the interfacial films [56,57].

E values (Fig. 4), that inform about the interactions between the adsorbed molecules, remained almost constant for both βlg and SPI interfacial films before and after the gastric stage, suggesting that the hydrolysis of the protein interfacial film did not significantly affect its elasticity. However, the results observed for the βlg interfacial film differ from that reported by Maldonado-Valderrama, et al. [32], as they reported a slight decrease in the dilatational modulus when βlg got adsorbed at an olive o/w interface under simulated gastric conditions, in the presence of pepsin. However, these results may be due to the differences in the ionic content of their SGF, as the presence of different contents of ionic salts does affect the interfacial behaviour of the adsorbed molecules [21,58]. It could be possible that the salts may have a larger effect than the pepsin hydrolysis in the dilatational modulus, by promoting the screening of the charges and the conformational change of the adsorbed protein [21]. Roth, et al. [59], reported that the screening of the protein surface charges promoted the increment in the cohesiveness of the interfacial films, then obstructing the penetration of low molecular weight emulsifiers to the interface. No significant changes were observed for SPI films (Table 2), which is in accordance with the lower degree of destabilization of SPI stabilized o/w emulsions when subjected to the gastric conditions [13]. Therefore, it is possible that SPI interfacial films could be less affected by pepsin activity than that formed by βlg.

E values for HPMC did not show any significant change after the subphase was exchanged (Table 2), suggesting that the degree of interaction between the adsorbed molecules of HPMC at the interface was not affected. Camino and Pilosof [38] showed that the values of the charge of the HPMC stabilized emulsions were similar, both in neutral and acid pH, thus suggesting that the degree of interactions between HPMC is not affected by pH changes (from control (pH 7.0) to gastric stage (pH 2.5)). Moreover, HPMC stabilized emulsions did not experimented significant changes under gastric digestion conditions due to the poor impact caused by the salts content and pepsin activity [13].

However, the low electrostatic repulsion or steric impediments between the adsorbed molecules may cause some degree of destabilization of protein emulsions under the simulated gastric conditions. In fact, an important increment in the oil droplets size distribution of these emulsions under gastric conditions was observed in a previous work [13]. This behaviour is also in accordance with previous reports that indicated that high salt concentrations increased the flocculation of βlg stabilized emulsions because the attractive interactions were more predominant than the electrostatic repulsions and, consequently, the interactions between the droplets increased [60–62].

3.1.3. Intestinal stages: SIF

As summarized in Table 1 the passage through the duodenal conditions was simulated in two successive steps: firstly, it was simulated the duodenal proteolysis of the protein interfacial films carried out by pancreatic proteases (duodenal stage 1 (SIF 1)). It was then incorporated the SIF in the presence of pancreatic lipase and BS during the subphase exchange to simulate the duodenal lipolysis process in all cases (duodenal stage 2 (SIF 2)).

3.2. Intestinal stage 1: SIF 1-duodenal proteolysis of protein interfacial films

The incorporation of the intestinal proteases (SIF 1) exerted a slight effect on the interfacial properties of both protein interfacial films (Figs. 1 and 2) with an increment in the γ values to 15.5 mN/m (Table 2). These results suggest that the hydrolysis of the adsorbed proteins resulted in a reduction of the interfacial coverage associated with the formation of products that may be desorbed from the o/w interface [36]. These authors have reported that the protein had already been partially hydrolyzed by pepsin which could again promote the exposure of trypsin and chymotrypsin susceptible sites.

Table 2 reflects a reduction of E in both interfacial films (although not statistically significant) indicating that the resulting interfacial structure was less compact, which could probably occur due to a degradation of the interfacial films caused by the duodenal enzymes.

However, few information exists in the literature in relation to the effects of the duodenal proteases on the interfacial properties of o/w interfaces.

