Food Research International 137 (2020) 109360

Contents lists available at ScienceDirect

Food Research International

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

Impact of whey protein/surfactant mixture and oil type on the gastrointestinal fate of emulsions: Ingredient engineering

Andresa Gomes[a], Ana Letícia Rodrigues Costa[b], Dayane Dias Cardoso[a], Guilherme de Figueiredo Furtado[a], Rosiane Lopes Cunha[a,*]

[a] Department of Food Engineering (DEA), Faculty of Food Engineering (FEA), University of Campinas (UNICAMP), Rua Monteiro Lobato, 80, Campinas, SP CEP: 13083-862, Brazil

[b] Department of Materials and Bioprocess Engineering, School of Chemical Engineering, University of Campinas, Av. Albert Einstein, 500, Campinas, SP CEP: 13083-852, Brazil

ARTICLE INFO

Keywords:
WPI/Tween 80 mixture
Sunflower oil
MCT oil
Controlled lipolysis
Ingredient engineering

ABSTRACT

The engineering of ingredients emerges as a strategy to design emulsified products aiming to control the lipid hydrolysis. In this context, oil-in-water (O/W) emulsions composed of different oil phases (Sunflower oil - LCT or NEOBEE® 1053 - MCT) and stabilized by whey protein isolate - WPI (1% w/w), Tween 80 – T80 (1% w/w) or varied ratios of WPI/T80 (0.9975%WPI/0.0025%T80; 0.75%WPI/0.25%T80; 0.5%WPI/0.5%T80 w/w) were produced and submitted to simulated gastrointestinal conditions. The lipolysis of LCT was influenced by the fatty acid chain length and emulsifier composition, while only the fatty acid chain length affected the lipolysis of MCT. The emulsions produced with LCT and 1%WPI or 09975%WPI/00025%T80 showed the highest release rate of free fatty acids (FFAs), but similar result was observed for the 0.5%WPI/0.5%T80 system. In the 0.5% WPI/0.5%T80 mixture, WPI and T80 worked together and achieved an improved performance during the gastric (stability similar as 1%T80 emulsion) and small intestinal phases (lipolysis similar as 1%WPI emulsion). The rational selection of ingredients is useful to design emulsions with improved performance as a delivery system since the emulsion structural stability during digestion, the oil type and interaction between lipase-interface had a marked impact on the efficiency of lipid digestion.

1. Introduction

Lipids play a central role in the sensory properties of food products. Some of these properties that are relevant to mention are consistency, creaminess and palatability, which are associated with pleasantness of food in the mouth and satiation sensory cue (Day et al., 2014; Lett, Norton, & Yeomans, 2016). Also, the vehiculation of functional lipophilic compounds in lipid matrices increases the water solubility of these bioactive compounds promoting an improvement of their bioaccessibility (Salvia-Trujillo et al., 2017). However, lipid is the macronutrient with the highest energy density and its overconsumption is often cited as a major cause of obesity and increased incidence of diabetes and heart diseases (Lett et al., 2016). In this way, designing food structures for functional benefits must consider the paradoxical role that lipids play in our diet.

Lipids are incorporated in many processed foods, such as oil-in-water (O/W) emulsion based systems (Guo, Ye, Bellissimo, Singh, & Rousseau, 2017). Emulsion microstructure exerts a significant impact on gastrointestinal fate and may, therefore, be manipulated to develop delivery systems with modulated lipid hydrolysis (lipolysis) rate, depending of the desired characteristics of the end product.

The engineering of ingredients emerges as a strategy to design emulsified products with characteristics directed to specific needs (Guo et al., 2017). Such products could be built by selecting a specific fitted combination of the lipid phase and emulsifier or a mixture of emulsifiers, such as protein/surfactant (Singh, Ye, & Ferrua, 2015). Proteins, like whey proteins, stabilize emulsions by electrostatic and/or steric repulsion mechanisms (Ozturk & McClements, 2016). However, proteins exhibit a slow diffusion into the oil–water interface, and are strongly dependent on environmental conditions such as temperature, pH and ionic strength, which may restrict their application in colloidal systems (McClements & Gumus, 2016). In the stomach, due to the low pH and presence of enzymes, the droplets of emulsions stabilized by WPI lose the stabilization mechanism promoted by electrostatic repulsion and become aggregated, as consequence of flocculation process (Golding et al., 2011). The weakening of the protein layer that

* Corresponding author.
E-mail address: rosiane@unicamp.br (R.L. Cunha).

https://doi.org/10.1016/j.foodres.2020.109360
Received 28 February 2020; Received in revised form 22 May 2020; Accepted 25 May 2020
Available online 10 June 2020

Table 1
Mean particle size (D_{43}) of the fresh emulsions and after the gastric and the intestinal steps produced with LCT or MCT oil and stabilized by WPI, WPI/Tween80 mixture or Tween 80.

Oil type	Composition of emulsifier (w/w)	Fresh D_{43} (μm) ± StDev[1]	Gastric D_{43} (μm) ± StDev[1]	Intestinal D_{43} (μm) ± StDev[1]
LCT	1%WPI	$1.55 \pm < 0.01^{aA***}$	$30.53 \pm 2.66^{aA*}$	$25.18 \pm 1.36^{aB**}$
	0.9975%WPI/0.0025%T80	$1.32 \pm < 0.01^{bA***}$	$30.81 \pm 2.21^{aA*}$	$21.67 \pm 6.46^{aB**}$
	0.75%WPI/0.25%T80	$1.29 \pm 0.07^{bA***}$	$13.20 \pm 0.89^{bA**}$	$20.33 \pm 3.80^{aB*}$
	0.5%WPI/0.5%T80	$1.24 \pm 0.14^{bA**}$	$1.37 \pm 0.19^{cA**}$	$23.72 \pm 3.69^{aB*}$
	1%T80	$1.22 \pm 0.05^{bA**}$	$1.32 \pm 0.02^{cA**}$	$20.94 \pm 2.52^{aB*}$
MCT	1%WPI	$1.42 \pm < 0.01^{aB***}$	$18.90 \pm 4.48^{aB**}$	$128.9 \pm 10.4^{aA*}$
	0.9975%WPI/0.0025%T80	$1.21 \pm 0.29^{bA*}$	$17.43 \pm 1.53^{aB**}$	$106.4 \pm 15.7^{abA*}$
	0.75%WPI/0.25%T80	$1.03 \pm 0.01^{bcB**}$	$8.51 \pm 2.57^{bB**}$	$111.5 \pm 14.6^{bA*}$
	0.5%WPI/0.5%T80	$1.00 \pm < 0.01^{cB**}$	$1.32 \pm 0.15^{cA**}$	$105.3 \pm 19.7^{bA*}$
	1%T80	$0.95 \pm 0.05^{cB**}$	$1.28 \pm 0.06^{cB**}$	$105.0 \pm 9.1^{bA*}$

[1] Standard deviation (StDev). Different letters indicate significant difference at $p < 0.05$. Small letters: differences in the same column among different emulsifier composition (1%WPI; 0.9975%WPI/0.0025%T80; 0.75%WPI/0.25%T80; 0.5%WPI/0.5%T80 or 1%T80) at the same oil phase. Capital letters: differences in the same column between LCT and MCT samples at the same emulsifier composition. Asterisk: differences in the same line among fresh emulsion and after the gastric and intestinal steps at the same emulsifier and oil composition.

surrounds the flocculated droplets may induce a further destabilization by coalescence, reducing the interfacial area accessible for lipolysis in the intestinal phase (Costa et al., 2020). Differently, Tween 80 (T80) is a nonionic surfactant that rapidly reduces interfacial tension between the phases, facilitating the emulsification process, even though it is a synthetic emulsifier. Emulsions stabilized by nonionic surfactants are not affected by the environmental conditions of the stomach, though this surfactant class offers some resistance to the lipase adsorption onto the droplets surface reducing the lipolysis rate (Yao et al., 2013). Therefore, we hypothesized, that the WPI/T80 mixture would achieve a greater performance than each emulsifier alone. In the mixture, T80 would improve the stability of emulsion during the gastric phase while the WPI would facilitate the adsorption of lipase onto the interface during the intestinal phase. Also, protein/surfactant mixture is a strategy to reduce the proportion of chemically synthetized compounds in food formulations (Gülseren & Corredig, 2014).

The oil phase also influences lipolysis since it may alter the interfacial layer composition and the emulsifier adsorption process onto the droplet surface (Gomes, Costa, & Cunha, 2018). Emulsions produced with medium chain triacylglycerols (MCTs) generally have a higher release rate of free fatty acids (FFAs) during intestinal phase than those with long chain triacylglycerols (LCTs). Nevertheless, LCT forms mixed micelles with larger hydrophobic domains than those formed by MCT. The hydrophobic domains are responsible for solubilization of the bioactive compound and for its subsequent adsorption in the intestinal mucosa (McClements, 2018).

In the last years some studies have evaluated the lipolysis in emulsions produced with different emulsifiers (Borreani, Leonardi, Moraga, Quiles, & Hernando, 2019; Costa et al., 2020; Hou, Liu, Lei, & Gao, 2014; Karthik & Anandharamakrishnan, 2016). Likewise, other studies have investigated the influence of different lipid phases on the lipid hydrolysis (Majeed et al., 2016; Salvia-Trujillo et al., 2019; Schoener, Zhang, Lv, Weiss, & McClements, 2019; Verkempinck et al., 2018). However, the effect of the interaction among protein/surfactant mixtures and lipid phases (chain length/saturation degree) on the behavior of the emulsions submitted to *in vitro* digestion has been little studied.

In this context, we produced O/W emulsions stabilized by WPI, T80 or WPI/T80 mixtures with different oil phases (Sunflower oil-LCT and NEOBEE® 1053-MCT) and we evaluate their behavior through the simulated gastrointestinal tract (GIT), considering two aspects: 1) emulsion stability during digestion steps and 2) lipolysis process. This information allowed us to better understand how the interaction between different ingredients (single emulsifier, mixture of emulsifiers, lipid phase) would affect the lipid hydrolysis.

