Journal of Functional Foods 41 (2018) 135–147

Contents lists available at ScienceDirect

Journal of Functional Foods

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

Kinetic approach to study the relation between *in vitro* lipid digestion and carotenoid bioaccessibility in emulsions with different oil unsaturation degree

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ARTICLE INFO

Chemical compounds:
Sucrose palmitate (PubChem CID: 9829755)
Sucrose stearate (PubChem CID: 9898327)
Lipase (PubChem CID: 54603431)
Bile salt (PubChem CID: 439520)
Oleic acid (PubChem CID: 445639)
Linoleic acid (PubChem CID: 5280450)
Linolenic acid (PubChem CID: 5280934)
α-carotene (PubChem CID: 4369188)
β-carotene (PubChem CID: 5280489)
Lycopene (PubChem CID: 446925)

Keywords:
Lipolysis
Emulsion
Unsaturation degree
Carotenoid
Bioaccessibility

ABSTRACT

Emulsions were prepared with oils (5% w/v) differing in unsaturation degree (olive, soybean or linseed oil) and sucrose ester (0.5% w/v) used as emulsifier. The oils studied were enriched with carotenoids from carrot or tomato purees. All emulsions were *in vitro* digested and characterised at the level of lipolysis and carotenoid micellarisation kinetics in the small intestinal phase. Olive oil emulsions led to a faster and more extensive lipolysis and carotenoid bioaccessibility compared to soybean and linseed oil emulsions. Monounsaturated fatty acids present in olive oil might be more hydrophobic in comparison to polyunsaturated fatty acids from soybean or linseed oil, leading to micelles with greater capacity of solubilising hydrophobic carotenoids. The obtained results evidence the potential of the oil unsaturation degree for modulating lipolysis and carotenoid bioaccessibility in the gastrointestinal tract and moreover, exemplify the relevance of a kinetic approach including modelling of different lipolysis species to quantitatively prove their interrelation.

1. Introduction

Lipids, proteins and carbohydrates are the most important energy nutrients for humans of which lipids contain the highest energy per weight (9 kcal/g). In this sense, lipids supply energy, but they are also important for structural support in tissues and provide essential fatty acids and lipid soluble micronutrients in the human body (Golding & Wooster, 2010). Carotenoids are hydrophobic micronutrients present in various fruits and vegetables and are associated with potential health promoting effects, like cataract and cancer prevention (Rao & Rao, 2007). In order to exert these possible health benefits, carotenoids must be absorbed into the body. In summary, carotenoids must be successively (i) released from their food matrix; (ii) dissolved in a lipid phase; (iii) incorporated in mixed micelles and (iv) absorbed into the blood stream (Deming & Erdman, 1999). Bioaccessibility refers to the first three steps, hence representing the ingested carotenoid fraction which is incorporated into mixed micelles and is available for absorption in the small intestine.

It can be hypothesised that by improving lipid digestion, carotenoid bioaccessibility can be favoured as both processes are strongly linked (Deming & Erdman, 1999). In the human diet, lipids are mostly present as oil-in-water (o/w) emulsions, such as soups and sauces. O/w emulsions are composed of oil droplets dispersed in water, whereby the oil-water interface is covered with a layer of emulsifier allowing the

Abbreviations: OO, olive oil; SO, soybean oil; LO, linseed oil; CEOO, carrot-enriched olive oil; CESO, carrot-enriched soybean oil; CELO, carrot-enriched linseed oil; TEOO, tomato-enriched olive oil; TESO, tomato-enriched soybean oil; TELO, tomato-enriched linseed oil; BAC, bioaccessibility

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https://doi.org/10.1016/j.jff.2017.12.030
Received 27 July 2017; Received in revised form 14 December 2017; Accepted 15 December 2017