3.3. Intestinal stage 2: SIF 2- duodenal lipolysis

As it was also observed during the digestion of the emulsions formed by these emulsifiers [13], the most important changes occurred after introducing the pancreatic lipase and BS under duodenal conditions (SIF 2 stage in Table 2). These results concur with other studies and suggest that, under the lipolysis conditions, the emulsions and o/w interfaces experiment the most dramatic changes [12,58,63,64]. These changes can be associated with important phenomena such as: BS adsorption to the interface, interactions between BS and partially digested interfacial films, adsorption and activity of pancreatic lipase, changes in the interfacial charges, accumulation of reaction products, etc. BS could also solubilize the lipolytic products in form of micelles. Thus, the resulting characteristics of the interface are very complex [20,65–67].

The changes in γ during the lipolysis (Figs. 1–3) can be analysed by distinguishing two different regions [32,42]: an important reduction of γ was first observed when exchanging the subphase; after that, a slower reduction rate of γ was observed approximately 1800 s later, that continued until the end of this step. The highest decrease in γ values observed at the beginning of this stage for all the systems could be associated with the rapid penetration of the interfacial films by BS which exhibit a high interfacial activity and small sizes with a fast diffusion from the subphase to the interface [23,68]. A rapid adsorption of BS under duodenal conditions was also observed at the interfaces formed by both intact proteins [23] and HPMC [35].

Maldonado-Valderrama, et al. [32] and Torcello-Gomez, et al. [42] suggested that the change in the slope of the decrease of the γ values could be due to saturation of the interface by BS and lipase, as well as the presence of the lipolysis products, such as FFA and monoglycerides, that can remain adsorbed at the interface due to their interfacial activity [69,70]. Consequently, the presence of these lipolytic products at the interface may inhibit the ability of lipase to contact and hydrolyse more lipids, thus retarding the lipolysis process. According to the literature, the increment in the number of FFA released from the emulsions considerably decreased at the end of the duodenal stage because their accumulation at the interface was promoted [13,20,71]. Furthermore, steric factors could also promote the inhibition of the interfacial anchoring of

lipase/colipase, as reported for experiments under static digestion systems.

Results in Table 2 show the strong decrease of E reaching values that were ranging in between those obtained for pure duodenal components and pure protein solutions, thus forming fluid interfacial films. These results indicate that the accumulation of BS and FFA in the interface reduced the degree of interaction in the interfacial films and correlate with the fact that these interfacial films were penetrated by BS upon the subphase exchange under the intestinal conditions [23,35]. These lower E values indicate the formation of a fluid interfacial network which could promote the high degree of destabilization of their emulsions as reported using the same emulsifiers [13,35].

However, the reduction of E values was smaller for SPI films than for βlg films, which indicates that SPI was less disrupted by the penetration of lipase/BS. A high resistance to BS displacement was also observed for non hydrolyzed SPI films [23].

3.4. Desorption stage: DES

This final stage consisted in exchanging the subphase by pure SIF (desorption stage (DES)) to remove any reversibly adsorbed material, such as lipolysis products, proteolysis products, BS or enzymes, from the o/w interface in order to obtain the characteristics of the remnant interfacial films after being exposed to the digestion process. A very quick increment in the γ values (Figs. 1–3) at the beginning of this stage was observed, as well as an increment of the viscoelastic parameters (Fig. 4), indicating that soluble products were eliminated from the interface. Although the γ values increased, they reached lower values than those corresponding to the pure o/w interface (24.0 mN/m), thus indicating the presence of irreversibly adsorbed interfacial materials which remained at the o/w interface such as lipolytic products (FFA and monoglycerides) as reported by Maldonado-Valderrama, et al. [32] for βlg and β-casein interfacial films [72].

The analysis of the increment of γ during the DES stage could help to quantify the resistance of the interfacial films to the remotion of the reversibly adsorbed materials from the o/w interface. The accumulation of materials at the interface has been reported to be one of the mechanisms involved in the modulation of lipase activity [34].