2. Material and methods

2.1. Material

The ingredients used to prepare the emulsions were polyoxyethylene sorbitan monooleate (Tween 80) obtained from Dinamica Quimica Contemporanea Ltda (Diadema, Brazil), whey protein isolate – WPI (approximately 90% w/w protein) kindly donated by Fonterra Co-operative Group Limited (Auckland, New Zealand) and ultrapure water from a Millipore Milli-Q system (resistivity 18.2 MΩ/cm). Sunflower oil - LCT (Bunge Alimentos S.A., Brazil) was purchased from the local market and the medium chain triacylglycerol - MCT (NEOBEE® 1053) was kindly donated by Stepan Lipid Nutrition (Northfield, USA). The fatty acid composition of oils was obtained by gas chromatograph with a capillary column–CGC Agilent 6850 Series GC System (Santa Clara, CA), after esterification (Hartman, 1973). LCT main fatty acid composition was 5.29% palmitic acid (16:0), 3.72% stearic acid (18:0), 41.48% oleic acid (18:1) and 47.64% linoleic acid (18:2). The main fatty acid composition of MCT was 51.41% caprylic acid (8:0) and 47.30% capric acid (10:0). All ingredients were used without further purification based on commercial products that are commonly used in industrial applications.

Bile extract porcine (B8631), pancreatin from porcine (P7545) and pepsin from porcine gastric mucosa (P6887) were purchased from Sigma-Aldrich (St. Louis, USA). The other reagents used in the experiments were analytical grade.

2.2. Emulsion preparation

Oil-in-water (O/W) emulsions were prepared using the same oil to aqueous phases weight ratio (10:90). The oil phase was composed of sunflower oil (LCT) or NEOBEE® 1053 (MCT), while the aqueous phase was composed of solutions with 1%WPI, WPI/T80 mixture (0.9975% WPI/0.0025%T80, 0.75%WPI/0.25%T80, 0.5%WPI/0.5%T80) or 1%T80 (w/w). The WPI/T80 ratios were chosen to verify the influence of the Tween 80 concentration above the critical micellar concentration- CMC (0.0025%) (Grigoriev, Derkatch, Krägel, & Miller, 2007). Each emulsifier was dissolved in water for 2 h using a magnetic stirrer at 25 °C, then, the solutions with different ratios of WPI/T80 were prepared. The emulsion was produced by pre-mixing the oil and aqueous phases using an Ultra Turrax model T18 (IKA, Staufen, Germany) for 3 min at 14,000 rpm, followed by homogenization at 50 MPa/5 MPa using a Panda 2KNS1001L double-stage homogenizer (Niro Soavi, Parma, Italy). The samples were passed through the homogenizer only once. The apparent viscosity of LCT/MCT emulsions (100 s^{-1}) varied from 1.55 to 1.65 (mPa.s) (Gomes et al., 2018). Emulsions were

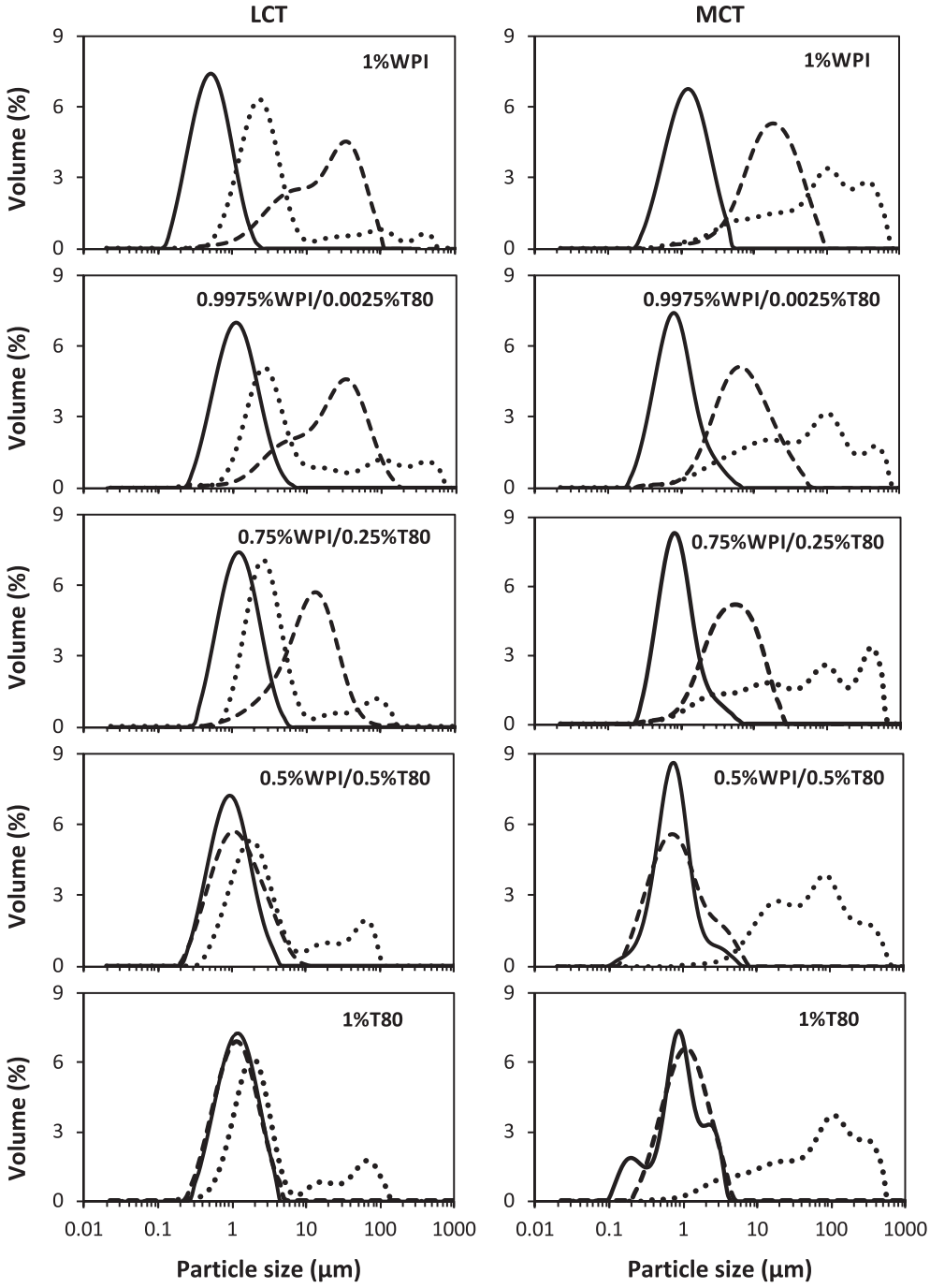

Fig. 1. Volume size distribution of the emulsions produced with LCT or MCT stabilized by WPI, WPI/Tween 80 mixture or Tween 80: (—) fresh emulsions; (− −) after gastric and (·····) intestinal phases.

evaluated by means of optical microscopy, mean droplet size (D_{43}), droplet size distribution, ζ-potential and *in vitro* digestibility. Measurements were performed just after emulsions preparation in duplicate and all measurements were performed three times.

2.3. Characterization of emulsions

2.3.1. Optical microscopy

Fresh emulsions (up to two hours after preparation) were examined in an optical microscope (Axio Scope.A1, Carl Zeiss, Germany) with 100x oil immersion objective lens. The images were captured with the software AxioVision Rel. 4.8 (Carl Zeiss, Germany).

2.3.2. Particle size distribution

The particle size distribution was determined by the static laser diffraction method using a Mastersizer 2000 (Malvern Instruments Ltd, Malvern, UK). The samples were dispersed in water and the rotational velocity was 1,750 rpm.

The average particle diameter was expressed as the mode of the size distribution curves (peak values) or as the volume-surface mean diameter (D_{43}) calculated by Eq. (1).