stability of the dispersed oil droplets. Improving carotenoid bioaccessibility can be achieved during processing by removing natural barriers and incorporating the carotenoids into the oil phase of o/w emulsions (Palmero et al., 2013; Mutsokoti, Panozzo, Musabe, Van Loey, & Hendrickx, 2015; McClements et al., 2015). Consequently, carotenoid bioaccessibility may be modulated by controlling the emulsion characteristics. It has been described that (micro)structural characteristics of o/w emulsions, such as emulsion droplet size, interfacial composition and lipid nature, may significantly impact their fate during gastrointestinal conditions, as it will determine their interaction with digestive enzymes (McClements, Decker, & Park, 2008; Singh, Ye, & Horne, 2009). For instance, emulsions with a smaller initial droplet size and therefore a higher active surface area, presented a faster and higher *in vitro* lipid digestion in comparison with emulsions with a larger droplet size (Salvia-Trujillo et al., 2017). Also the emulsifier type used to stabilize emulsions has shown to play an important role on the behaviour of emulsions during their passage through the gastrointestinal tract (McClements et al., 2008; Verkempinck et al., 2018). With regard to the lipid nature, extensive research has been focused on studying the influence of the fatty acyl chain length of the carrier oil on the *in vitro* lipid digestion or carotenoid bioaccessibility (Huo, Ferruzzi, Schwartz, & Failla, 2007; Nagao, Kotake-Nara, & Hase, 2013). In all mentioned studies, lipids with longer fatty acyl chains led to a higher carotenoid bioaccessibility in comparison with medium or short chain fatty acyl chains. Longer fatty acids are more hydrophobic, so formed mixed micelles had a more hydrophobic core which facilitated carotenoid micellarisation. In addition, mixed micelles composed of longer fatty acids tend to be larger (Christensen, Schultz, Mollgaard, Kristensen, & Mullertz, 2004) and are thus able to solubilize more hydrophobic material, such as carotenoids. In addition, the interest of using medium or short chain triglycerides for human consumption is limited due to their poor nutritional value. Within oils with long chain triacylglycerols (TAG), which are the majority of commonly used edible oils in food products, little is known about the possible influence of the saturation degree on their behaviour during digestion, and results published so far are rather inconsistent. In this sense, Huo et al. (2007) did not see any effect of the saturation degree of the added oils on the α- and β-carotene incorporation into mixed micelles. On the contrary, Gleize et al. (2013) and Failla, Chitchumronchokchai, Ferruzzi, Goltz, and Campbell (2014) saw a negative correlation between oils saturation degree and carotenoid bioaccessibility. Research of Nagao et al. (2013) concluded that monounsaturated fatty acids (MUFA) are more efficient in enhancing β-carotene bioaccessibility than polyunsaturated fatty acids (PUFA) as PUFA are more prone to oxidation than MUFA. Unambiguous information is required to make clear conclusions in this research field. In this work, a kinetic rather than a single point end level approach (used so far in this research field) is elaborated in order to better understand the link between the unsaturation degree of emulsified oils, lipid digestion and carotenoid bioaccessibility. The kinetics thereby focus on lipid hydrolysis and carotenoid micellarisation in the small intestine. With regard to lipid digestion, not only the amount of free fatty acids (FFA) are quantified, but different lipid digestion species including TAG, diacylglycerol (DAG) and monoacylglycerol (MAG), both in the digest as well as in the micellar fraction. Moreover, the data of these multiple lipolysis products obtained by the kinetic digestion study were modelled. This allows to quantitatively elucidate the relationship between lipid digestion and micelle formation. Furthermore, this study aimed to link the evolution of the lipid digestion reaction to the (micro) structural changes occurring along the digestive tract. Finally, bioaccessibility of lipophilic carotenoids was investigated in addition to lipid digestion, as it is hypothesised that these two processes might be strongly linked. The kinetic approach allows to evaluate this hypothesis throughout the whole digestion process based on the resulting kinetic parameters and not based on single end values. To the best of our knowledge, the presented work is the first lipid digestion study performing a kinetic approach whereby multiple lipid digestion species were quantified to evaluate the effect of the oil unsaturation degree on both the kinetics of lipid digestion as well as carotenoid bioaccessibility. The obtained data of the current kinetic study can eventually contribute to the development of predictive mathematical (*in silico*) models.

2. Material and methods

Simple o/w emulsions were prepared with different oil types, being olive oil (rich in MUFA), soybean and linseed oil (rich in PUFA) with different unsaturation degree while having a similar chain length (Section 2.3). All emulsions were subjected to an *in vitro* digestion procedure using a kinetic approach (Section 2.4). The digested samples are referred as 'digest', while the aqueous fraction harvested after ultracentrifugation of the digest is referred as 'micelles'. All o/w emulsions and digests were characterised in terms of particle size, lipid and carotenoid content (Sections 2.5–2.7). The concentration of multiple lipid species and carotenoids in the micelle fractions was determined and used to monitor the micelle formation and composition as well as the carotenoid micellarisation (Sections 2.6 and 2.7). The evaluation of lipid digestion in both carrot- and tomato-based emulsions, can be seen as independent validation studies of the impact of oil type on this reaction.

