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Interactions of β-carotene with WPI/Tween 80 mixture and oil phase: Effect on the behavior of O/W emulsions during *in vitro* digestion

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ABSTRACT

This study investigated the impact of adding β-carotene on the structure of fresh O/W emulsions with different oil phase (sunflower oil-LCT or NEOBEE®1053-MCT) and emulsifiers (WPI, Tween 80 – T80 or WPI/T80 mixture). In this sense, the behavior of emulsions through the gastrointestinal tract, the stability and bioaccessibility of β-carotene were also assessed. The β-carotene reduced the interfacial tension of the LCT/MCT-water systems. The addition of β-carotene promoted an increase of viscoelasticity of LCT/MCT-T80 (0.5% WPI/0.5% T80 and 1% T80 w/w) interfaces, but an increase of WPI content reduced the viscoelasticity of interfacial layers (LCT/MCT-1% WPI). These changes in the interface properties influenced the mean droplet size and ζ-potential of the fresh emulsions. LCT systems presented similar bioaccessibility/stability of β-carotene. However, β-carotene entrapped within protein-coated MCT droplets was more stable than within T80-MCT systems. Our results show that β-carotene interacted with other ingredients of emulsions changing their properties and behavior under gastrointestinal tract as well as the stability/bioaccessibility of β-carotene.

1. Introduction

Carotenoids are lipophilic pigments mainly found in fruits and vegetables, which are beneficial for human health if consumed at appropriate levels. β-carotene is a carotenoid widely used in supplemented food and pharmaceutical products due to its strong antioxidant capacity and high pro-vitamin A activity. However, the absorption of β-carotene from both natural and processed foods is inefficient, which can be attributed to several causes: entrapment within plant tissues, low solubility in water and potential degradation during the gastrointestinal digestion (Allahdad, Varidi, Zadmard, Saboury, & Haertlé, 2019; Boon, McClements, Weiss, & Decker, 2010).

The vehiculation of β-carotene within oil phase of oil-in-water (O/W) emulsions has indicated to be an effective strategy to enhance its physicochemical stability and bioaccessibility (Sharif et al., 2017; Wei, Tong, Dai, Wang, et al., 2020). Furthermore, previous studies have shown that the bioactive compound functionality may be optimized by designing the emulsion properly. It involves the rational choice of ingredients since the combination of the type of emulsifier and lipid affects the emulsion structure and, therefore, its behaviour through the gastrointestinal tract (Salvia-Trujillo et al., 2017; Sharif et al., 2017). However, the bottleneck in these approaches is the lack of attention to the ability of bioactive compound to interact chemically and/or physically with other ingredients of the emulsified system. If the structutre of the emulsion is modified, the stability and/or tune bioaccessibility can be compromised, as well as the functional claim of the bioactive compound. Therefore, the aim of this study was to investigate the impact of adding β-carotene on the structure of fresh O/W emulsions, built with different ingredients, and during their passage through the gastrointestinal tract. The effects of the bioactive compound-emulsifier-oil interactions on the stability and bioaccessibility of β-carotene were also assessed.

A non-ionic surfactant (Tween 80) and a protein (whey protein) were selected to represent food-grade emulsifiers with different structure and interfacial properties. Tween 80 (T80) is a chemically synthesized low molecular weight surfactant that can rapidly adsorb onto the droplet surface and reduce the interfacial tension. Tween 80-stabilized interfacial layer shows high kinetic stability under gastric

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conditions, although this emulsifier hinders the access of lipase to oil droplet surface in the intestinal phase (Mun, Decker, & McClements, 2007; Vinarov et al., 2012). Differently, globular proteins, like WPI, are natural molecules that slowly decrease the interfacial tension, due to their high molecular weight and complex structure. Despite the low adsorption velocity, the proteins show antioxidant activity, form a viscoelastic interfacial layer and their adsorption is an extremely difficult process to reverse (Wilde, Mackie, Husband, Gunning, & Morris, 2004). Emulsions stabilized with WPI are sensitive to stomach conditions, however protein-interfaces can be easily replaced by lipase (Golding et al., 2011; Mun et al., 2007). In a previous work, emulsions stabilized by WPI/T80 mixture (0.5% WPI/0.5% T80 w/w) achieved a greater performance through the gastrointestinal tract (stability and lipolysis rate) than the systems stabilized by each emulsifier alone (Gomes, Costa, Cardoso, Furtado, & Cunha, 2020). The combination of emulsifiers improves the kinetic stability of emulsions under varied environmental conditions, which is a premise for controlling gastrointestinal digestion (Wei et al., 2019; Wei, Tong, Dai, Ma, et al., 2020). Thus, we hypothesized, that the vehiculation of β-carotene in mixed interfaces would improve its physicochemical stability and bioaccessibility. Moreover, we identified that the nature of the carrier oil also influence the interfacial composition and the gastrointestinal fate of an emulsion (Gomes et al., 2020; Gomes, Costa, & Cunha, 2018). Emulsions produced with medium chain triacylglycerols (MCT) show a higher lipolysis rate, nevertheless, systems composed of long chain triacylglycerol (LCT) form mixed micelles with higher width of the hydrophobic domains, responsible for the bioactive compound solubilization (McClements, 2018).

All things considered, the performance of β-carotene undergoing *in vitro* digestibility was evaluated in emulsions with different: i) oil phase - sunflower (LCT) or NEOBEE® 1053 (MCT) oil and either ii) emulsifiers - WPI, Tween 80 or WPI/Tween 80 mixture. The results of this study can effectively contribute to figure out the role of the bioactive compound on the emulsion structure, oil digestion and the own bioaccessibility.

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), with approximately 90% w/w protein, kindly donated by Fonterra Cooperative 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 in the local market whereas the medium-chain triacylglycerol - MCT (NEOBEE® 1053) was kindly donated by Stepan Lipid Nutrition (Northfield, USA). Sunflower oil 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 NEOBEE® 1053 was 51.41% caprylic acid (8:0) and 47.30% capric acid (10:0) (Gomes et al., 2020). All ingredients were used without further purification based on commercial products that are commonly used in industrial applications.

β-carotene (C9750) (≥93% purity), pepsin from porcine gastric mucosa (P6887), pancreatin from porcine 8 × USP (P7545) were purchased from Sigma-Aldrich (St. Louis, USA). The bile extract porcine (SC-214601) was purchased from Santa Cruz Biotechnology (Dallas, USA). The other reagents were analytical grade.

2.2. Preparation of the oil and aqueous phases

The aqueous phase was composed of solution of whey protein isolate (WPI), Tween 80 (T80) or WPI/T80 mixture. Initially each emulsifier was dissolved in water for 2 h using a magnetic stirrer at 25 °C (1% WPI or 1% T80 w/w) and then the solution of WPI/T80 mixture was prepared (0.5%/0.5% w/w of WPI/T80). The oil phase was prepared by dispersing β-carotene in LCT or MCT (0.25 μg/g oil) in the absence of light, with mild heating (15 min at 42 ± 2 °C) and then stirring at room temperature for about 12 h in order to ensure full dissolution.

2.3. Characterization of the oil and aqueous phase

2.3.1. Interfacial tension and dilational rheology

The initial interfacial tension experiments were performed between water or emulsifier aqueous solution (1% WPI, 0.5% WPI/0.5% T80 or 1% T80 w/w) and oil phase enriched with β-carotene. All the measurements were performed at 25 °C using a tensiometer Tracker-S (Teclis, France) by the pendant droplet method.