$$D_{43} = \frac{\sum n_i d_i^4}{\sum n_i d_i^3} \quad (1)$$

where n_i is the number of droplets with diameter d_i

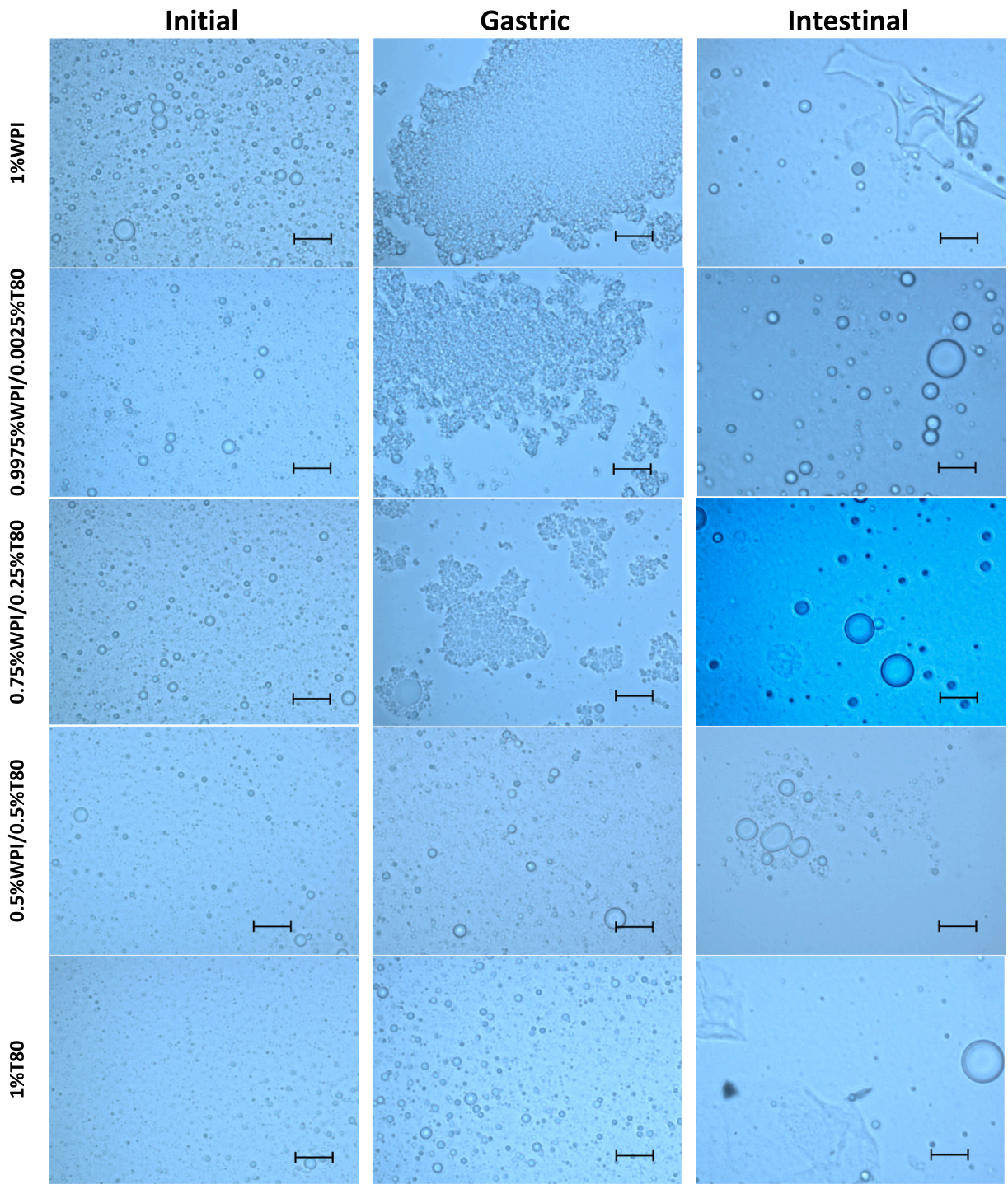

Fig. 2. Optical micrographs of the O/W emulsions produced with LCT and stabilized by 1%WPI, 0.9975%WPI/0.0025%T80, 0.75%WPI/0.25%T80, 0.5%WPI/0.5%T80 or 1%T80: fresh and after gastric and intestinal phases. Scale bar = 10 μm.

2.3.3. ζ-potential

The determination of ζ-potential of the samples dispersed in water (0.001%) was performed at 25 °C using a Zetasizer Nano-ZS (Malvern Instruments, UK). The electrophoretic mobility was obtained by Laser Doppler Anemometry technique and the mathematical model proposed by Smoluchowski was used to convert electrophoretic mobility measurements in ζ-potential values. The ζ-potential was measured to provide indirect information about changes in the interfacial composition during different steps of the *in vitro* digestion model.

2.3.4. In vitro digestion of emulsions and fatty acid release

The emulsions were digested by subjecting them to sequential incubation in simulated gastric fluid (SGF) and then simulated intestinal fluid (SIF) using the slight modified *in vitro* digestion protocol (Minekus et al., 2014; Brodkorb et al., 2019) where, according to the authors, the mouth phase can be eliminated for liquid samples. The samples were placed in a stirred (100 rpm) double jacketed reaction vessel maintained at 37 ± 1 °C (Mun, Park, Kim, & McClements, 2016). Then, 60 mL of each sample was incubated for 2 h with 60 mL of SGF at pH 3 (SGF contained 6.9 mmol L^{-1} of KCl, 0.9 mmol L^{-1} KH_2PO_4, 25.0 mmol L^{-1} $NaHCO_3$, 47.2 mmol L^{-1} NaCl, 0.1 mmol L^{-1}

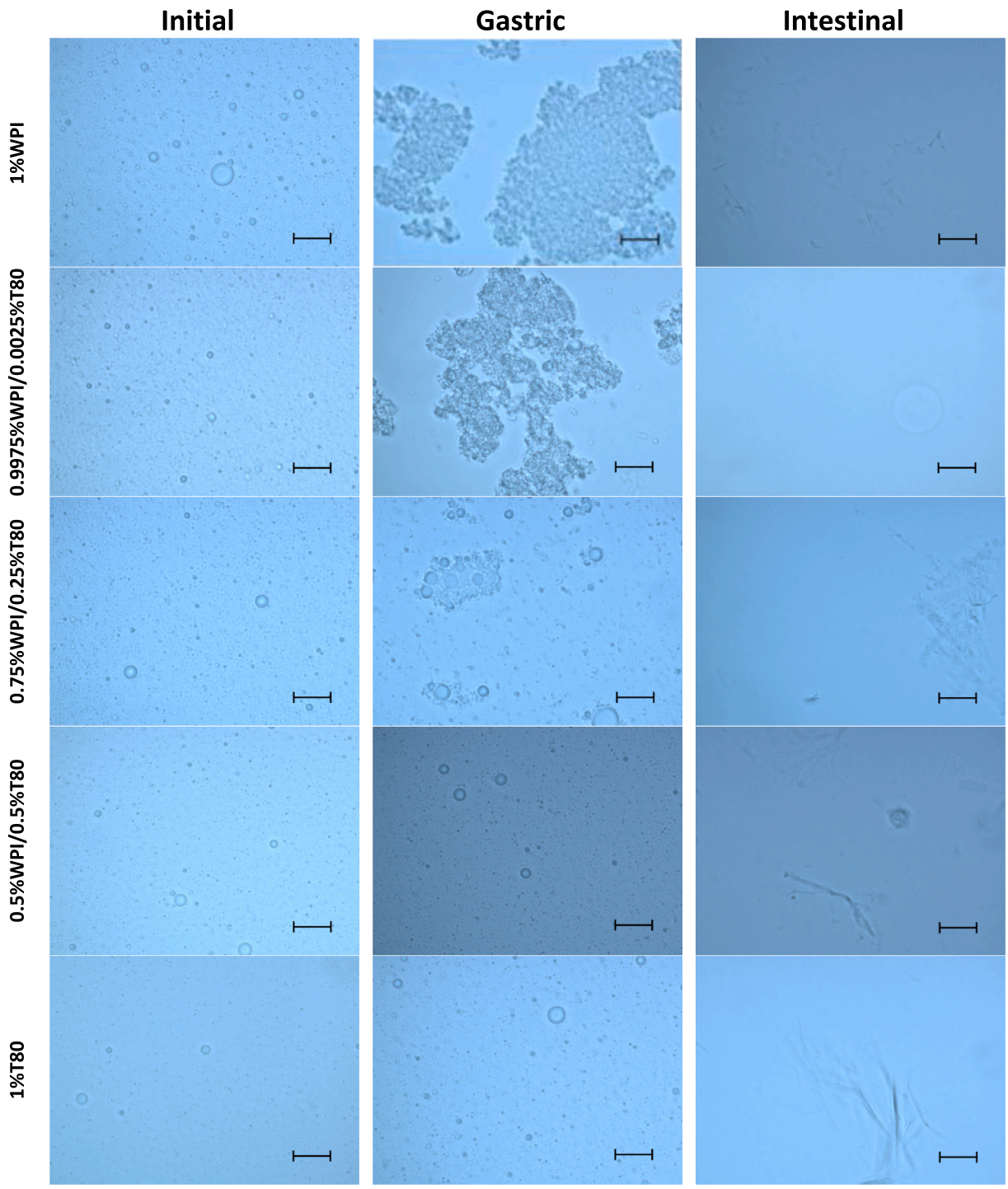

Fig. 3. Optical micrographs of the O/W emulsions produced with MCT and stabilized by 1%WPI, 0.9975%WPI/0.0025%T80, 0.75%WPI/0.25%T80, 0.5%WPI/0.5%T80 or 1%T80: fresh and after gastric and intestinal phases. Scale bar = 10 μm.

$MgCl_2(H_2O)_6$, 0.5 mmol L^{-1} $(NH_4)_2CO_3$, 0.15 mmol L^{-1} $CaCl_2(H_2O)_2$ and 9.6 mL of fresh pepsin dispersion (25,000 U mL^{-1})). After 2 h of incubation in SGF, 20 mL of sample was collected for immediate characterization (Sections 2.3.1, 2.3.2 and 2.3.3). Then, the sample + SGF was mixed (1:1) with SIF. The temperature was adjusted to 37 ± 1 °C and the pH was adjusted to 7 with 1 M NaOH. The SIF contained 6.8 mmol L^{-1} KCl, 0.8 mmol L^{-1} KH_2PO_4, 85.0 mmol L^{-1} $NaHCO_3$, 38.42 mmol L^{-1} NaCl, 0.33 mmol L^{-1} $MgCl_2(H_2O)_6$, 0.6 mmol L^{-1} $CaCl_2(H_2O)_2$, 70.72 g L^{-1} of bile salts and 25 mL of fresh pancreatin dispersion (800 U mL^{-1} based on trypsin activity).

During intestinal digestion, the pH was maintained at 7.0 by the addition of 1 M NaOH, through an automatic titration unit (pH-stat T50 titrator, Metler Toledo, Mississauga, Canada). The volume of NaOH added to the samples was measured every 1 min and used to calculate the concentration of free fatty acids (FFA) released in the reaction vessel. FFA released were calculated using Eq. (2), taking into account the number of moles of NaOH required to neutralize the FFA that could be produced from the triacylglycerols if they were completely digested (assuming the generation of 2 FFAs per triacylglycerol molecule by the action of lipase) (Li & McClements, 2010). After 2 h of incubation in SIF, the samples were taken for structural characterization (Sections 2.3.1, 2.3.2 and 2.3.3).