The final γ values (Table 2) show that the increment of γ did occur in the following order: βlg &z.rsquo; HPMC &z.rsquo; SPI. These results could indicate that SPI formed the most resistant interfaces to the remotion of the reversible adsorbed materials followed by HPMC and finally by βlg, that presented the highest increment of γ. This behaviour indicates that SPI and HPMC could develop stronger interactions with the reversible adsorbed materials than βlg, retarding their degree of desorption.

The knowledge of the capacity of the emulsifiers to interact with BS supposes a special interest since it is a key mechanism to modulate the rate and extent of lipolysis [34]. One of the key roles of BS is to prepare the O/W interface for a better access of the lipolytic enzymes to the lipid substrates and then incorporate the lipolytic products into BS micelles that would be transported in the aqueous medium and absorbed to the mucosa of the small intestine [68]. Therefore, if the adsorbed or unadsorbed emulsifiers are capable to interact with BS, a reduction of the ability/availability of BS to improve the lipase activity could occur, thus promoting the accumulation of inhibitory lipolysis products at the O/W interface.

The hypothesis that lipid digestion and lipid absorption could be controlled by the selection of suitable (adsorbed or unabsorbed) emulsifiers which impact in the activity of BS has been previously indicated. Previous reports studying the susceptibility of the interfacial films to be displaced by BS have demonstrated that the displacement could depend on the molecular feature of each emulsifier, as well as on the interfacial coverage [34]. Maldonado-Valderrama, et al. [56] investigated the competitive displacement of βlg by BS from air-water and O/W interfaces under *in vitro* duodenal digestion suggesting that the BS penetrate into, weaken and break up the βlg interfacial networks via an orogenic mechanism [73]. Sarkar, et al. [40] also suggested that BS adsorbed at the emulsion droplet surfaces pushing off the milk protein from the interface. These displacements indicate the lower resistant to the interfacial films and could be related with the higher degree of lipolysis obtained by the emulsions stabilized by these emulsifiers. In conclusion milk proteins itself do not suppose an effective barrier to lipolysis which was attributed to their low resistance to the proteases action or to penetration/displacement carried out by BS [34].

The lowest increment in γ obtained for SPI (Fig. 2) and HPMC (Fig. 3) could indicate the higher affinity between these emulsifiers and BS, which may reduce their desorption degree. This is in accordance with previous results obtained when analyzing the competitive and sequential adsorption between non hydrolyzed proteins (βlg, egg white proteins and soy proteins) and BS under duodenal conditions [23] establishing that SPI resisted the BS displacement, being the only protein that could compete with BS for the interface.

Recent works have demonstrated that the interactions of BS with surface active or non-surface active polysaccharides can decrease the BS content and hence potentially delay or inhibit the lipolysis. Soluble dietary fibers (glucan and arabinoxylan) could interact with BS micelles either by forming dynamic complexes with the micelles or by trapping BS micelles in aggregated structures [74]. Moreover, strong evidences on the interactions between BS and HPMC were demonstrated, as BS were bound or "sequestered" by HPMC, mainly by interactions with the hydrophobic core of HPMC, thus being partially screened their charge [35,75].

This cooperative performance between soy protein and HPMC with BS suggests the existence of favorable molecular interactions. In addition, HPMC stabilized emulsions presented a similar degree of lipolysis than SPI stabilized emulsions, with a much lower extent of lipolysis than that of βlg stabilized emulsions [13].

The capacity of SPI and HPMC to interact with BS has also been associated with the lower plasma total and LDL cholesterol detected in hyper-cholesterolemic humans and laboratory animals. As indicated by Golding and Wooster [76], 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, not only the accumulation of lipolytic products at the interfaces will result in a self-regulation of fat digestion but also prevent the re-absorption of BS from the small intestine into the enterohepatic circulation [77].

4. Conclusions

The evolution of the interfacial tension throughout the whole simulated gastrointestinal transit and the mechanical properties of the interfacial layer after each digestion stage as determined in the Octopus tensiometer could be successfully related to the evolution of emulsions stabilized with the same emulsifiers.