Dilational dynamic experiments were performed using a tensiometer Tracker-S (Teclis, France) at 25 °C subjecting the droplet interface to very low sinusoidal compression and expansion of the surface area A. The measurement of the interfacial ditational modulus E^* (mN/m) (Eq. (1)) is a result of a dilational change of interfacial tension γ (N/m) as a consequence of a small change in interfacial area A (m^2) (Lucassen & Van Den Tempel, 1972). A deformation of 6% at a frequency of 0.1 Hz was chosen to perform the measurements of dilational rheology. Firstly, an oil droplet was generated, and the interfacial tension was measured for 30 min. After that, five sinusoidal oscillation cycles were performed followed by a time corresponding to 180 cycles without any oscillation for 9.5 h.

$$E^* = \frac{d\gamma}{d\ln A} = E' + iE'' \quad (1)$$

Analysis of interfacial tension and dynamic dilational modulus between the aqueous and oil phases without β-carotene, were also performed in order to understand the effect of the addition of β-carotene on the characteristics of interfacial layers. Furthermore, we also carried out the interfacial tension measurements between water and oil phase without β-carotene as a control.

2.4. Emulsion preparation

Oil-in-water (O/W) emulsions were prepared using the same oil to aqueous phases weight ratio (1:9). The emulsion was produced by premixing the oil and aqueous phases (described in section 2.2) 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). Emulsions were evaluated by means of optical microscopy, mean droplet size (D_{43}), droplet size distribution, ζ-potential and *in vitro* digestibility right after their preparation. The emulsions were produced in duplicate and characterized in three replicates.

Emulsions with oil phase composed of only LCT or MCT, without β-carotene, were prepared, and used as control samples, in order to verify the effect of the addition of β-carotene on the structure of fresh emulsions and after passing through the gastric and intestinal steps.

2.5. Characterization of emulsions

2.5.1. Droplet size distribution

The droplet size distribution was determined by the laser diffraction method using a Mastersizer 2000 (Malvern Instruments Ltd, Malvern, UK) (Lin, Liang, Zhong, Ye, & Singh, 2018). The samples were dispersed in water and the rotational velocity was set to 1750 rpm. The mean droplet size was expressed as the volume-surface mean diameter (D_{43}) estimated according to Eq. (2) and polydispersity index (*Span*) was calculated according to Eq. (3).

$$D_{43} = \frac{\sum n_i d_i^4}{\sum n_i d_i^3} \quad (2)$$

$$Span = \frac{d_{(90)} - d_{(10)}}{d_{(50)}} \quad (3)$$

where n_i is the number of droplets with diameter d_i and $d_{(10)}$, $d_{(50)}$ and $d_{(90)}$ are the diameters at 10%, 50% and 90% of cumulative volume, respectively.

2.5.2. Fluorescence microscopy

Initially, the emulsions were stained with a solution containing the fluorescent dye Red Nile. The microstructure of the emulsions was observed in a fluorescence microscope (Axio Scope.A1, Carl Zeiss, Germany) using 100x oil immersion objective lens coupled with filter set 43 (excitation: BP545/25; beam splitter: FT 570; emission BP 605/70). The images were captured with the software ZEN (Carl Zeiss, Germany).

2.5.3. ζ-potential

The determination of ζ-potential of the samples dispersed in water (0.001% v/v) was performed at 25 °C using the equipment Zetasizer Nano-ZS (Malvern Instruments, UK) according to the description in our previous report (Gomes et al., 2020). The electrophoretic mobility was obtained by Laser Doppler Anemometry technique and the mathematical Smoluchowski model was used to convert electrophoretic mobility measurements in ζ-potential values.

2.5.4. Kinetic stability - laser scanning turbidimetry

Emulsion stability was monitored with the optical scanning instrument Turbiscan ASG (Formulaction, France). Emulsions freshly prepared were placed in flat-bottomed cylindrical glass tubes (140 mm high, 16 mm diameter) and the first measurement of backscattered light intensity was performed (0 days). The tubes were stored at 25 °C for 7 days before the second measurement of backscattered light intensity which was obtained at wavelength of 880 nm. Emulsion destabilization was analyzed using backscattering (BS) profiles at different sample height (mm). A sample height of 0 mm corresponds to the bottom of the measurement cell.

Turbiscan Stability Index (TSI) was used to quantify the destabilization process of the emulsions. This index is calculated as the sum of all the destabilization processes in the measuring cell according to Eq. (4) (Trujillo-Cayado, Alfaro, Muñoz, Raymundo, & Sousa, 2016).

$$TSI = \sum_j |scan_{ref}(h_j) - scan_i(h_i)| \quad (4)$$

where $scan_{ref}$ and $scan_i$ are the initial backscattering value and the backscattering value after 7 days of storage, respectively, h_j is the given height in the measuring cell and TSI is the sum of all the scan differences from the bottom to the top of the vial.

2.5.5. Encapsulation and loading efficiency

Encapsulation efficiency (EE %) is defined as the ratio between the amount of β-carotene effectively retained in the emulsions ($\beta - \text{carotene}_{emulsion}$) and the quantity of β-carotene in the oil phase ($\beta - \text{carotene}_{oilphase}$), according to Eq. (5).

$$EE(\%) = \frac{\beta - \text{carotene}_{emulsion}}{\beta - \text{carotene}_{oil\ phase}} \times 100\% \quad (5)$$

Loading efficiency (LE %) can be calculated by the amount of the β-carotene effectively retained in emulsion ($\beta - \text{carotene}_{emulsion}$) divided by the total emulsion weight, according to Eq. (6). The methods of separation and quantification of β-carotene in both oil phase and emulsions are described in section 2.5.6.1.

$$LE(\%) = \frac{\beta - \text{carotene}_{emulsion}}{total\ emulsion\ weight} \times 100\% \quad (6)$$

2.5.6. In vitro digestion of emulsions

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 of Minekus et al. (2014) where, according to the authors, the mouth step can be eliminated for liquid samples. The samples were placed in a stirred (100 rpm) double jacketed reaction vessel maintained at 37 ± 1 °C. 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} $MgCl_2(H_2O)_6$, 0.5 mmol L^{-1} $(NH_4)_2CO_3$, 0.15 mmol L^{-1} $CaCl_2(H_2O)_2$ and fresh pepsin dispersion (25,000 U mL^{-1}) (enzymatic activity 542.35 ± 38.55 U/mg). After 2 h of incubation in SGF, 20 mL of sample was collected for immediate characterization (sections 2.5.1, 2.5.2 and 2.5.3). Then sample + SGF was mixed (1:1) with SIF. The temperature was adjusted to 37 ± 1 °C and 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 fresh pancreatin dispersion (800 U mL^{-1}) based on trypsin activity (enzymatic activity 5.69 ± 0.20 U/mg). 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. (7), 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) (Liu, Ma, Zhang, Gao, & McClements, 2017). After 2 h of incubation in SIF, samples were taken for structural characterization (sections 2.5.1, 2.5.2 and 2.5.3) and quantification of the β-carotene (section 2.5.6.1).

$$\%FFA = \frac{V_{NaOH} x M_{NaOH} x MW_{lipid}}{2 x W_{lipid}} \quad (7)$$

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 (867 g/mol) or MCT oil (492 g/mol) and W_{lipid} is the weight of lipid initially present in the reaction vessel (g).