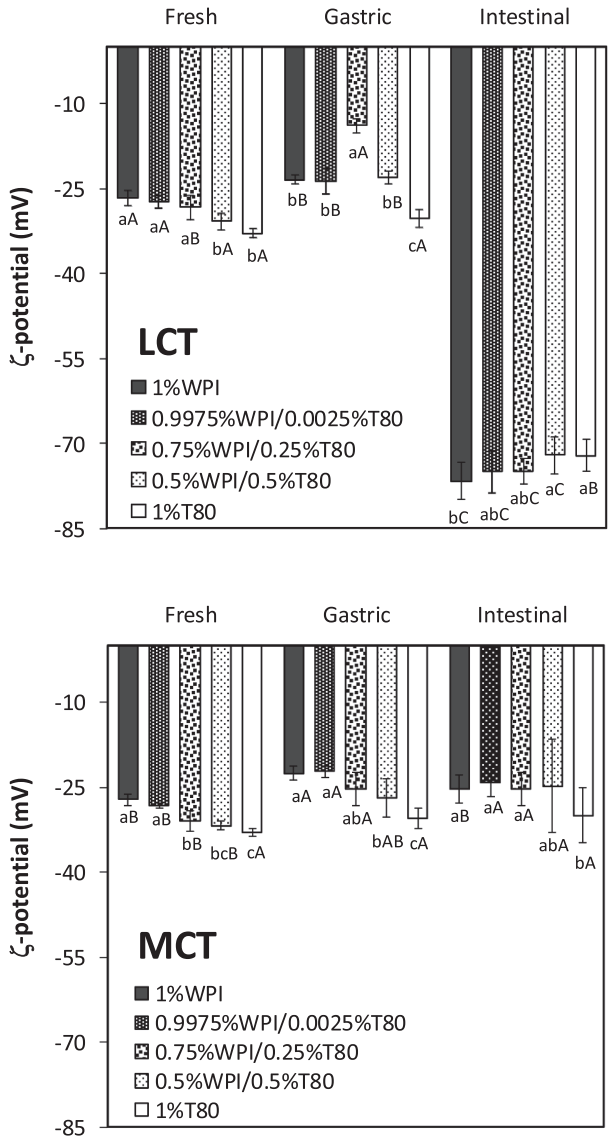

Fig. 4. Behavior of ζ-potential of the O/W emulsions, produced with LCT or MCT as oil phase and stabilized by 1%WPI, 0.9975%WPI/0.0025%T80, 0.75% WPI/0.25%T80, 0.5%WPI/0.5%T80 or 1%T80: after preparation (fresh) and after gastric and intestinal phases. Different letters indicate significant difference at $p < 0.05$. Small letters: differences between emulsifier composition (1% WPI, 0.9975%WPI/0.0025%T80, 0.75%WPI/0.25%T80, 0.5%WPI/0.5%T80 or 1%T80) at the same oil phase. Capital letters: differences between fresh emulsion and after the gastric and intestinal steps at the same emulsifier composition.

$$\%FFA = \frac{V_{NaOH} \times M_{NaOH} \times MW_{lipid}}{2 \times W_{lipid}} \quad (2)$$

where V_{NaOH} is the volume (L) of NaOH, M_{NaOH} is the molarity of NaOH (M) , MW_{lipid} is the average molecular weight of sunflower oil – LCT (867 g/mol) or MCT oil (492 g/mol) and W_{lipid} is the weight of lipid initially present in the reaction vessel (g).

The digestion process and the analyzes to characterize the samples after the stomach and intestinal stages of the GIT model were performed in duplicate. All measurements were performed three times.

3. Results and discussion

3.1. Evaluation of structural changes during in vitro digestion

The physical stability of the emulsions was evaluated before and after passing through the simulated gastrointestinal tract (GIT), based on the analysis of particle size, particle size distribution, ζ–potential, microstructure and macroscopic appearance after each stage of the GIT model. Two factors were examined to understand the destabilization of emulsions during digestion and how it affects the surface area available for lipolysis using mixed WPI/Tween 80 emulsions: 1) emulsifier composition, and 2) oil phase composition.

3.1.1. Fresh emulsions

All evaluated conditions led to the formation of emulsions with small droplet sizes ($D_{43} < 1.55$ μm) and most of them presented monomodal distribution (Table 1 and Fig. 1). Microscopy images (Figs. 2 and 3) confirmed the small oil droplets distributed evenly by the emulsions.

Emulsions formulated with LCT presented a larger D_{43} than those stabilized by MCT, except for systems stabilized by 0.9975%WPI/0.0025%T80 (w/w). This result can be associated with the viscosity and the hydrophobicity of the dispersed phase. The LCT presents higher viscosity (around 42.6 mPa.s) than MCT (around 30 mPa.s), hindering the droplet breakup into smaller sizes in the systems formed by LCT (Chiplunkar & Pratap, 2016; Walker, Gumus, Decker, & McClements, 2017). The LCT systems also present an initial interfacial tension slightly higher due to its higher hydrophobicity. Furthermore, the newly formed interface of LCT droplets needs a higher amount of emulsifier to be saturated, due to the greater degree of unsaturation of the LCT fatty acid chains, slowing the formation of the layer around the droplet. These characteristics result in a higher interfacial pressure for LCT systems compared to the MCT ones as observed in our previous study (Gomes et al., 2018). It should be noted that the interfacial pressure is opposed to droplet deformation and rupture (Gomes et al., 2018; Qian & McClements, 2011).

The addition of T80 promoted a decrease of D_{43} value (0.0025%T80 w/w), regardless of the oil used, which could be attributed to differences in interfacial properties of the emulsifiers (McClements & Gumus, 2016). Proteins are less efficient at forming droplets with reduced diameter because they form a solid viscoelastic interfacial film which is more resistant to disruption (Wilde, Mackie, Husband, Gunning, & Morris, 2004). Furthermore, WPI presents slower adsorption on the droplet surface than Tween 80 and, therefore, more re-coalescence of droplets could occur during the homogenization process (Wilde et al., 2004). Otherwise, T80 has better surface activity property compared to WPI and forms a fluid adsorbed layer around the droplet. This fluidity allows the rapid migration of T80 from more concentrated regions to reduced concentration areas (Gibbs-Marangoni mechanism) facilitating the production of droplets with small diameters (Kotsmar et al., 2008). The increase of T80 concentration in the emulsifier mixture (0.25%T80 w/w) resulted in a further decrease of D_{43} only in MCT systems. The D_{43} values did not present significative differences to 0.5%WPI/0.5%T80 and 1%T80 LCT/MCT systems, indicating that the increase of the T80 concentration above 0.5% (w/w) is not capable to promote an additional reduction of the droplet size.

All fresh emulsions presented negative ζ-potential (pH 6.6 – 6.8), with the magnitude of their ζ-potential depending on the composition of the interfacial layer (Fig. 4). The LCT/MCT emulsions stabilized by 1%T80 or 0.5%T80/0.5%WPI (w/w) presented similar ζ-potential (around −33 mV). Although Tween 80 is a nonionic surfactant, some molecules such as ions OH^- coming from the water, and free fatty acids from the commercial emulsifier, or from the oil, may adsorb on the interface explaining the negative ζ-potential of the droplets (Chang & McClements, 2016; Hsu & Nacu, 2003).

The reduction of T80 in the emulsifier mixture resulted in a decrease

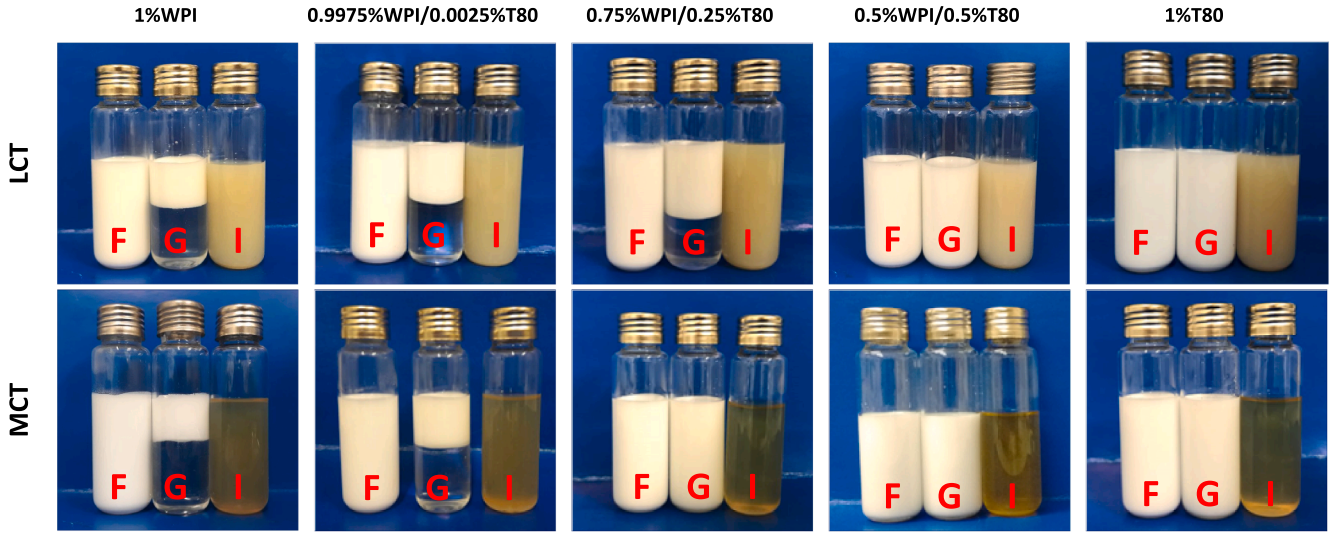

Fig. 5. Visual aspect of the LCT/MCT emulsions stabilized by 1%WPI, 0.9975%WPI/0.0025%T80, 0.75%WPI/0.25%T80, 0.5%WPI/0.5%T80 or 1%T80: fresh emulsions (F) and after gastric (G) and intestinal (I) phases.

of the ζ-potential, which reached similar values, around − 27 mV, in LCT/MCT systems with 1%WPI and 0.9975%WPI/0.0025%T80 (w/w). Protein-coated oil droplets showed negative ζ-potential values because the solution pH was above the WPI isoelectric point (pI ≈ 5.1) (Park, Mun, & Kim, 2018).

3.1.2. Emulsions under simulated gastric conditions

The microstructure of fresh emulsions was changed after the gastric step. Such changes were dependent on the emulsifier and oil composition.

The droplet size distribution curves of LCT/MCT emulsions stabilized by the prevailed WPI content (1%WPI, 0.9975%WPI/0.0025%T80 and 0.75%WPI/0.25%T80 w/w) became broader and droplet size increased considerably during the gastric phase (Table 1 and Fig. 1). Microscopy images indicated extensive droplet aggregation and the presence of some large individual oil droplets for both LCT/MCT systems (Figs. 2 and 3). The aggregation process was confirmed by the visual aspect of these emulsions, which showed phase separation after the stomach phase (Fig. 5). The microscopy image of MCT-0.75%WPI/0.25%T80 system showed smaller clusters, and the phase separation was not observed for this system.