The changes observed during the desorption stage of interfacial lipolysis indicated that lipolysis products would be eliminated more easily from the interface formed by βlg than from those formed by HPMC or SPI, in agreement with the degree of lipolysis of those emulsions reported in a previous work [13]. Thus, the results of the present work seem to be consistent with the relation between a reduced lipolysis and a lower withdrawal of the materials, such as BS micelles, from the o/w interface.

This work evidences the crucial role of the interfacial region in the digestion process where the interactions of emulsifiers and BS would influence their availability to remove lipolysis products, thus being a key factor to control the rate and extent of lipolysis.

Acknowledgments

This work has been supported by Universidad de Buenos Aires (Grant Number 20020130100524), Consejo Nacional de Investigaciones Científicas y Técnicas, Agencia Nacional de Promoción Científica y Tecnológica (Grant Number: PICT-2014-3668) and BEC.AR.

References

[1] S.S. Narine, A.G. Marangoni, Relating structure of fat crystal networks to mechanical properties: a review, Food Res. Int. 32 (1999) 227–248.
[2] B.S. Ghotra, S.D. Dyal, S.S. Narine, Lipid shortenings: a review, Food Res. Int. 35 (2002) 1015–1048.
[3] M.C. Michalski, C. Genot, C. Gayet, C. Lopez, F. Fine, F. Joffre, J.L. Vendeuvre, J. Bouvier, J.M. Chardigny, K. Raynal-Ljutovac, Multiscale structures of lipids in foods as parameters affecting fatty acid bioavailability and lipid metabolism, Prog. Lipid Res. 52 (2013) 354–373.
[4] J. Marciani, R. Faulks, M.S.J. Wickham, D. Bush, B. Pick, J. Wright, E.F. Cox, A. Fillery-Travis, P.A. Gowland, R.C. Spiller, Effect of intragastric acid stability of fat emulsions on gastric emptying plasma lipid profile and postprandial satiety, Br. J. Nutr. 101 (2009).
[5] H. Singh, A. Ye, D. Horne, Structuring food emulsions in the gastrointestinal tract to modify lipid digestion, Prog. Lipid Res. 48 (2009) 92–100.
[6] D.J. McClements, E. Decker, Y. Park, Controlling lipid bioavailability through physicochemical and structural approaches, Food Sci. Nutr. 49 (2009) 48–67.
[7] R. Weiss, J. Dziura, S. Burgert, W. Tamborlane, S. Taksali, C. Yeckel, K. Allen, M. Lopes, M. Savoye, J. Morrison, R. Sherwin, S. Caprio, Obesity and the metabolic syndrome in children and adolescents, N. Engl. J. Med. 350 (2004) 2362–2374.
[8] H. García-Alcala, D. Cuevas-Ramos, C. Genestier-Tamborero, O. Hirales-Tamez, P. Almeda-Valdés, R. Mehta, C. Aguilar-Salinas, Significant increment in the prevalence of overweight and obesity documented between 1994 and 2008 in Mexican college students, diabetes, Metab. Syndr. Obes.: Targets Ther. 3 (2010) 79–85.
[9] World Health Organization, World Report in Obesity and Overweight, 2009.
[10] A. Sarkar, D.S. Horne, H. Singh, Pancreatin-induced coalescence of oil-in-water emulsions in an in vitro duodenal model, Int. Dairy J. 20 (2010) 589–597.
[11] A. Helbig, E. Silletti, E. Timmerman, R.J. Hamer, H. Gruppen, In vitro study of intestinal lipolysis using pH-stat and gas chromatography, Food Hydrocoll. 28 (2012) 10–19.
[12] U. Lesmes, D.J. McClements, Controlling lipid digestibility: response of lipid droplets coated by β-lactoglobulin-dextran Maillard conjugates to simulated gastrointestinal conditions, Food Hydrocoll. 26 (2012) 221–230.
[13] F.A. Bellesi, M.J. Martinez, V.M. Pizones Ruiz-Henestrosa, A.M.R. Pilosof, Comparative behavior of protein or polysaccharide stabilized emulsion under in vitro gastrointestinal conditions, Food Hydrocoll. 52 (2016) 47–56.
[14] P.J. Wilde, R.J. Ridout, A.R. Mackie, M.S.J. Wickham, R.M. Faulks, Modulating lipid delivery in food emulsions, in: Q. Huang, P. Given, M. Qian (Eds.), Micro/Nanoencapsulation of Active Food Ingredients, 2007, pp. 67–88.