2.5.6.1. β-carotene quantification by HPLC. For β-carotene extraction, an aliquot of the digesta (sample + FIS) fluid was collected and centrifuged (16,873 × g, 5418R Eppendorf®, Hamburg, Germany) at 37 °C for 40 min. After centrifugation, the raw digesta sample was separated into an opaque sediment top phase and an aqueous bottom phase. The aqueous phase containing the solubilized β-carotene was separated and assumed to represent the micellar fraction (Liu et al., 2017). The β-carotene was extracted from the emulsion, the micelle and raw digesta fractions by liquid–liquid extraction using absolute ethanol-hexane mixture. In flasks, 3 mL of the raw digesta or micellar fraction or 0.5 mL of emulsion was added to 2 mL of absolute ethanol and 3 mL of hexane. The systems were shaken every 10 min for a period of 20 min and the extraction was repeated three times. Subsequently, the phases containing hexane were removed from the extraction system, combined, and diluted with hexane in volumetric flasks. β-carotene content in the sample was quantified by an HPLC-DAD (Waters, Alliance E2695, Milford, USA) according to Brasili et al. (2017) with some modification. β-carotene was separated in a fused-core C18 column (Kinetex, 100 × 4.6 mm i.d.; 2.6 μm; Phenomenex, Torrance, USA) using a mobile phase of methanol (A) and *tert*-methyl butyl ether (B) and the following gradient: 0 min, 95% A; 18 min, 80% A; 23 min, 95% A. The temperature and flow rate were 29 °C and 0.9 mL/min, respectively. The results were calculated using a β-carotene calibration

curve (R^2 = 0.9997).

The stability of β-carotene initially encapsulated in the O/W emulsions, after passing through the simulated gastrointestinal tract, as well as its bioaccessibility (Liu et al., 2017) were calculated by Eq. (8) and Eq. (9), respectively

$$\text{Stability}(\%) = \frac{C_{digesta\ phase}}{C_{initial\ emulsion}} \times 100\% \quad (8)$$

$$\text{Bioaccessibility}(\%) = \frac{C_{micellar\ phase}}{C_{initial\ emulsion}} \times 100\% \quad (9)$$

where $C_{digesta\ phase}$, $C_{micellar\ phase}$ and $C_{initial\ emulsion}$ are the contents of β-carotene in the digesta phase, micellar fraction and initial emulsion, respectively.

2.6. Statistical analysis

Analysis of variance (ANOVA) was performed using Minitab 16® software and the significant differences ($p < 0.05$) between the treatments were evaluated using Tukey analysis.

3. Results and discussion

3.1. Effect of the addition of β-carotene on the structure of the fresh O/W emulsions

All evaluated conditions led to the formation of β-carotene emulsions with small droplets size (average diameter < 1.67 μm) and monomodal distribution (Table 1 and Fig. 1S). Microscopy images (Fig. 2S) confirmed the small oil droplets distributed evenly by the emulsions.

The addition of β-carotene influenced the mean droplet size (D_{43}) of all fresh emulsions. It was expected that the increase of T80 concentration would promote a considerable decrease of D_{43} values due its lower molecular weight and faster adsorption than WPI as observed by Gomes et al. (2018). However, β-carotene LCT/MCT emulsions stabilized with T80 (0.5% WPI/0.5% T80 and 1% T80 w/w) showed larger droplet size than emulsions without this bioactive compound. On the other hand, a decrease of the D_{43} values was observed for the β-carotene LCT/MCT emulsions stabilized by only 1% WPI (Table 1). These results suggest that β-carotene changed the interface properties in a different way, depending on the interfacial layer composition. Thus, analyzes of interfacial tension and complex viscoelastic modulus were performed to better investigate the effect of β-carotene on interfaces.

The addition of β-carotene promoted a reduction of interfacial tension in systems composed of only water-LCT/MCT (Fig. 1), indicating that this bioactive compound has some surface activity and could facilitate the breakup of droplets in both LCT and MCT systems. β-carotene also promoted a decrease of interfacial tension in LCT systems with 1% WPI, 0.5% WPI /0.5% T80 or 1% T80 (w/w) and in MCT-1% WPI system (Fig. 1), but this reduction was not significant in MCT-0.5% WPI /0.5% T80 and MCT-1% T80 interfaces.

The drop in the interfacial tension explains the smaller droplet sizes of the LCT/MCT β-carotene emulsions stabilized by 1% WPI (w/w) (Table 1), but complex viscoelastic modulus measurements were needed to elucidate the increased droplet sizes of LCT/MCT β-carotene emulsions stabilized with T80 (0.5% WPI/0.5% T80 and 1% T80 w/w). The presence of β-carotene altered the complex viscoelastic modulus values of the interfaces differently, depending on their composition.

In an emulsified system, most of the surfactants are located at the interface, due to their amphiphilic character. The hydrophilic heads of the WPI and T80 are positioned inside the aqueous phase, while their hydrophobic tails are realigned in the oil phase (Gomes et al., 2018), where the β-carotene is solubilized. The β-carotene molecules, driven by their hydrophobic nature, must move towards the interface and interact with the hydrophobic chains of both surfactants forming the

Table 1
Mean droplet size (D_{43}), *Span*, TSI values, β-carotene encapsulation efficiency (EE) and β-carotene loading efficiency (LE) of the fresh emulsions produced with LCT or MCT and stabilized by 1% WPI, 0.5% WPI/0.5% T80 mixture or 1% T80.

Oil Type	Emulsifier composition (w/w)	Fresh				Fresh β-carotene			
		D_{43} (μm)	*Span*	TSI	D_{43} (μm)	*Span*	TSI	EE %	LE % (x 10^3)
LCT	1% WPI	$1.55 \pm < 0.01^{aA*}$	$1.67 \pm < 0.01^{bB*}$	$6.85 \pm 0.35^{aA*}$	$1.37 \pm 0.05^{cA**}$	$1.79 \pm 0.39^{aB*}$	$5.30 \pm 0.26^{bA**}$	85.62 ± 5.94^{aA}	2.140 ± 0.148^{aA}
	0.5% WPI/0.5% T80	$1.24 \pm 0.14^{bA**}$	$1.81 \pm 0.13^{aA*}$	$6.20 \pm 0.14^{bA**}$	$1.61 \pm 0.07^{bA*}$	$1.75 \pm 0.11^{aB*}$	$6.95 \pm 0.07^{aA*}$	85.42 ± 2.04^{aA}	2.135 ± 0.051^{aA}
	1% T80	$1.22 \pm 0.05^{bA**}$	$1.76 \pm 0.05^{aA*}$	$4.82 \pm 0.11^{cA**}$	$1.67 \pm 0.02^{aA*}$	$1.72 \pm < 0.01^{aA**}$	$5.90 \pm 0.36^{bA*}$	86.81 ± 1.54^{aA}	2.170 ± 0.038^{aA}
MCT	1% WPI	$1.42 \pm < 0.01^{aB*}$	$2.46 \pm 0.18^{aA*}$	$3.90 \pm 0.44^{aB**}$	$1.36 \pm 0.01^{aA**}$	$2.47 \pm 0.31^{aA*}$	$3.77 \pm 0.06^{aB*}$	83.54 ± 2.31^{aA}	2.088 ± 0.057^{aA}
	0.5% WPI/0.5% T80	$1.00 \pm < 0.01^{bB**}$	$1.77 \pm 0.09^{bA**}$	$3.03 \pm 0.28^{bB**}$	$1.32 \pm 0.04^{bB*}$	$2.44 \pm 0.06^{aA*}$	$3.68 \pm 0.22^{aB*}$	83.93 ± 1.85^{aA}	2.098 ± 0.046^{aA}
	1% T80	$0.95 \pm 0.05^{cB**}$	$1.93 \pm 0.35^{bA*}$	$2.90 \pm 0.30^{bB**}$	$1.26 \pm 0.01^{cB*}$	$1.50 \pm 0.02^{bB**}$	$3.38 \pm 0.19^{aB*}$	84.27 ± 2.17^{aA}	2.107 ± 0.054^{aA}

Mean values ± standard deviation. Different letters indicate significant difference at $p < 0.05$. Small letters: differences in the same column between samples with different emulsifier composition at the same oil phase. Capital letters: differences in the same column between LCT and MCT samples at the same emulsifier composition. Asterisk: differences between O/W emulsion and O/W β-carotene-emulsion at the same emulsifier and oil phase composition.