In the stomach phase, the systems were submitted to the environmental conditions of very low pH (pH = 3) and high ionic strength (≈100 mV). During the rapid acidification of the systems, pH values around the isoelectric point (pI ≈ 5.1) of WPI were reached. In this condition, the protein layer had no longer enough repulsive forces to avoid the aggregation of the droplets (Singh & Ye, 2013; Zhang et al., 2016). However, the droplets remained flocculated even at pH below the isoelectric point (pH = 3), when the WPI molecules are positively charged. The high ionic strength of the medium leads to the shielding of electrical charges on the adsorbed protein molecules because proteins are capable of binding ions (particularly calcium). The ion protein interactions enhance intermolecular association and, hence, increase the droplet package (Singh & Ye, 2013). Thus, the emulsions stabilized by WPI showed extensive droplet flocculation with a densely packed network gel-like structure (Figs. 2 and 3).

In addition to very low pH and high ionic strength, the systems were exposed to the pepsin action. Proteins undergo conformational changes (exposition of hydrophobic groups) to adsorb onto the oil–water interface, enhancing the accessibility of pepsin to specific peptide bonds (Singh & Ye, 2013; Zhang et al., 2016). Hydrolysis of the WPI interfacial layer produces small peptides that form a weak and more susceptible to rupture interfacial film (Schröder, Berton-Carabin, Venema, & Cornacchia, 2017), favoring the coalescence by the proximity of the aggregated droplets. Therefore, partial WPI hydrolysis may diminish the protective effect of interfacial layer, predisposing the droplets to a bridging-flocculation mechanism (van Aken, Bomhof, Zoet, Verbeek, & Oosterveld, 2011).

The destabilization by coalescence was more intense in MCT droplets (Figs. 2 and 3), which was attributed to the greater susceptibility of WPI to proteolysis on the MCT droplet surface than on the more hydrophobic LCT interface. At the more hydrophobic interface, the protein exposes more hydrophobic residues (Gomes et al., 2018), however, most of them are embedded in the oil phase hindering the access of pepsin. Furthermore, the protein molecules are closer to each other on the LCT interface also making the pepsin action more difficult (Sakuno, Matsumoto, Kawai, Taihei, & Matsumura, 2008).

The presence of T80, even at low concentration, hampered the flocculation (Table 1) and coalescence processes of droplets (Figs. 2 and 3). The T80 promoted the displacement of the WPI molecules from the droplet surface (competitive adsorption) creating a protein/surfactant mixed interface (Gomes et al., 2018; Maldonado-Valderrama & Patino, 2010). In this way, the presence of T80 in interfacial layer made the system more resistant to destabilization since non-ionic surfactants are highly stable under stomach conditions (Espert, Salvador, & Sanz, 2019).

Emulsions stabilized by higher T80 concentrations (≥ 0.5% w/w) showed the smallest structural changes after experiencing the simulated stomach conditions (Table 1 and Figs. 1–3). These systems did not show changes in the droplet size demonstrating the strong influence of T80 regardless of the oil phase. T80 has a big polyoxyethylene head providing a high steric repulsion among the oil droplets avoiding their aggregation and coalescence (Espert et al., 2019).

The LCT systems with concentration of T80 ranging from 1 to 0.5% (w/w) and the MCT systems ranging from 1 to 0.25%T80 (w/w) did not present phase separation (Fig. 5). Such results show that the concentration of T80 required to avoid phase separation (drastic reduction of flocculation droplets) was much higher for the LCT emulsions. The proteins interact more strongly with the LCT interface hampering their replacement by T80 at the interface (Gomes et al., 2018).

These results show that the replacement of T80 by WPI up to 0.5% (w/w), for both oils, LCT or MCT, was efficient to avoid the aggregation of droplets under gastric conditions and produced emulsions with similar characteristics to those stabilized only with T80. This may have important consequences for designing emulsions that are able to induce satiety and improve the bioaccessibility of lipophilic functional

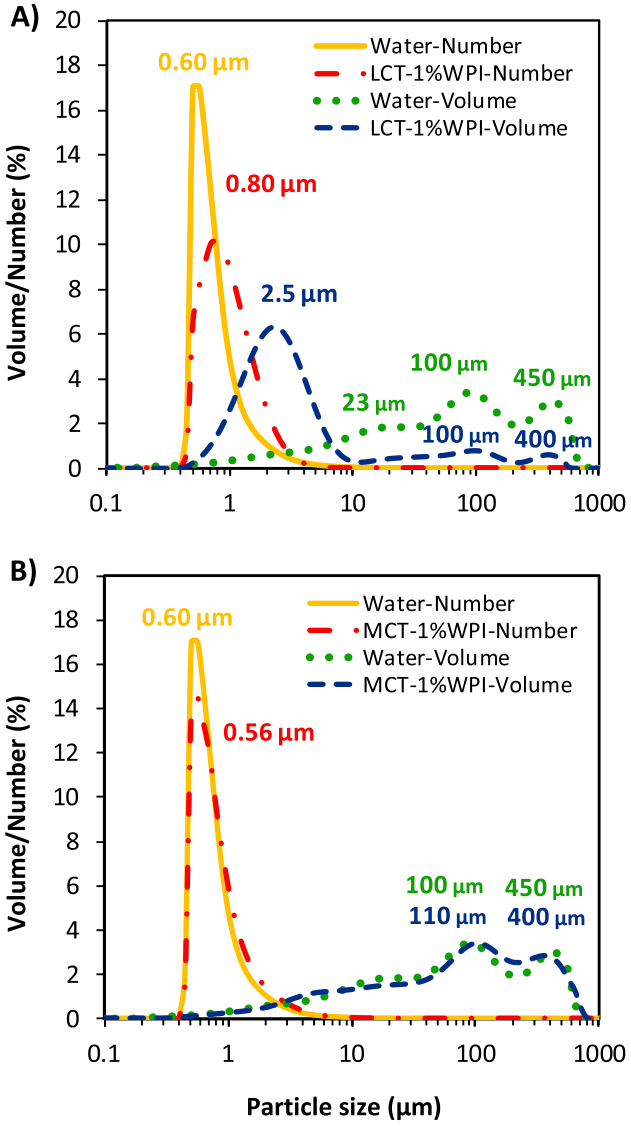

Fig. 6. Number and Volume size distribution and modes of particle size distribution curves after intestinal step: water (control sample) or emulsions stabilized with 1%WPI and produced with A) LCT or B) MCT.

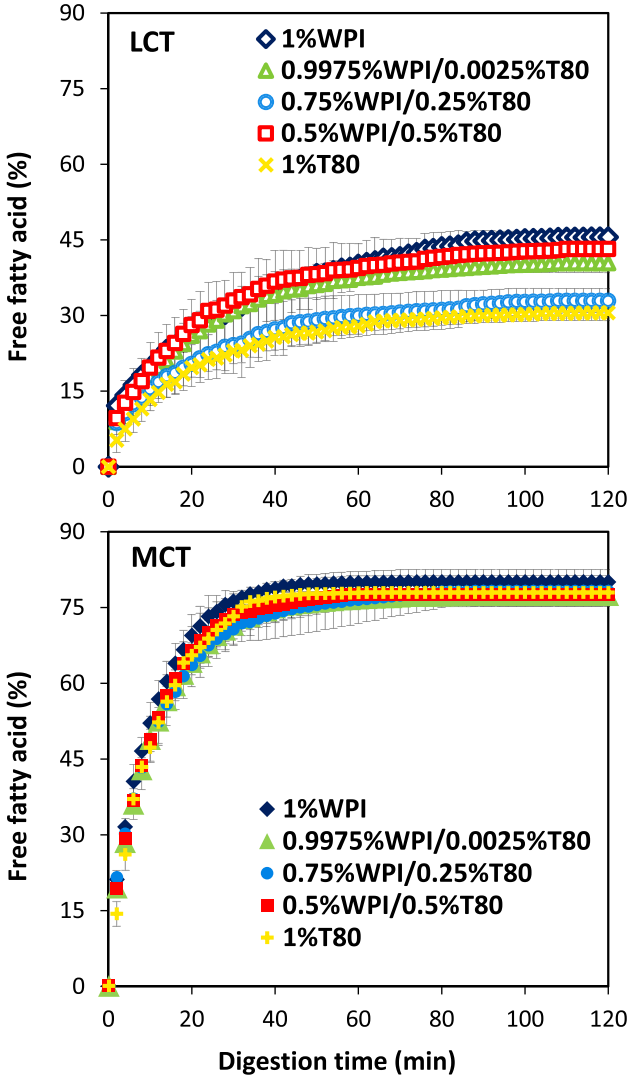

Fig. 7. FFAs released under simulated intestinal conditions as a function of time of the O/W emulsions, produced with LCT or MCT as oil phase and stabilized by WPI, WPI-Tween 80 mixture or Tween 80.

compounds. The gastric stable emulsions have shown slower rate of gastric emptying and higher lipid hydrolysis rate (Lett et al., 2016).

After the gastric phase, the ζ-potential was measured to provide with indirect information about changes in the interfacial composition. The ζ-potential became less negative after the stomach phase for LCT/MCT systems stabilized by 1%WPI, 0.9975%WPI/0.0025%T80, 0.75% WPI/0.25%T80 and for 0.5%WPI/0.5%T80 LCT system.

This effect is attributed to the susceptibility of WPI to strong changes of environmental conditions (low pH and high ionic strength) and the adsorption of charged species from gastric fluid on the emulsifier layer, resulting in electrostatic screening effects (Chang & McClements, 2016). Also, the WPI molecules present in the interfacial layer lose some negatively charged residues as a result of the pepsin action. The proteolysis facilitates the displacement of some protein molecules and/or peptides from the interface allowing the adsorption of other positive charged species, with surface active, coming from the gastric juice (Schröder et al., 2017). The ζ-potential of MCT-0.5%WPI/0.5%T80 and LCT/MCT-1%T80 systems remained unchanged, reaffirming the ability of T80 to maintain emulsion stability in gastric conditions.

Table 2
Free fatty acid release (FFA) during the intestinal stage from O/W emulsions produced with LCT or MCT oil and stabilized by WPI, WPI/Tween80 mixture or T80.