[15] D.J. McClements, Y. Li, Structured emulsion-based delivery systems: controlling the digestion and release of lipophilic food components, Adv. Colloid Interface Sci. 159 (2010) 213–228.
[16] B. Zeeb, C.L. Lopez-Pena, J. Weiss, D.J. McClements, Controlling lipid digestion using enzyme-induced crosslinking of biopolymer interfacial layers in multilayer emulsions, Food Hydrocoll. 46 (2015) 125–133.
[17] D.J. McClements, Encapsulation, protection, and release of hydrophilic active components: potential and limitations of colloidal delivery systems, Adv. Colloid Interface Sci. 219 (2015) 27–53.
[18] S. Mun, E.A. Decker, D.J. McClements, Influence of emulsifier type on in vitro digestibility of lipid droplets by pancreatic lipase, Food Res. Int. 40 (2007) 770–781.
[19] S. Sandra, E. Decker, D. McClements, Effect of interfacial protein cross-linking on the in vitro digestibility of emulsified corn oil by pancreatic lipase, J. Agric. Food Chem. 56 (2008) 7488–7494.
[20] D.J. McClements, Y. Li, Review of in vitro digestion models for rapid screening of emulsion-based, Food Funct. 1 (2010) 32–59.
[21] J. Maldonado-Valderrama, R. Miller, V.B. Fainerman, P.J. Wilde, V.J. Morris, Effect of gastric conditions on β-lactoglobulin interfacial networks: influence of the oil phase on protein structure, Langmuir 26 (2010) 15901–15908.
[22] H. Singh, A. Sarkar, Behaviour of protein-stabilised emulsions under various physiological conditions, Adv. Colloid Interface Sci. 165 (2011) 47–57.
[23] F.A. Bellesi, V.M. Pizones Ruiz-Henestrosa, A.M.R. Pilosof, Behavior of protein interfacial films upon bile salts addition, Food Hydrocoll. 36 (2014) 115–122.
[24] A. Lindahl, A.-L. Ungell, L. Knutson, H. Lennernäs, Characterization of fluids from the stomach and proximal jejunum in men and women, Pharm. Res. 14 (1997) 497–502.
[25] S.N. Murthy, J. Kostman, V.P. Dinoso, Effect of pH, substrate, and temperature on tryptic activity of duodenal samples, Dig. Dis. Sci. 25 (1980) 289–294.
[26] E. Bauer, S. Jakob, R. Mosenthin, Principles of physiology of lipid digestion, J. Anim. Sci. 18 (2005) 282–295.
[27] H. Mu, C.E. Hoy, The digestion of dietary triacylglycerols, Prog. Lipid Res. 43 (2004) 105–133.
[28] S. Mun, E.A. Decker, Y. Park, J. Weiss, D.J. McClements, Influence of interfacial composition on in vitro digestibility of emulsified lipids: potential mechanism for chitosan's ability to inhibit fat digestion, Food Biophys. 1 (2006) 21–29.
[29] S.J. Hur, E.A. Decker, D.J. McClements, Influence of initial emulsifier type on microstructural changes occurring in emulsified lipids during in vitro digestion, Food Chem. 114 (2009) 253–262.
[30] Z. Wang, M.A. Neves, L.-J. Yin, I. Kobayashi, K. Uemura, M. Nakajima, In vitro gastrointestinal digestibility of soybean oil-in-water emulsion droplets stabilized by polyglycerol esters of fatty acid, Food Sci. Technol. Res. 18 (2012) 149–156.
[31] T.J. Wooster, L. Day, M. Xu, M. Golding, S. Oiseth, J. Keogh, P. Clifton, Impact of different biopolymer networks on the digestion of gastric structured emulsions, Food Hydrocoll. 36 (2014) 102–114.
[32] J. Maldonado-Valderrama, J.A. Holgado-Terriza, A. Torcello-Gomez, M.A. Cabrerizo-Vilchez, In vitro digestion of interfacial protein structures, Soft Matter 9 (2013) 1043–1053.
[33] J. Maldonado-Valderrama, A. Torcello-Gómez, T. del Castillo-Santaella, J.A. Holgado-Terriza, M.A. Cabrerizo-Vílchez, Subphase exchange experiments with the pendant drop technique, Adv. Colloid Interface Sci. 222 (2015) 488–501.