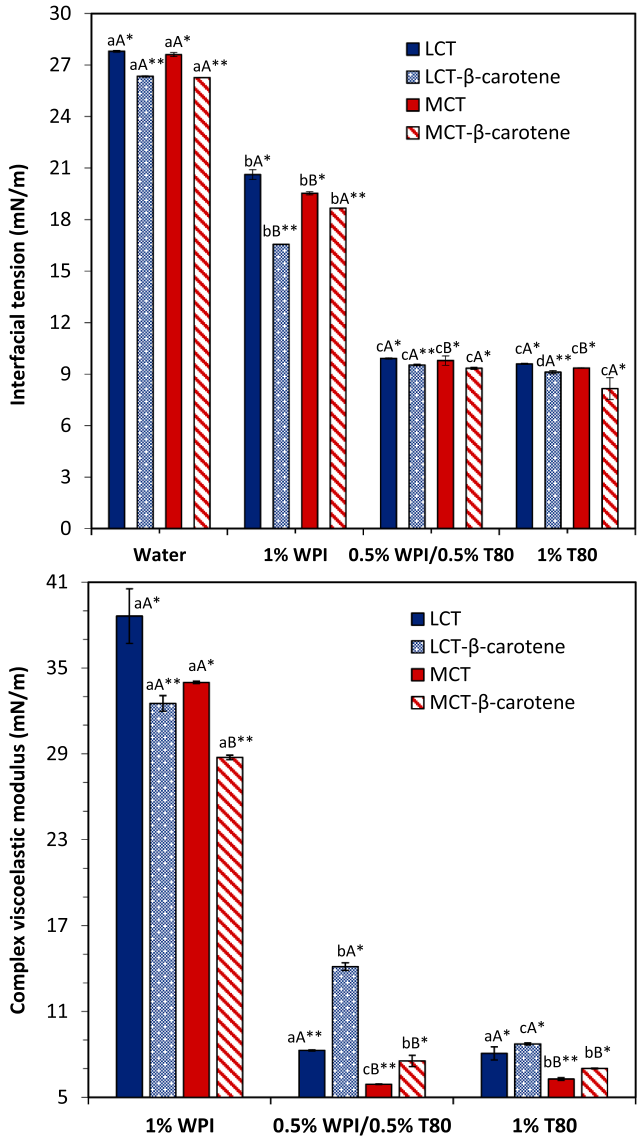

Fig. 1. Influence of β-carotene on the initial interfacial tension and complex dilational viscoelastic modulus: oil phase (LCT or MCT without or with β-carotene) and aqueous phase composition (water, 1% WPI, 0.5% WPI/0.5% T80 or 1% T80). Different letters indicate significant difference at $p < 0.05$. Small letters: differences between systems with different aqueous phase at the same oil phase. Capital letters: differences between emulsions produced with LCT and MCT or LCT-β-carotene and MCT-β-carotene at the same aqueous phase. Asterisk: differences between LCT and LCT-β-carotene or MCT and MCT-β-carotene at the same aqueous.

WPI/T80-β-carotene complexes. Although the β-carotene can bind to both surfactants through hydrophobic interactions, the complexes β-carotene-WPI and β-carotene-T80 are very distinct since that the WPI is a globular protein with high molecular weight while the T80 is a straight molecule with low molecular weight. Furthermore, the interfacial layers composed of WPI, WPI-T80 and T80 show different characteristics as result of the structure molecular of each surfactant and interaction between them at interface, which also affect emulsifier-β-carotene interaction. Following adsorption onto the oil–water interface, protein molecules interact through a combination of forces/bonds forming an interfacial network of highly entangled and cross-linked molecules, as can be demonstrated by high complex viscoelastic modulus (E*) values (Fig. 1) (Wan et al., 2014). A decrease in E* was observed in the presence of β-carotene suggesting that this carotenoid is located between protein molecules, decreasing the interaction between them, and reducing the viscoelastic character and the organization of the interfacial film. In contrast, T80 interfacial layer is loose and show low E* due to the low interaction between the surfactant molecules (Grigoriev, Derkatch, Kragel, & Miller, 2007). The addition of β-carotene promoted a slight increase of E*, which may be the result of the interaction between β-carotene and the hydrophobic tails of neighboring Tween 80 molecules. Similar behavior was observed to WPI/T80 interface, indicating that the mixed adsorption interfacial layers were composed predominantly by T80 (Fig. 1). Therefore, β-carotene promoted a decrease of the viscoelasticity in WPI interfaces (1% WPI w/w), while this bioactive compound caused an increase of the viscoelasticity in interfaces containing Tween 80. 1% WPI-β-carotene interfacial films became more susceptible to break up and T80-stabilized β-carotene layers offered greater resistance to disruption during the homogenization process, promoting the production of droplets with larger diameters (Dickinson, 2001).

The addition of β-carotene promoted the same effect in both LCT and MCT systems, however LCT/MCT 1% WPI emulsions presented similar mean droplet size while MCT emulsions stabilized by 0.5% WPI/0.5% T80 and 1% T80 showed smaller droplet size than LCT emulsions with the same emulsifier composition. These results could be associated with the lower viscosity and hydrophobicity of MCT than LCT as well as the different structure of their triacylglycerols. Proteins undergo more conformational rearrangements to anchor at the more hydrophobic LCT interface (Gomes et al., 2018; Maldonado-Valderrama & Patino, 2010) and, therefore, provide more hydrophobic groups into oil phase to interact with β-carotene promoting a further reduction of interfacial tension and droplet diameter in LCT β-carotene system (from 1.55 μm to 1.37 μm) compared to MCT β-carotene system (from 1.42 μm to 1.36 μm). In addition to the higher hydrophobicity, LCT chain is more complex as it is formed by unsaturated fatty acids (C18:1 and C18:2), which have bends in the molecular structure, while the fatty acids of MCT are saturated (C8:0 and C10:0) and show straight molecular structure. Thus, systems composed of LCT and/or WPI show interfaces (films) with a more interlaced structure and, hydrophobic interactions with rigid rod-like β-carotene molecules can promote a greater disruption of these films (Frank, Young, Britton, & Cogdell, 2004). On the other hand, MCT and/or T80 films show a simpler structure and the addition of β-carotene does not seem to affect their organization as deeply. This hypothesis is corroborated by the results of interfacial tension and complex viscoelastic modulus of the MCT/T80 interfaces, which showed small changes after the addition of β-carotene. In the mixed and T80 interfaces, in which the interfacial films seem to be dominated by T80, the viscosity of the oil phase had a greater influence on the droplet size than the interfacial properties. The lower viscosity of MCT (around 30 mPa.s) than LCT (around 42.6 mPa.s) facilitates the breakdown of MCT droplets in smaller sizes (Chiplunkar & Pratap, 2016; Walker, Gumus, Decker, & McClements, 2017).