Oil type	Eomposition of emulsifier (w/w)	Release of FFAs (%)
LCT	1%WPI	45.56 ± 1.05^{aB}
	0.9975%WPI/0.0025%T80	40.39 ± 1.89^{aB}
	0.75%WPI/0.25%T80	32.90 ± 2.41^{bB}
	0.5%WPI /0.5%T80	43.18 ± 1.36^{aB}
	1%T80	30.52 ± 1.51^{bB}
MCT	1%WPI	80.06 ± 2.46^{aA}
	0.9975%WPI/0.0025%T80	77.46 ± 0.56^{aA}
	0.75%WPI/0.25%T80	78.70 ± 3.65^{aA}
	0.5%WPI/0.5%T80	77.61 ± 2.10^{aA}
	1%T80	77.98 ± 2.68^{aA}

Different letters indicate significant difference at $p < 0.05$. Small letters: differences in the same column among different emulsifier composition (1%WPI; 0.9975%WPI/0.0025%T80; 0.75%WPI/0.25%T80; 0.5%WPI/0.5%T80 or 1%T80) at the same oil phase. Capital letters: differences in the same column between LCT and MCT samples at the same emulsifier composition.

3.1.3. Emulsions under in vitro intestinal conditions

After being exposed to the simulated intestinal conditions, all

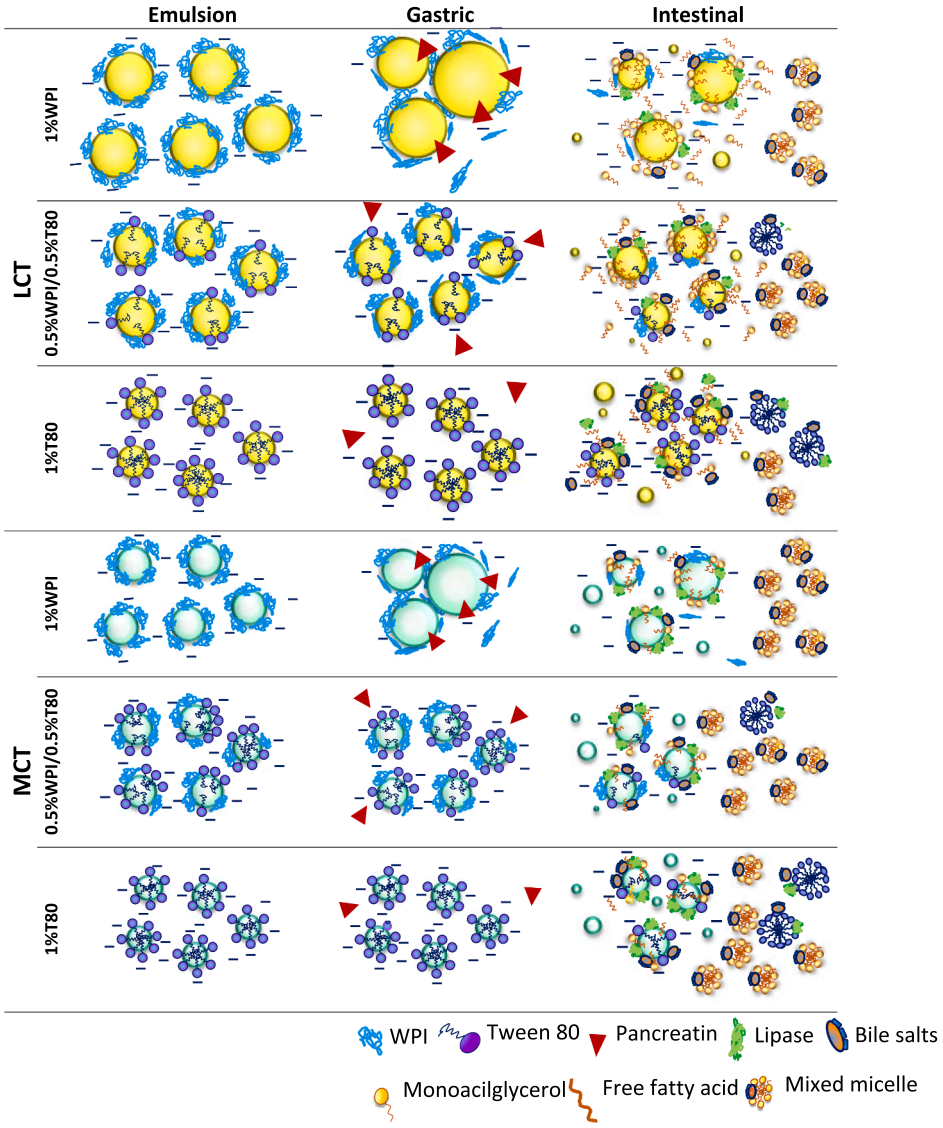

Fig. 8. Schematic representation of the physicochemical mechanism involved in the *in vitro* digestion steps of the emulsions composed of LCT or MCT and stabilized by 1%WPI, 0.5%WPI/0.5%T80 or 1%T80.

systems showed changes in the particle size (Figs. 2 and 3) with evidence of smaller and bigger particles in the size distribution curves (Fig. 1). These results indicate that the emulsions underwent several physicochemical modifications, as a result of lipid digestion, which promoted a considerable reduction in their physical stability.

In the intestine, the emulsions were exposed to different environmental conditions, such as high pH (7.0) and ionic strength (≈140 mM) and the presence of bile salts and enzymes (Singh & Ye, 2013). Bile salts adsorb onto the droplet surface and replace at least part of the emulsifier layer, due to their high surface activity (Bellesi, Martinez, Pizones Ruiz-Henestrosa, & Pilosof, 2016). Once at the oil droplet interface, the pancreatic lipase is capable to convert the triacylglycerols (LCT or MCT oils) in free fatty acids (FFAs) and monoacylglycerols (MAGs), which are also able to replace the emulsifiers from interface (Qian, Decker, Xiao, & McClements, 2012). On the one hand, the removal of these digestion products (FFAs and MAGs) from the droplet surface leads to a reduction of the droplet diameter. On the other hand, the lipid digestion is an interfacial process capable of changing the composition, microstructure and properties of the layer of emulsifiers. Thus, the emulsifying layer is weakened, allowing the droplet size to increase due to coalescence (Yang & McClements, 2013).

The emulsifier composition was not able to change the particle size of LCT/MCT systems, however the D_{43} values were affected by the oil phase composition. The MCT systems showed larger particles than the LCT systems. Fig. 1 shows the particle size distribution curves in LCT systems. The first peak, located in a region of smaller size, corresponds to the undigested oil droplets and the tail, which is in the region of larger particle sizes, represents the particles from LCT digestion and gastrointestinal fluids. The particles formed during MCT digestion showed multimodal distribution curves, shifted to the right towards larger sizes. The behavior of the distribution curves indicates that the MCT hydrolysis was superior to the LCT (Fig. 1). Long chain FFAs generated from digestion of LCT tend to accumulate at the oil–water interface due to their major affinity for lipid phase, hampering the lipase action by steric hindrance In contrast, medium chain FFAs move towards the surrounding aqueous phase due to their lower hydrophobicity (Qian et al., 2012; Yang & McClements, 2013). The facilitated lipase access to the MCT oil droplets may have resulted in higher rate of destabilization of droplets by coalescence.

The high polydispersity of the size distribution curves is attributed to the complex composition of the intestinal phase (undigested lipid droplets and colloidal structures such as micelles, vesicles, protein particles, insoluble bile, and calcium salts). These colloidal structures which are resulted of the interaction among the ingredients of the

emulsions and the compounds coming from the gastrointestinal fluids, may influence the particle size and the particle size distribution (Park et al., 2018).

The above results provided information on the influence of oil phase composition on particle size and particle size distribution after the intestinal step (digesta). However, a complementary analysis was necessary to deeply understand the contribution of gastrointestinal fluids (insoluble bile, enzymes, and calcium salts) on these properties. Therefore, *in vitro* digestion experiments using water as sample (control) were performed and particle size distribution curves (volume and number) obtained were compared with the distribution curves of the digested emulsion samples. Fig. 6 shows the mode values and number/volume size distribution curves of the water system (control) compared to the LCT or MCT systems stabilized by 1%WPI. Interestingly, all WPI/T80 ratios of LCT or MCT systems presented similar behavior. The control and 1%WPI-LCT systems showed a different volume and number size distribution. The size distribution curves of the 1%WPI-LCT system showed two peaks: one of undigested oil droplets (first peak) and other of the remaining particles of gastrointestinal fluid (Table 1). The microscopy images confirmed the presence of undigested LCT droplets after intestinal step (Fig. 2). Differently, the number/volume size distribution curves of the control and 1%WPI-MCT systems were remarkably similar, indicating the extensive lipolysis of the MCT droplets. The absence of MCT droplets in the microscopy images (Fig. 3) is aligned with the overlapping distribution curves (control and 1% WPI-MCT samples). This outcome reveals a predominant contribution of the particles from gastrointestinal fluid in the distribution curves of MCT samples, justifying their higher D_{43} values in comparation to LCT samples.

The lipid digestion also affected ζ-potential of samples after being submitted to the simulated intestinal conditions (Fig. 4). All samples showed negative values of ζ-potential, which may be attributed to the presence of various particles in the digesta (such as undigested oil droplets, undigested proteins, micelles and vesicles). These particles contain anionic species coming from the digestion of the original emulsions (e.g., peptides, free fatty acids, or phospholipids) or from the gastrointestinal fluids (e.g., bile and calcium salts) (Zhang, Zhang, Zhang, Decker, & McClements, 2015).

The ζ-potential magnitude was affected by oil chain length, in the same way as the droplet size, as resulted of oil droplet hydrolysis. Emulsions formed by long chain triacylglycerols-LCT showed higher negative ζ-potential (from -76.68 ± 3.24 to -72.08 ± 3.29 mV) than those containing medium chain triacylglycerols-MCT (from -29.91 ± 4.79 to -24.08 ± 2.65 mV), which could be associated to the hydrophobicity of the free fatty acids from digestion of triacylglycerols. Free fatty acids produced from MCT digestion may migrate rapidly from the droplet surface into surrounding aqueous phase. However, more hydrophobic FFAs released from LCT digestion tend to accumulate at the oil–water interface contributing to the increase of the ζ-potential of these systems (Zhang et al., 2015).