[34] A.M.R. Pilosof, Potential impact of interfacial composition of proteins and polysaccharides stabilized emulsions on the modulation of lipolysis. The role of bile salts, Food Hydrocoll. 68 (2017) 178–185.
[35] V.M. Pizones Ruiz-Henestrosa, F.A. Bellesi, N.A. Camino, A.M.R. Pilosof, The impact of HPMC structure in the modulation of in vitro lipolysis: the role of bile salts, Food Hydrocoll. 62 (2017) 251–261.
[36] T. del Castillo-Santaella, E. Sanmartín, M.A. Cabrerizo-Vílchez, J.C. Arboleya, J. Maldonado-Valderrama, Improved digestibility of β-lactoglobulin by pulsed light processing: a dilatational and shear study, Soft Matter 10 (2014) 9702–9714.
[37] D.J. Carp, G.B. Bartholomai, A.M.R. Pilosof, A kinetic model to describe liquid drainage from soy protein foams over an extensive protein concentration range, LWT –Food Sci. Technol. 30 (1997) 253–258.
[38] N.A. Camino, A.M.R. Pilosof, Hydroxypropylmethylcellulose at the oil–water interface. Part II. Submicron-emulsions as affected by pH, Food Hydrocoll. 25 (2011) 1051–1062.
[39] J. Bahtz, D. Knorr, C. Tedeschi, M.E. Leser, B. Valles-Pamies, R. Miller, Adsorption of octanoic acid at the water/oil interface, Colloids Surf. B: Biointerfaces 74 (2009) 492–497.
[40] A. Sarkar, D.S. Horne, H. Singh, Interactions of milk protein-stabilized oil-in-water emulsions with bile salts in a simulated upper intestinal model, Food Hydrocoll. 24 (2010) 142–151.
[41] M.J. Martinez, G. Martos, E. Molina, A.M. Pilosof, Reduced beta-lactoglobulin IgE binding upon in vitro digestion as a result of the interaction of the protein with casein glycomacropeptide, Food Chem. 192 (2016) 943–949.
[42] A. Torcello-Gomez, J. Maldonado-Valderrama, A.B. Jodar-Reyes, M.A. Cabrerizo-Vilchez, A. Martin-Rodriguez, Pluronic-covered oil-water interfaces under simulated duodenal conditions, Food Hydrocoll. 34 (2014).
[43] A. Berthold, H. Schubert, N. Brandes, L. Kroh, R. Miller, Behaviour of BSA and of BSA-derivatives at the air/water interface, Colloids Surf. A: Physicochem. Eng. 301 (2007) 16–22.
[44] V.N. Kazakov, V.B. Fainerman, P.G. Kondratenko, A.F. Elin, O.V. Sinyachenko, R. Miller, Dilational rheology of serum albumin and blood serum solutions as studied by oscillating drop tensiometry, Colloids Surf. B: Biointerfaces 62 (2008) 77–82.
[45] N.A. Camino, O.E. Pérez, C.C. Sanchez, J.M. Rodriguez Patino, A.M.R. Pilosof, Hydroxypropylmethylcellulose surface activity at equilibrium and adsorption dynamics at the air–water and oil–water interfaces, Food Hydrocoll. 23 (2009) 2359–2368.
[46] V.M. Pizones Ruiz-Henestrosa, Optimización De Las Propiedades Interfaciales Y Espumantes De Las Globulinas De Soja, Departamento de Ingeniería Química, Universidad de Sevilla, España, 2008.
[47] F. MacRitchie, Protein adsorption/desorption at fluid interfaces, Colloids Surfaces 41 (1989) 25–34.
[48] V.J. Morris, A.P. Gunning, Microscopy, microstructure and displacement of proteins from interfaces: implications for food quality and digestion, Soft Matter 4 (2008) 943–951.
[49] J. Maldonado-Valderrama, P.J. Wilde, F. Mulholland, V.J. Morris, Protein unfolding at fluid interfaces and its effect on proteolysis in the stomach, Soft Matter 8 (2012) 4402–4414.
[50] A. Macierzanka, A.I. Sancho, E.N.C. Mills, N.M. Rigby, A.R. Mackie, Emulsification alters simulated gastrointestinal proteolysis of β-casein and β-lactoglobulin, Soft Matter 5 (2009) 538–550.
[51] J. Mouecoucou, C. Villaume, C. Sanchez, L. Mejean, Beta-lactoglobulin/polysaccharide interactions during in vitro gastric and pancreatic hydrolysis assessed in dialysis bags of different molecular weight cut-offs, Biochim. Biophys. Acta 22 (2004) 105–112.
[52] A. Mackie, A. Macierzanka, Colloidal aspects of protein digestion, Curr. Opin. Colloid Interface Sci. 15 (2010) 102–108.