All fresh O/W emulsions showed good kinetic stability after 7 days of storage (low TSI values), which is associated to the small droplet size, unimodal droplet size distribution and low *Span* values (Table 1 and Fig. 1S). The kinetic stability of emulsions was affected by β-carotene and oil type. MCT systems were more stable than LCT systems and the addition of β-carotene slightly improved the kinetic stability of the emulsions stabilized by 1% WPI (w/w) while that the presence of this bioactive compound reduced the stability of 0.5% WPI/0.5% T80 and 1% T80 emulsions. The addition of β-carotene affected the D_{43} values as previously discussed, and it is known that the droplet diameter is directly associated to emulsion stability. Emulsions with larger droplet diameter and lower dispersed phase density (LCT systems) showed higher TSI values. Destabilization of O/W emulsions is related to the migration velocity of oil droplets towards the emulsion surface (creaming process), which depends directly on the droplet size and the density difference between continuous and disperse phases according to the Stokes law (Tadros, 2013).

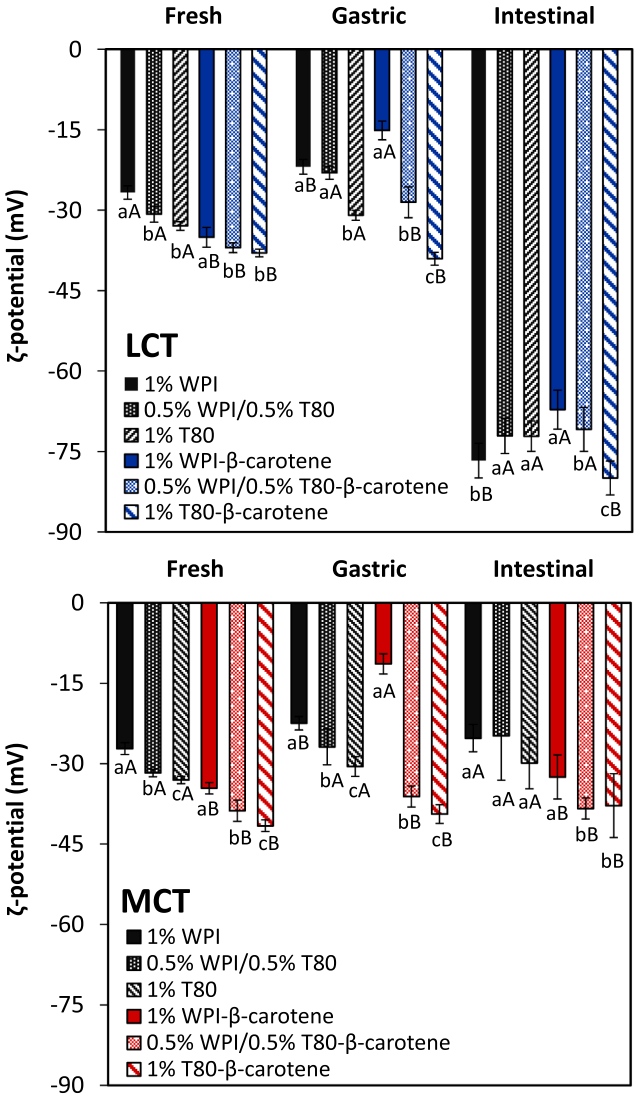

Fig. 2. Behavior of ζ-potential of the O/W emulsions without or with β-carotene, produced with LCT or MCT as oil phase and stabilized by 1% WPI, 0.5% WPI/0.5% T80 or 1% T80: after preparation (fresh) and after gastric and intestinal phases. Different letters indicate significant difference at $p < 0.05$. Small letters: differences of ζ-potential between samples with different emulsifier composition (1% WPI, 0.5% WPI/0.5% T80 or 1% T80) at the same oil phase composition (without or with β-carotene). Capital letters: differences of ζ-potential between samples at the same emulsifier composition with distinct oil phase composition.

The efficiency of encapsulation (EE) and loading (LE) of β-carotene in emulsions with different formulations is shown in Table 1. All systems showed similar EE and LE indicating that the interfacial composition did not affect the amount of β-carotene entrapped in the oil droplets. Despite the greater solubility of β-carotene in MCT than LCT, the oil type also did not impact the efficiency of encapsulation and loading of β-carotene since we used a much lower concentration than the solubility of this bioactive compound in LCT (Roohinejad et al., 2015).

All the initial β-carotene emulsions presented high negative ζ-potential, which may be attributed to the electrical characteristics of the adsorbed emulsifiers onto the interface (Fig. 2). WPI coated droplets present a high negative ζ-potential at neutral pH since this pH condition is well above of the WPI isoelectric point (pI ~ 5.1) (Park, Mun, & Kim, 2018). Tween 80 is a nonionic surfactant and does not show surface charge, however, negatively charged molecules, such as ions OH^- coming from water and free fatty acids released from the emulsifier or the oil, can adsorb onto the T80 interface resulting in decreased ζ-potential values (Qian, Decker, Xiao, & McClements, 2012a). β-carotene emulsions presented higher negative ζ-potential (from −34.5 to −41.5 mV) than emulsions prepared in the same conditions without this bioactive compound (from −26.6 to −33.0 mV). The presence of β-carotene may have promoted a thickening of the interfacial layer and changed the ζ-potential values. These results indicate that there were interactions between β-carotene and both emulsifiers, WPI and Tween 80, and that such interactions could have been able to modify the characteristics of interfaces. However, ζ-potential measurements provide only qualitative and indirect information about the characteristics of interfacial layer (Bhattacharjee, 2016). Emulsions are complex systems and, therefore, ζ-potential values are not only a reflection of repulsive forces, but also of attractive forces between different compounds (emulsifier, oil, and carotene).

The β-carotene interacted with other ingredients of the emulsions (WPI, T80, LCT and MCT) changing the characteristics of the interface and the average droplet size. Such changes affect the behavior of the systems through the gastrointestinal tract, since they can influence the emulsion stability and the action of digestive enzymes on the interface and, therefore, the formation of micelles, bioaccessibility and the physicochemical properties of β-carotene.

3.2. *Effect of β-carotene on the behavior of emulsions under simulated gastric conditions*

Only slight changes were observed in the mean droplet size after fresh β-carotene emulsions had been passed through the simulated stomach, except for the emulsions stabilized by only WPI. LCT/MCT β-carotene systems with 1% WPI exhibited a large increase in mean droplet size (D_{43} = 20.99–25.89 μm) and broader droplet size distributions (Table 2 and Fig. 1S). These results agree with microscopy

Table 2
Mean droplet size (D_{43}) of the emulsions after the gastric and intestinal steps, without or with β-carotene, produced with LCT or MCT oil and stabilized by 1% WPI, 0.5% WPI/0.5% T80 mixture or 1% T80 (w/w).