3.2. Influence of the oil and emulsifier composition on the lipid digestion

The lipid digestion profiles of LCT/MCT emulsions followed similar trends. In the initial period (first 30 min), a rapid increase in the release of FFAs was observed, followed by a more gradual increase at longer times (from 30 to 60 min) until a relatively constant final value was reached (Fig. 7). However, the lipid hydrolysis kinetics was influenced by the oil phase type. The LCT systems showed lower release of free fatty acids that MCT systems (Table 2). At the end of the digestion, the amount of FFAs released from the LCT and MCT emulsions were 30–45% and 77–45%, respectively. Such behavior could be attributed to the unsaturation degree, chain length and fatty acid composition of LCT and MCT oils.

Sunflower oil (LCT) is composed primarily of triacylglycerols-TAGs (98–99%) with a high concentration of linoleic acid (47.64%), followed by oleic acid (41.48%), which are polyunsaturated and monounsaturated fatty acids, respectively (Gomes et al., 2018). The presence of double bonds in an unsaturated fatty acid produces bends its chain, causing a steric hindrance effect that protects ester bond (Sun et al., 2015). Also, as mentioned above, FFAs released from LCT-hydrolysis have higher affinity for lipid phase tending to remain at the oil–water interface restricting further lipase activity (Han et al., 2019; Li & McClements, 2010). Differently, MCT is mainly composed of caprylic acid – C8:0 (51.41%) and capric acid – C10:0 (47.30%), which are saturated fatty acids (straight molecule). The lower hydrophobicity of medium chain FFAs promoted their fast displacement towards the surrounding aqueous medium. Such characteristics may have facilitated the access of lipase to MCT droplet surface (Han et al., 2019; Li & McClements, 2010).

The distinct lipolysis rate of the LCT and MCT systems may be identified by analyzing the appearance of digesta samples (Fig. 5). The LCT digestion resulted in more turbid samples indicating an incomplete lipid hydrolysis. Such samples presumably have the presence of non-digested droplets, incompletely micellar-solubilized lipids, and the co-existence of micellar, liquid crystalline and liposomal or vesicular structures. In a different way, the samples from MCT digestion were more translucid due to the more intense lipolysis and probably the micellar solubilization of the medium chain FFAs (Sek, Porter, Kaukonen, & Charman, 2002).

Some molecules generated by lipolysis may present surface activity (such as fatty acids or fatty acid salts, monoacylglycerols and diacylglycerols) and may have an adverse effect on the interfacial biocatalysis. Molecules formed by fatty acid with long chain and with high unsaturation degree present more surface activity. Therefore, these molecules may stay longer at the interface competing with lipase, which could also contribute to a slower lipolysis of LCT emulsions compared to the MCT emulsions (Reis et al., 2008; Verkempinck et al., 2018).

The MCT hydrolysis was not affected by emulsifier composition (Fig. 7 and Table 2). All MCT systems released a similar amount of FFAs. As aforementioned, the lower surface activity and hydrophobicity of fatty acids of MCT favored the hydrolyze process of this oil (Sek et al., 2002).

In LCT emulsions, the system stabilized by prevailed WPI content (1% WPI and 0.9975%WPI/0.0025%T80) showed the highest release of FFAs (45.56–40.39%). During the small intestinal phase, the protein-interface is exposed to the presence of bile salts and enzymes, such as proteases and lipases, initiating a series of processes. First, pancreatic proteases (trypsin and chymotrypsin) promote the further proteolysis of the adsorbed proteins/peptides on the droplet surface making the interface even weaker (Nik, Corredig, & Wright, 2010). Second, simultaneously to protein hydrolysis, the interface is also altered by bile salts and pancreatic lipases. The high surface activity of bile salts allows an efficient replacement of proteins/peptides from the interface, especially β-Lactoglobulin molecules or peptides (Maldonado-Valderrama et al., 2008; Singh & Sarkar, 2011). Bile salt-rich interface favors the adsorption of co-lipase and its complexation with lipase. Last, once onto the interface, the lipase cleaves the lipids molecules forming 2-monoglycerides and free fatty acids (Singh & Ye, 2013). Therefore, the hydrolyzed protein-interface facilitates the replacement by surface activity molecules (bile salts and complex lipase/co-lipase) explaining the higher release of FFAs in the protein-LCT system (Mat, Le Feunteun, Michon, & Souchon, 2016). It is important to highlight that lipolysis could have been more intense. The flocculation and coalescence of droplets in the gastric step decreased the area to the access to bile salts and lipase to the lipid phase in the intestine (Figs. 2 and 3), reducing the extent of lipolysis (Costa et al., 2020; Golding & Wooster, 2010).

The increase of T80 concentration decreased the FFAs release in the LCT systems. The systems stabilized by 0.75%WPI/0.25%T80 and 1%T80 released around 33% and 31% of FFAs, respectively (Fig. 7 and Table 2). Small molecule surfactants, such as Tween 80 may inhibit the

lipase action, even when the lipase is complexed with co-lipase. T80 molecules, which have higher surface activity than lipase, form a relatively dense interfacial layer around the droplets that avoid the direct contact between this enzyme and the emulsified oils (Yao et al., 2013). Therefore, besides hindering the adsorption of lipase, T80 in the concentration of 0.25% (w/w) did not prevent the flocculation and the coalescence of droplets in the stomach (Fig. 2). The droplet destabilization promoted the decrease of the interfacial area to lipase action and consequently contributed to the reduced extent of lipolysis. Furthermore, the free Tween 80 micelles in the aqueous phase interact with the bile acids and lipase enzyme, avoiding their adsorption onto the oil droplet surface (Yao et al., 2013).

Interestingly, LCT systems with 0.5%WPI/0.5%T80 and 1%WPI had a similar free fatty acid release rate (43%). This result suggests that WPI and T80 worked in cooperation at the 1:1 ratio (0.5%WPI/0.5%T80 system), obtaining an improved performance. In the 0.5%WPI/0.5%T80 mixture, T80 promoted the stability of emulsion during the gastric phase, avoiding a reduction of the interfacial area accessible for lipase action in the intestinal phase. The WPI, in turn, facilitated the lipase adsorption onto the oil droplet allowing an improved lipid hydrolysis. Our results indicate that lipolysis during *in vitro* digestion was modulated by both emulsifier/protein and lipid properties, as presented in the schematic representation shown in Fig. 8. Moreover, 0.5%WPI/0.5%T80 system showed good stability under environmental changes (pH, ionic strength) and it was produced with the partial replacement of the synthetic surfactant by a natural emulsifier, attending the consumer demand for healthier products.

4. Conclusion

We observed a correlation between the oil and emulsifier composition and the extent of lipolysis. Our results indicate that lipid digestion could be modulated by the ability of lipase to adsorb on the oil droplet surface, which is influenced by the droplet interfacial area, the interfacial composition and the type of emulsified oil. Oil type and emulsifier composition influenced the droplet size of the fresh emulsions and their behavior in the gastric and small intestinal phases. Emulsions with a higher WPI concentration were more sensitive to the stomach phase and those with interfaces dominated by T80 were more resistant to lipase action in the intestine. The hydrolysis of LCT was influenced by the fatty acid chain length and interfacial layer composition while only the fatty acid chain length affected the lipolysis of MCT. In an unexpected way, a beneficial effect, coming from collaborative work between WPI and T80, was obtained with 0.5%WPI/0.5%T80 mixture in LCT system. This system showed the same release of FFAs than the 1%WPI system, besides presenting a good stability under changing environmental conditions.

The combination of the surfactant/protein ratio and the oil type could be used to design emulsions with specific structures. The control of the emulsions structure seems to be the key point to modulate the lipid digestion. Thus, our results are an additional tool for the rational selection of ingredients, with the objective of improving the performance of emulsions that, in addition to being used as delivery systems of bioactive functional compounds, can induce satiety.

CRediT authorship contribution statement

Andresa Gomes: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data curation, Writing - original draft, Visualization, Project administration, Funding acquisition. **Ana Letícia Rodrigues Costa:** Formal analysis, Writing - review & editing. **Dayane Dias Cardoso:** Formal analysis, Data curation. **Guilherme de Figueiredo Furtado:** Investigation, Methodology, Formal analysis. **Rosiane Lopes Cunha:** Conceptualization, Methodology, Resources, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This study was financed by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brazil (CAPES) – Finance Code 001. Gomes thanks CNPq-Brazil (140705/2015-5 and 156024/2018-7), Costa thanks CAPES (88887.473207/2020-00), Furtado thanks FAPESP (2017/08130-1) and Cardoso thanks CNPq-Brazil (PIBIC-137438/2017-6) for the fellowship. Cunha thanks CNPq- Brazil (307168/2016-6) for the productivity grant.

References

Bellesi, Martinez, Pizones Ruiz-Henestrosa, & Pilosof (2016). Comparative behavior of protein or polysaccharide stabilized emulsion under *in vitro* gastrointestinal conditions. *Food Hydrocolloids, 52*, 47–56.

Borreani, Leonardi, Moraga, Quiles, & Hernando (2019). How do different types of emulsifiers/stabilizers affect the *in vitro* intestinal digestion of O/W emulsions? *Food Biophysics, 14*(3), 313–325.

Brodkorb, Egger, Alminger, Alvito, Assunção, Ballance, ... Recio (2019). INFOGEST static *in vitro* simulation of gastrointestinal food digestion. *Nature Protocols, 14*(4), 991–1014.

Chang, & McClements (2016). Influence of emulsifier type on the *in vitro* digestion of fish oil-in-water emulsions in the presence of an anionic marine polysaccharide (fucoidan): Caseinate, whey protein, lecithin, or Tween 80. *Food Hydrocolloids, 61*, 92–101.

Chiplunkar, & Pratap (2016). Utilization of sunflower acid oil for synthesis of alkyd resin. *Progress in Organic Coatings, 93*, 61–67.

Costa, Gomes, Furtado, Tibolla, Menegalli, & Cunha (2020). Modulating *in vitro* digestibility of Pickering emulsions stabilized by food-grade polysaccharides particles. *Carbohydrate Polymers, 227*, 115344.

Day, Golding, Xu, Keogh, Clifton, & Wooster (2014). Tailoring the digestion of structured emulsions using mixed monoglyceride–caseinate interfaces. *Food Hydrocolloids, 36*, 151–161.