[53] A. Malaki Nik, A.J. Wright, M. Corredig, Surface adsorption alters the susceptibility of whey proteins to pepsin-digestion, J. Colloid Interface Sci. 344 (2010) 372–381.
[54] M. Fathi, Á. Martín, D.J. McClements, Nanoencapsulation of food ingredients using carbohydrate based delivery systems, Trends Food Sci. Technol. 39 (2014) 18–39.
[55] D. Mudgil, S. Barak, Composition, properties and health benefits of indigestible carbohydrate polymers as dietary fiber: a review, Int. J. Biol. Macromol. 61 (2013) 1–6.
[56] J. Maldonado-Valderrama, N.C. Woodward, A.P. Gunning, M.J. Ridout, F.A. Husband, A.R. Mackie, V.J. Morris, P.J. Wilde, Interfacial characterization of β-lactoglobulin networks: displacement by bile salts, Langmuir 24 (2008) 6759–6767.
[57] A.R. Mackie, A.P. Gunning, M.J. Ridout, P.J. Wilde, J. Rodriguez Patino, In situ measurement of the displacement of Protein ilms from the air/water interface by surfactant, Biomacromolecules 2 (2001) 1001–1006.
[58] H. Singh, A. Ye, Structural and biochemical factors affecting the digestion of protein-stabilized emulsions, Curr. Opin. Colloid Interface Sci. 18 (2013) 360–370.
[59] S. Roth, B.S. Murray, E. Dickinson, Interfacial shear rheology of aged and heat-treated beta-lactoglobulin films: displacement by nonionic surfactant, J. Agric. Food Chem. 48 (2000) 1491–1497.
[60] T.J. Wooster, M.A. Augustin, The emulsion flocculation stability of protein–carbohydrate diblock copolymers, J. Colloid Interface Sci. 313 (2007) 665–675.
[61] S. Tcholakova, N.D. Denkov, D. Sidzhakova, B. Campbell, Effect of thermal treatment, ionic strength, and pH on the short-term and long-term coalescence stability of beta-lactoglobulin emulsions, Langmuir 22 (2006) 6042–6052.
[62] H.J. Kim, E.A. Decker, D.J. McClements, Impact of protein surface denaturation on droplet flocculation in hexadecane oil-in-water emulsions stabilized by β-lactoglobulin, J. Agric. Food Chem. 50 (2002) 7131–7137.
[63] A. Malaki Nik, A.J. Wright, M. Corredig, Impact of interfacial composition on emulsion digestion and rate of lipid hydrolysis using different in vitro digestion models, Colloids Surf. B: Biointerfaces 83 (2011) 321–330.
[64] A. Sarkar, K.K.T. Goh, R.P. Singh, H. Singh, Behaviour of an oil-in-water emulsion stabilized by β-lactoglobulin in an in vitro gastric model, Food Hydrocoll. 23 (2009) 1563–1569.