Oil Type	Emulsifier composition (w/w)	Gastric	Gastric β-carotene	Intestinal	Intestinal β-carotene
		D_{43} (μm)	D_{43} (μm)	D_{43} (μm)	D_{43} (μm)
LCT	1% WPI	$30.53 \pm 2.66^{aA*}$	$22.87 \pm 2.14^{aA**}$	$25.2 \pm 1.4^{aB**}$	$49.2 \pm 14.4^{aB*}$
	0.5% WPI/0.5% T80	$1.37 \pm 0.19^{bA**}$	$1.72 \pm 0.08^{bA*}$	$23.7 \pm 3.7^{abB**}$	$67.0 \pm 12.2^{aB*}$
	1% T80	$1.32 \pm 0.02^{bA**}$	$1.68 \pm 0.01^{bA*}$	$20.9 \pm 2.5^{bB**}$	$45.5 \pm 13.8^{aB*}$
MCT	1% WPI	$18.90 \pm 4.48^{aB*}$	$18.68 \pm 2.46^{aB*}$	$128.9 \pm 10.4^{aA**}$	$156.5 \pm 21.3^{aA*}$
	0.5% WPI/0.5% T80	$1.32 \pm 0.15^{bA*}$	$1.39 \pm 0.14^{bB*}$	$105.3 \pm 19.7^{bA**}$	$163.1 \pm 15.9^{aA*}$
	1% T80	$1.28 \pm 0.06^{bB*}$	$1.26 \pm 0.03^{bB*}$	$105.0 \pm 9.1^{bA**}$	$142.2 \pm 21.6^{aA*}$

Mean values ± standard deviation. Different letters indicate significant difference at $p < 0.05$. Small letters: differences in the same column between samples with different emulsifier composition at the same oil phase. Capital letters: differences in the same column between LCT and MCT samples at the same emulsifier composition. Asterisk: differences between O/W emulsion and O/W β-carotene-emulsion at the same emulsifier and oil phase composition.

images that show an extensive droplet aggregation (Fig. 2S). Such behavior may be attributed to the weakening of the electrostatic repulsion between the droplets (very low pH and high ionic strength) and the partial hydrolysis of the WPI coating by pepsin, predisposing the droplets to flocculation (Xu et al., 2014). The slight change in D_{43} values of the systems stabilized with 0.5% WPI/0.5% T80 suggest that the presence of T80 improved the stability of the droplets, even in the presence of WPI. T80 provides a high steric repulsion among oil droplets and it is highly stable under gastric conditions, such as low pH, high ionic strength and presence of enzymes, avoiding the aggregation and coalescence of droplets (Golding et al., 2011). It is interesting observe the values of D_{43} of the 1% T80 systems maintained unchanged indicating that the addition of β-carotene improved the stability of T80 interfacial film under gastric conditions (Tables 1 and 2).

The systems without or with β-carotene presented some differences in the D_{43} values after gastric step. β-carotene 1% WPI systems showed lower D_{43} values while the T80 β-carotene systems presented higher D_{43} values in comparison with the systems with the same composition without β-carotene. These results could be related to the mean droplet size of the initial emulsions, without or with β-carotene. Furthermore, the hydrophobic interactions between β-carotene and WPI could have hinder the access of pepsin to specific peptide bonds of WPI decreasing protein hydrolysis and size of clusters produced by bridging-flocculation of droplets.

All β-carotene-systems showed negative ζ-potential values after passing through the gastric step, with the ζ-potential decreasing in the following order for both oils: Tween 80 (around −39 mV), WPI/Tween 80 mixture (from −28.5 to −36.2 mV), WPI (from −11.5 to −15.2 mV) (Fig. 2). The ζ-potential values remained practically unchanged under gastric conditions for the emulsions stabilized by only T80, however, they presented smaller values for the WPI systems. Furthermore, β-carotene emulsions with 1% WPI presented lower ζ-potential (from −11.5. to −15.0 mV) than emulsions prepared in the same conditions without this bioactive compound (around −22 mV). The β-carotene can form stable-surface activity complexes with the WPI through hydrophobic interactions (Allahdad et al., 2019). β-carotene-WPI bindings can result in conformational changes in the secondary and tertiary structures of proteins which can alter the interactions between adsorbed WPI onto the interface and molecules from simulated gastric fluid/pepsin (Allahdad et al., 2019; Li, Wang, Chen, & Lu, 2015). Such interactions could explain the more pronounced ζ-potential decrease in systems with higher WPI concentration (1% w/w).

The ζ-potential was influenced by the oil type in β-carotene systems stabilized by 0.5% WPI/0.5% T80 (w/w). MCT β-carotene system did not present significant change in the ζ-potential value (around −36.1 mV) while a considerable decrease was observed in LCT β-carotene-system (around −28.5 mV). WPI exposes more hydrophobic peptides to interact with LCT interface, hindering the displacement of WPI molecules from interface by T80 (Maldonado-Valderrama & Patino, 2010). This effect allows the LCT interface stabilized by WPI/T80 mixture to have a higher amount of WPI compared to less hydrophobic MCT interfaces (Gomes et al., 2018). The additional amount of WPI in LCT interface could have interacted with β-carotene resulting in a decreased ζ-potential.

3.3. Effect of β-carotene on the behavior of emulsions under in vitro intestinal conditions

The largest change in the mean particle size occurred when the samples were submitted to simulated intestinal conditions (Table 2). There were no significant differences in D_{43} values for different interfacial layer compositions, however, the particles present in LCT digesta were smaller than those contained in MCT digesta. These results could be related to the extensive MCT oil droplets digestion (Section 3.3.1).

We used the distribution curves to compare the effect of adding β-carotene on the particle size of the digesta, as the curves are in the region of larger particles and, therefore, small alterations in them can mean major changes in the D_{43} values. As shown in Fig. 1S, the curves of particle size distribution of digested MCT and MCT β-carotene emulsions were very similar, indicating the little influence of addition of β-carotene in MCT systems and the extensive lipolysis in both systems. On the other hand, LCT β-carotene systems showed the first peak of the size distribution curves shifted towards larger particle sizes. This behavior could indicate a greater coalescence of oil droplets with β-carotene, which may lead to less lipid digestion due to the smaller interfacial area available for the action of digestive enzymes. Furthermore, the β-carotene molecule is too large, requiring the formation of larger structures, such as micelles or vesicles, in which the bioactive fits inside their hydrophobic interior (McClements, 2018).

After passing through the intestinal step, β-carotene emulsions presented higher negative ζ-potential (Fig. 2). This effect can be attributed to the adsorption of anionic-species with surface activity from simulated gastrointestinal fluid (e.g., bile salts, lipases) or generated during triacylglycerol digestion (e.g., free fatty acids). The ζ-potential values were higher for the particles contained in the LCT digesta than those present in the MCT digesta. MCT is composed of medium chain fatty acids that tend to leave the interface and move into the aqueous phase whereas the sunflower oil contains long chain acids that tend to accumulate at oil–water interface increasing the ζ-potential (Sek, Porter, Kaukonen, & Charman, 2002). The presence of β-carotene did not have relevant effect on the ζ-potential values of the systems after the intestinal step.

3.3.1. Oil digestion

The kinetics of lipid hydrolysis was influenced by the oil type. The lipolysis curves of the MCT systems without or with β-carotene showed three stages: a fast-initial reaction rate up to 30 min that markedly slowed down until reach a relatively constant final value after 60 min of digestion (Fig. 3). Otherwise, the lipid digestion curves of LCT systems without or with β-carotene presented only two stages: a rapid increase in the release of FFAs up to 60 min followed by a more gradual increase at longer times without to reach an equilibrium value. At the end of the digestion, MCT emulsions showed more pronounced lipolysis than LCT systems.