Espert, Salvador, & Sanz (2019). Rheological and microstructural behaviour of xanthan gum and xanthan gum-Tween 80 emulsions during *in vitro* digestion. *Food Hydrocolloids, 95*, 454–461.

Golding, & Wooster (2010). The influence of emulsion structure and stability on lipid digestion. *Current Opinion in Colloid & Interface Science, 15*, 90–101.

Golding, Wooster, Day, Xu, Lundin, Keogh, & Clifton (2011). Impact of gastric structuring on the lipolysis of emulsified lipids. *Soft Matter, 7*, 3513–3523.

Gomes, Costa, & Cunha (2018). Impact of oil type and WPI/Tween 80 ratio at the oil-water interface: Adsorption, interfacial rheology and emulsion features. *Colloids and Surfaces B: Biointerfaces, 164*, 272–280.

Grigoriev, Derkatch, Krägel, & Miller (2007). Relationship between structure and rheological properties of mixed BSA/Tween 80 adsorption layers at the air/water interface. *Food Hydrocolloids, 21*, 823–830.

Gülseren, & Corredig (2014). Interactions between polyglycerol polyricinoleate (PGPR) and pectins at the oil–water interface and their influence on the stability of water-in-oil emulsions. *Food Hydrocolloids, 34*, 154–160.

Guo, Ye, Bellissimo, Singh, & Rousseau (2017). Modulating fat digestion through food structure design. *Progress in Lipid Research, 68*, 109–118.

Han, Gu, Zhang, Shang, Yan, McClements, ... Xiao (2019). Bioaccessibility and cellular uptake of β-carotene in emulsion-based delivery systems using scallop (Patinopecten yessoensis) gonad protein isolates: Effects of carrier oil. *Food & Function, 10*(1), 49–60.

Hartman (1973). Rapid preparation of fatty acid methyl esters from lipids. *LAB.PRACT. 22*(7), 475–476.

Hou, Liu, Lei, & Gao (2014). Investigation into the *in vitro* release properties of β-carotene in emulsions stabilized by different emulsifiers. *LWT – Food Science and Technology, 59*, 867–873.

Hsu, & Nacu (2003). Behavior of soybean oil-in-water emulsion stabilized by nonionic surfactant. *Journal of Colloid and Interface Science, 259*, 374–381.

Karthik, & Anandharamakrishnan (2016). Enhancing omega-3 fatty acids nanoemulsion stability and in-vitro digestibility through emulsifiers. *Journal of Food Engineering, 187*, 92–105.

Kotsmar, Grigoriev, Xu, Aksenenko, Fainerman, Leser, & Miller (2008). Equilibrium of adsorption of mixed milk protein/surfactant solutions at the water/air interface. *Langmuir, 24*(24), 13977–13984.

Lett, Norton, & Yeomans (2016). Emulsion oil droplet size significantly affects satiety: A pre-ingestive approach. *Appetite, 96*, 18–24.

Li, & McClements (2010). New mathematical model for interpreting pH-stat digestion profiles: Impact of lipid droplet characteristics on *in vitro* digestibility. *Journal of Agricultural and Food Chemistry, 13*, 8085–8092.

Majeed, Antoniou, Hategekimana, Sharif, Haider, Liu, ... Zhong (2016). Influence of

carrier oil type, particle size on *in vitro* lipid digestion and eugenol release in emulsion and nanoemulsions. *Food Hydrocolloids, 52*, 415–422.
Maldonado-Valderrama, & Patino (2010). Interfacial rheology of protein–surfactant mixtures. *Current Opinion in Colloid & Interface Science, 15*(4), 271–282.
Maldonado-Valderrama, Woodward, Gunning, Ridout, Husband, Mackie, ... Wilde (2008). Interfacial characterization of β-lactoglobulin networks: Displacement by bile salts. *Langmuir, 24*, 6759–6767.
Mat, Le Feunteun, Michon, & Souchon (2016). *In vitro* digestion of foods using pH-stat and the INFOGEST protocol: Impact of matrix structure on digestion kinetics of macronutrients, proteins and lipids. *Food Research International, 88*, 226–233.
McClements (2018). Enhanced delivery of lipophilic bioactives using emulsions: A review of major factors affecting vitamin, nutraceutical, and lipid bioaccessibility. *Food & Function, 9*(1), 22–41.
McClements, & Gumus (2016). Natural emulsifiers — Biosurfactants, phospholipids, biopolymers, and colloidal particles: Molecular and physicochemical basis of functional performance. *Advances in Colloid and Interface Science, 234*, 3–26.
Minekus, Alminger, Alvito, Ballance, Bohn, Bourlieu, ... Brodkorb (2014). A standardised static *in vitro* digestion method suitable for food-an international consensus. *Food & Function, 5*, 1113–1124.
Mun, Park, Kim, & McClements (2016). Influence of methylcellulose on attributes of β-carotene fortified starch-based filled hydrogels: Optical, rheological, structural, digestibility, and bioaccessibility properties. *Food Research International, 87*, 18–24.
Nik, Corredig, & Wright (2010). Changes in WPI-stabilized emulsion interfacial properties in relation to lipolysis and ß-carotene transfer during exposure to simulated gastric-duodenal fluids of variable composition. *Food Digestion, 1*, 14–27.
Ozturk, & McClements (2016). Progress in natural emulsifiers for utilization in food emulsions. *Current Opinion in Food Science, 7*, 1–6.
Park, Mun, & Kim (2018). Emulsifier dependent *in vitro* digestion and bioaccessibility of β-carotene loaded in oil-in-water emulsions. *Food Biophysics, 13*(2), 147–154.
Qian, Decker, Xiao, & McClements (2012). Nanoemulsion delivery systems: Influence of carrier oil on β-carotene bioaccessibility. *Food Chemistry, 135*(3), 1440–1447.
Qian, & McClements (2011). Formation of nanoemulsions stabilized by model food-grade emulsifiers using high-pressure homogenization: Factors affecting particle size. *Food Hydrocolloids, 25*(5), 1000–1008.
Reis, Miller, Leser, Watzke, Fainerman, & Holmberg (2008). Adsorption of polar lipids at the water–oil interface. *Langmuir, 24*(11), 5781–5786.
Sakuno, Matsumoto, Kawai, Taihei, & Matsumura (2008). Adsorption and structural change of β-lactoglobulin at the diacylglycerol–water interface. *Langmuir, 24*(20), 11483–11488.
Salvia-Trujillo, Verkempinck, Sun, Van Loey, Grauwet, & Hendrickx (2017). Lipid digestion, micelle formation and carotenoid bioaccessibility kinetics: Influence of emulsion droplet size. *Food Chemistry, 229*, 653–662.
Salvia-Trujillo, Verkempinck, Zhang, Van Loey, Grauwet, & Hendrickx (2019). Comparative study on lipid digestion and carotenoid bioaccessibility of emulsions, nanoemulsions and vegetable-based in situ emulsions. *Food Hydrocolloids, 87*, 119–128.
Schoener, Zhang, Lv, Weiss, & McClements (2019). Fabrication of plant-based vitamin D3-fortified nanoemulsions: Influence of carrier oil type on vitamin bioaccessibility. *Food & Function, 10*(4), 1826–1835.
Schröder, Berton-Carabin, Venema, & Cornacchia (2017). Interfacial properties of whey protein and whey protein hydrolysates and their influence on O/W emulsion stability. *Food Hydrocolloids, 73*, 129–140.
Sek, Porter, Kaukonen, & Charman (2002). Evaluation of the in-vitro digestion profiles of long and medium chain glycerides and the phase behaviour of their lipolytic products. *Journal of Pharmacy and Pharmacology, 54*(1), 29–41.
Singh, & Sarkar (2011). Behaviour of protein-stabilised emulsions under various physiological conditions. *Advances in Colloid and Interface Science, 165*(1), 47–57.
Singh, & Ye (2013). Structural and biochemical factors affecting the digestion of protein-stabilized emulsions. *Current Opinion in Colloid & Interface Science, 18*(4), 360–370.
Singh, Ye, & Ferrua (2015). Aspects of food structures in the digestive tract. *Current Opinion in Food Science, 3*, 85–93.
Sun, Xia, Zheng, Qiu, Zhang, McClements, & Xiao (2015). Nanoemulsion-based delivery systems for nutraceuticals: Influence of carrier oil type on bioavailability of pterostilbene. *Journal of Functional Foods, 13*, 61–70.
van Aken, Bomhof, Zoet, Verbeek, & Oosterveld (2011). Differences in *in vitro* gastric behaviour between homogenized milk and emulsions stabilised by Tween 80, whey protein, or whey protein and caseinate. *Food Hydrocolloids, 25*(4), 781–788.
Verkempinck, Salvia-Trujillo, Moens, Carrillo, Van Loey, Hendrickx, & Grauwet (2018). Kinetic approach to study the relation between *in vitro* lipid digestion and carotenoid bioaccessibility in emulsions with different oil unsaturation degree. *Journal of Functional Foods, 41*, 135–147.
Walker, Gumus, Decker, & McClements (2017). Improvements in the formation and stability of fish oil-in-water nanoemulsions using carrier oils: MCT, thyme oil, & lemon oil. *Journal of Food Engineering, 211*, 60–68.
Wilde, Mackie, Husband, Gunning, & Morris (2004). Proteins and emulsifiers at liquid interfaces. *Advances in Colloid and Interface Science, 108–109*, 63–71.
Yang, & McClements (2013). Vitamin E bioaccessibility: Influence of carrier oil type on digestion and release of emulsified α-tocopherol acetate. *Food Chemistry, 141*(1), 473–481.
Yao, Wang, Fang, Phillips, Jiang, Hu, ... Tian (2013). Impact of surfactants on the lipase digestibility of gum arabic-stabilized O/W emulsions. *Food Hydrocolloids, 33*(2), 393–401.
Zhang, Zhang, Zhang, Decker, & McClements (2015). Influence of emulsifier type on gastrointestinal fate of oil-in-water emulsions containing anionic dietary fiber (pectin). *Food Hydrocolloids, 45*, 175–185.
Zhang, Zhang, Zou, Xiao, Zhang, Decker, & McClements (2016). Enhancement of carotenoid bioaccessibility from carrots using excipient emulsions: Influence of particle size of digestible lipid droplets. *Food & Function, 7*(1), 93–103.