[65] S. Gallier, H. Tate, H. Singh, In vitro gastric and intestinal digestion of a walnut oil body dispersion, J. Agric. Food Chem. 61 (2013) 410–417.
[66] A. Malaki Nik, A.J. Wright, M. Corredig, Interfacial design of protein-stabilized emulsions for optimal delivery of nutrients, Food Funct. 1 (2010) 141–148.
[67] H. Singh, A. Sarkar, Behaviour of protein-stabilised emulsions under various physiological conditions, Adv. Colloid Interface Sci. 165 (2011) 47–57.
[68] J. Maldonado-Valderrama, P. Wilde, A. Macierzanka, A. Mackie, The role of bile salts in digestion, Adv. Colloid Interface Sci. 165 (2011) 36–46.
[69] P. Reis, K. Holmberg, H. Watzke, M.E. Leser, R. Miller, Lipases at interfaces: a review, Adv. Colloid Interface Sci. 147–148 (2009) 237–250.
[70] E. Troncoso, J.M. Aguilera, D.J. McClements, Fabrication, characterization and lipase digestibility of food-grade nanoemulsions, Food Hydrocoll. 27 (2012) 355–363.
[71] M. Espinal-Ruiz, F. Parada-Alfonso, L.-P. Restrepo-Sánchez, C.-E. Narváez-Cuenca, D.J. McClements, Impact of dietary fibers [methyl cellulose, chitosan, and pectin] on digestion of lipids under simulated gastrointestinal conditions, Food Funct. 5 (2014) 3083–3095.
[72] T. del Castillo-Santaella, J. Maldonado-Valderrama, M.A. Cabrerizo-Vilchez, C. Rivadeneira-Ruiz, D. Rondon-Rodriguez, M.J. Galvez-Ruiz, Natural inhibitors of lipase: examining lipolysis in a single droplet, J. Agric. Food Chem. 63 (2015) 10333–10340.
[73] N.C. Woodward, A.P. Gunning, A.R. Mackie, P.J. Wilde, V.J. Morris, Comparison of the orogenic displacement of sodium caseinate with the caseins from the Airâ̂Water interface by nonionic surfactants, Langmuir 25 (2009) 6739–6744.
[74] P. Gunness, B.M. Flanagan, J.P. Mata, E.P. Gilbert, M.J. Gidley, Molecular interactions of a model bile salt and porcine bile with (1,3:1,4)-β-glucans and arabinoxylans probed by 13C NMR and SAXS, Food Chem. 197 (2016) 676–685 (Part A).
[75] A. Torcello-Gómez, T.J. Foster, Influence of interfacial and bulk properties of cellulose ethers on lipolysis of oil-in-water emulsions, Carbohydr. Polym. 144 (2016) 495–503.
[76] M. Golding, T.J. Wooster, The influence of emulsion structure and stability on lipid digestion, Curr. Opin. Colloid Interface Sci. 15 (2010) 90–101.
[77] L. Chiappisi, M. Gradzielski, Co-assembly in chitosan–surfactant mixtures: thermodynamics, structures, interfacial properties and applications, Adv. Colloid Interface Sci. 220 (2015) 92–107.