Differences in lipid hydrolysis might be attributed to chain size, unsaturation degree and surface activity of the fatty acids that compose the LCT and MCT oils. FFAs and 2-monoacylglycerols from LCT digestion have considerably higher interfacial activity than the same molecules coming from MCT digestion (Zhu, Ye, Verrier, & Singh, 2013). Long chain FFAs also tend to accumulate at the oil–water interface due to their major affinity for oily phase while medium chain FFAs move towards the surrounding aqueous phase due their lower hydrophobicity (Sek et al., 2002). Therefore, molecules from LCT hydrolysis can keep longer at the interface competing with the lipase and/or hampering this enzyme access by steric hindrance. Furthermore, sunflower oil is composed of PUFAs, which have a bended structure promoting a more intense steric hindering effect that protects TAGs from lipolysis (Verkempinck et al., 2018).

The emulsifier type exerted an appreciable impact on LCT digestion, with the percentage of free fatty acid released decreasing in the following order: WPI (46–52%), WPI-T80 blend (35–43%), T80 (28–30%) (Fig. 3). Tween 80 interfacial layer offers more resistance to displacement by bile salts, which limits the subsequent adsorption of the lipase/co-lipase complex necessary to perform the oil digestion. The T80 can bind to bile salt molecule in the medium preventing its adsorption at the interface (Vinarov et al., 2012). Otherwise, bile salts have an increased ability to displace proteins from oil-water interface because they can penetrate and disrupt the interfacial layer, facilitating the access to lipase and resulting in a higher release rate of FFAs in WPI systems (Golding et al., 2011).

The addition of β-carotene did not affect the release of FFAs of the 1% WPI or 0.5% WPI/0.5% T80 MCT systems. A similar amount of FFAs

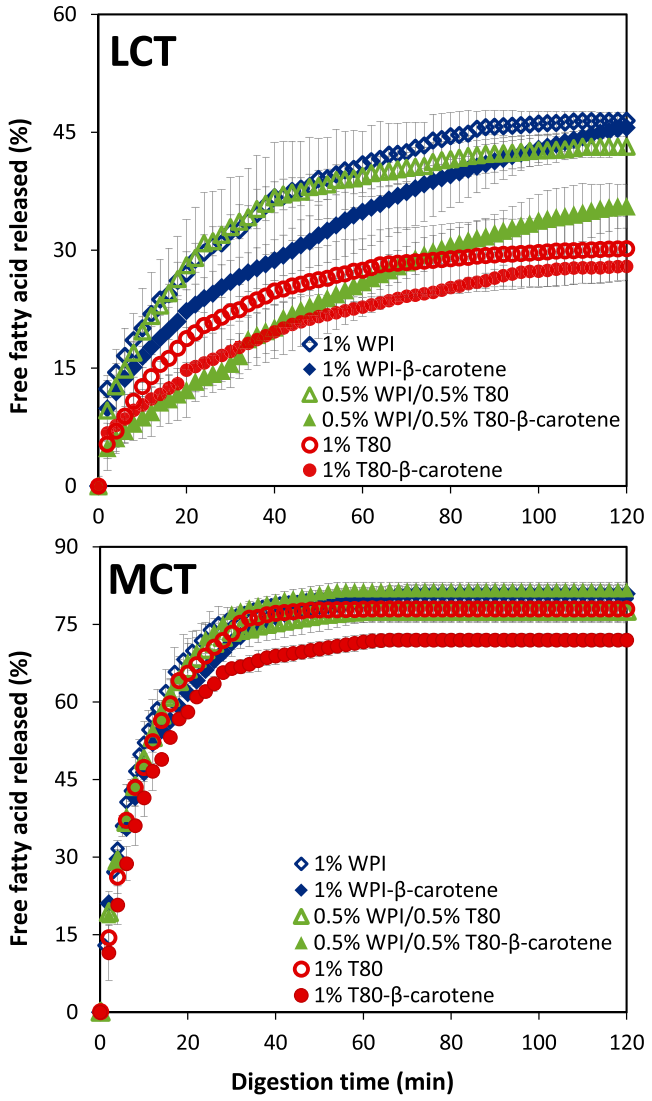

Fig. 3. FFAs released under simulated intestinal conditions as a function of time of the O/W emulsions without or with β-carotene, produced by LCT or MCT as oil phase and stabilized by WPI, WPI/Tween 80 mixture or Tween 80.

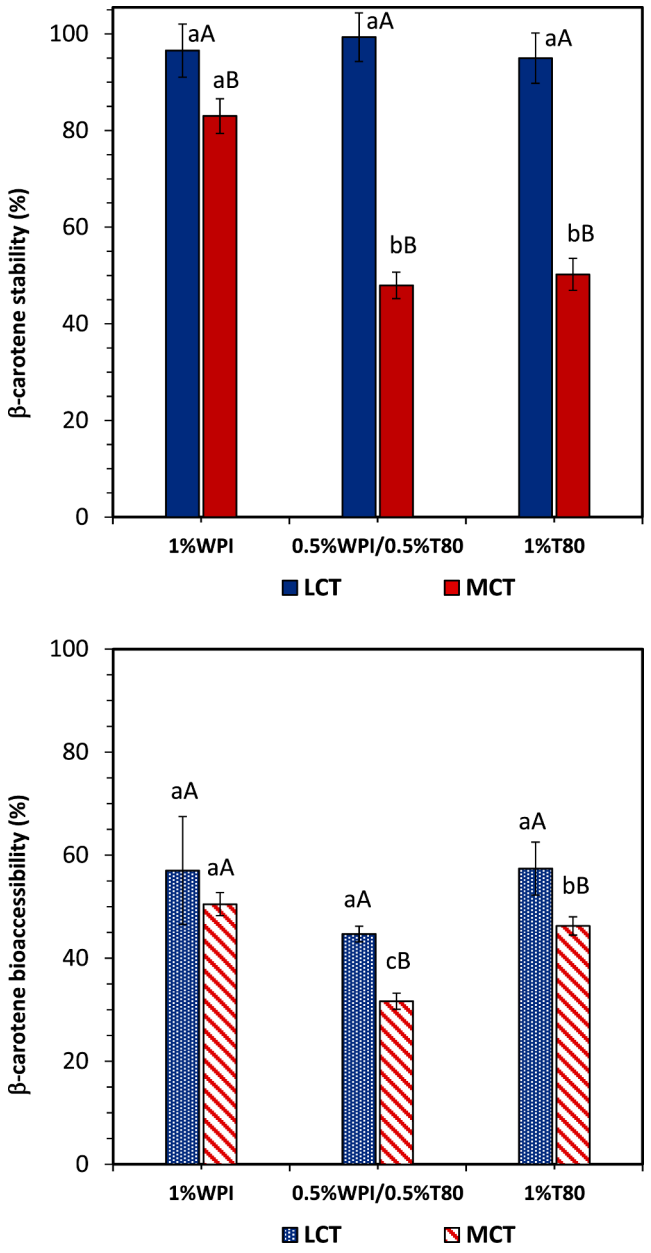

Fig. 4. Influence of oil type and emulsifier composition on the stability, through the simulated gastrointestinal tract, and bioaccessibility of β-carotene initially encapsulated within O/W emulsions produced with LCT or MCT and stabilized by 1% WPI, 0.5%WPI/0.5%T80 or 1%T80. Different letters indicate significant difference at $p < 0.05$. Small letters: differences of Stability/Bioaccessibility between samples stabilized by 1% WPI, 0.5% WPI/0.5% T80 mixture or 1% T80 at the same oil type. Capital letters: differences of Stability/Bioaccessibility between emulsions produced with LCT or MCT at the same emulsifier composition.

was released in LCT and LCT β-carotene systems, however, the presence of β-carotene promoted a delay in lipolysis suggesting that the lipase access to oil droplet surface was hindered by WPI-β-carotene complex. A decrease of lipid digestion was observed in LCT systems stabilized with 0.5% WPI/0.5% T80 or 1% T80 and 1% T80 MCT-system indicating that the T80/β-carotene interaction also made the interfacial layer more resistant to the action of digestive enzymes and bile salts, reducing the lipolysis rate.

3.3.2. Stability and bioaccessibility of β-carotene after in vitro digestion

The effect of interfacial layer composition and oil type on the stability under gastrointestinal conditions and bioaccessibility of β-carotene is shown in Fig. 4. In general, LCT systems offered higher protection and bioaccessibility to β-carotene than MCT systems. As mentioned earlier, long chain FFAs tend to accumulate onto interface while medium chain FFAs have a tendency to move towards the aqueous phase (Sek et al., 2002). The presence of different molecules onto the LCT-interface, such as FFAs and 2-monoacylglycerols, can hinder the precipitation of FFAs and bile salts by Ca^{2+}, and, therefore the formation of insoluble calcium soaps and calcium-bile acid complexes. In contrast, the FFAs of MCT digestion are in the aqueous phase, which make them more available for complexation with Ca^{2+}. The precipitation of FFAs and bile salts by calcium is considered the main reason for the decrease in the number of mixed micelles and the width of hydrophobic domains within the micelles, leading to reduction of the solubility of lipophilic compounds in mixed micelles (Lin et al., 2018). Furthermore, mixed micelles assembled from long chain FFAs generally have greater solubilization capacity of lipophilic bioactive compounds than those built from medium chain FFAs, because LCT mixed micelles have wider nonpolar regions (McClements, 2018). Such effects could have contributed to the higher β-carotene bioaccessibility in LCT systems.

Regarding the higher β-carotene stability in LCT systems, there are

some possible reasons for this phenomenon. First, the incomplete lipolysis in LCT systems allowed that a greater amount of β-carotene remained trapped in the undigested oil droplets, protected from pro-oxidant molecules and environmental conditions that could degrade it. Also, the mean droplet size was larger in LCT emulsions than MCT ones. It means that the interfacial area was smaller for LCT systems, which can also lead to a slower β-carotene degradation rate (Qian, Decker, Xiao, & McClements, 2012b).

In LCT systems, the stability and bioaccessibility of β-carotene were similar for 1% WPI, 0.5% WPI/0.5% T80 and 1% T80, despite the difference in the rate and extent of lipid digestion among these samples (Fig. 3). As previously observed by Giang et al. (2016) only a fraction of hydrolyzed products may be recovered within the micellar phase. Otherwise, MCT system stabilized by 1% WPI showed higher stability and bioaccessibility than MCT systems with 0.5% WPI/0.5% T80 and 1% T80. Such results indicate that, in addition to the oil type, the emulsifier nature may have a pronounced influence on the bioaccessibility, and chemical stability of the β-carotene trapped in the MCT emulsions.

β-carotene is sensitive to environmental stress and oxidation is identified as the main cause of its degradation and loss of health-related properties (Boon et al., 2010). The incorporation of this bioactive in the oil phase of the emulsions protect it from degradation for two reasons: i) physical barrier offered by the interfacial film which limits the diffusion of oxygen, pro-oxidant molecules or free radicals into the droplet core to interact with β-carotene; and ii) antioxidant property of interfacial layer (Rodriguez-Concepcion et al., 2018).

The emulsifiers of the Tween class have very low antioxidant activity justifying the lower β-carotene content in the micellar and digesta phases of the 0.5% WPI/0.5% T80 and 1% T80 MCT systems (Rodriguez-Concepcion et al., 2018). Otherwise, the higher concentration of β-carotene in 1% WPI MCT system can be related to the action of this protein in different ways. Proteins are effective antioxidants that can act by chelating transition metals or as free radical scavengers. The major protein fractions of WPI, β-lactoglobulin and α-lactoglobulin, have cysteyl residues, disulphide bonds and thiol groups that are capable of scavenging free radicals at the oil–water interface and in the aqueous phase, inhibiting the lipid oxidation (Berton-Carabin, Ropers, & Genot, 2014). Also, the thicker protein interfacial layer may act as a physical barrier hindering the contact between β-carotene and pro-oxidant molecules present in the aqueous phase (Qian et al., 2012b). Lastly, as mentioned before, the three main protein fractions of WPI (β-lactoglobulin, α-lactalbumin and bovine serum albumin-BSA) can form stable complexes with β-carotene protecting this carotenoid from degradation (Allahdad et al., 2019; Li et al., 2015). Furthermore, the hydrophobic binding pockets enable the protein to increase the apparent solubility of carotenoid in plasma and modulate their delivery to cells and *in vivo* tissues (Li et al., 2015).

Regarding the nature of the oil phase, LCT contains a high proportion of unsaturated fatty acids, such as oleic and linoleic acids, which can react with oxygen, pro-oxidants or free radicals working as antioxidants to protect β-carotene from degradation. This antioxidant effect may have contributed to avoid the β-carotene degradation, even on T80 stabilized systems, which resulted in a higher β-carotene concentration in both phases for all LCT systems. On the other hand, MCT oil is not able to offer protection to β-carotene, as it is composed mainly of saturated fatty acids. Therefore, the β-carotene chemical stability in MCT systems was influenced only by the interfacial layer composition.

4. Conclusion

The β-carotene interacted with other ingredients of the emulsified system, modifying its features, such as interfacial layer, mean droplet size and ζ-potential, which influenced the amount of β-carotene within mixed micelles.

LCT emulsions stabilized by WPI, WPI/T80 mixture or T80 proved to be an effective encapsulation and release system of β-carotene. All LCT-systems presented similar stability and bioaccessibility of β-carotene. The β-carotene encapsulated in MCT-system with 0.5% WPI/0.5% T80 and 1% T80 tended to chemically degrade under simulated digestive system conditions, which resulted in a lower stability and bioaccessibility of β-carotene. On the other hand, the β-carotene entrapped within protein-coated MCT droplets was more stable to chemical degradation, suggesting that emulsions produced with WPI may offer better chemical stability to β-carotene. In this way, the chemical stability of the bioactive must be considered before selecting ingredients that will compose the emulsion-based delivery systems.

Our results show the importance of knowing the interaction of the bioactive with the oil and the emulsifier, to carefully select the appropriate ingredients when designing emulsions in order to improve the stability and bioaccessibility of the bioactive compound.

CRediT authorship contribution statement

Andresa Gomes: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data curation, Writing - original draft, Writing - review & editing, Visualization, Project administration. **Ana Letícia Rodrigues Costa:** Investigation, Formal analysis, Data curation. **Dayane Dias Cardoso:** Formal analysis, Data curation. **Grazielle Náthia-Neves:** Methodology, Formal analysis, Data curation. **M. Angela A. Meireles:** Methodology, Data curation. **Rosiane Lopes Cunha:** Conceptualization, Methodology, Resources, Writing - review & editing, Supervision, 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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2020.128155.

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