2.1. Materials

All used analytical or HPLC-grade chemicals and reagents were purchased from Sigma Aldrich (Diegem, Belgium), except for KCl, $MgCl_2(H_2O)_6$, NaOH, heptane, methanol, methyl-tert-butyl-ether and ethyl acetate (Acros Organics, Geel, Belgium); $NaHCO_3$, NaCl, H_2SO_4, KH2PO4, ethanol, acetone and trimethylamine (Fisher Scientific, Merelbeke, Belgium); HCl, diethylether and iso-propanol (VWR, Leuven, Belgium); acetone (Carlo Erba, Val-de-Reuil, France); $CaCl_2(H_2O)_2$ (Chem Lab, Zedelgem, Belgium) and lipid standards (Larodan, Solna, Sweden). Orange carrots (*Daucus carota* cv. Nerac) and red tomatoes (*Solanum lycopersicum,* a Spanish variety) were bought in a shop and stored at 4 °C until use. Olive oil was bought in a local shop while soybean and linseed oil were kindly donated by Vandemoortele (Ghent, Belgium). The linseed oil is a blend of 70% sunflower oil and 30% linseed oil to make the oil more oxidatively stable. Olive oil is mainly composed of oleic acid (MUFA), soybean oil of linoleic acid (PUFA) and linseed oil of linoleic and linolenic acid (PUFA). The specific composition of each oil is given in Table A (supplementary data). Sucrose ester (hydrophilic-lipophilic balance of 11) was kindly donated by Sisterna (Roosendaal, The Netherlands).

2.2. Preparation of carotenoid-enriched oil

Three different oils (olive, soybean and linseed oil) were separately enriched with carotenoids from carrot or tomato purees. Carrots mainly contain α-carotene and β-carotene, whereas tomatoes are rich in *trans* lycopene and β-carotene. This enrichment resulted in 6 different studied oils: carrot-enriched olive oil (CEOO), tomato-enriched olive oil (TEOO), carrot-enriched soybean oil (CESO), tomato-enriched soybean oil (TESO), carrot-enriched linseed oil (CELO) and tomato-enriched linseed oil (TELO). The transfer of other lipid soluble components present in carrots and tomatoes was considered to be negligible. It was specifically chosen to work with carotenoids transferred from a food matrix to the oil phase, rather than using pure carotenoids in order to approach a real system whereby carotenoids might already be transferred to the oil phase during food preparation prior to digestion.

To obtain the carrot-enriched oils, the procedure described by Palmero et al. (2013) was followed. Carrot pieces were mixed with demineralised water (1:1 w/w) for 1 min in a kitchen blender (Warington Commercial, Torrington, CT, USA). The carrot puree was homogenised at 100 MPa for 1 cycle (Panda 2 K, Gea Niro Soavi, Parma,

Italy) to break up the cellular structure and release the carotenoids entrapped in the chromoplasts, and was thereafter added to an oil phase (5:1 w/w). The carotenoid transfer to the oil phase was favoured by rotating the puree-oil mixture at room temperature for 5 h. Subsequently, the mixture was centrifuged at 8740*g* at 4 °C to recover the enriched oil. The enriched oils were stored at −80 °C in dark tubes flushed with nitrogen.

Tomato-enriched oils were obtained following the method previously described by Mutsokoti et al. (2015). In a first step, tomato pieces were mixed for 1 min with a kitchen blender (Warington Commercial, Torrington, CT, USA) and subsequently the tomato puree was sieved (pore size of 1 mm) to remove remaining peel and seeds. Next, the sieved puree was homogenised at 100 MPa for 1 cycle (Pony NS2006L, Gea Niro Soavi, Düsseldorf, Germany). To enrich the oil, the tomato puree was mixed with oil (5:1 w/w) using a kitchen blender and was thereafter homogenised at 100 MPa for 1 cycle. The tomato-enriched oil was recovered and stored the same way as described above.

2.3. Preparation of oil-in-water emulsions

Firstly, coarse o/w emulsions were prepared by mixing 5% of enriched oil (w/v) to a water phase containing 0.5% of emulsifier (w/v) (sucrose ester, hydrophilic-lipophilic balance value of 11) at 9500 rpm for 10 min (Ultra-Turrax T25, IKA, Staufen, Germany). O/w emulsions were further stabilised by homogenising the coarse emulsions at 100 MPa for 1 cycle (Pressure Cell Homogenizer, Stansted Fluid Power Ltd., UK) and was thereafter stored at 4 °C, protected from light and oxygen. All emulsions were stable in terms of particle size for at least 3 days (data not shown).

2.4. In vitro digestion

This study aimed to investigate the time dependency of *in vitro* lipolysis and the micellarisation of multiple lipid digestion products and carotenoids of o/w emulsions prepared with oils differing in unsaturation degree. For this, the static *in vitro* digestion method of Minekus et al. (2014) was used as modified by Mutsokoti et al. (2017), consisting on a gastric and small intestinal phase.

The gastric phase was mimicked by adding 10 mL of gastric fluids to 10 mL of emulsion (ratio 1:1). The gastric fluids consisted of simulated gastric fluid (composed as described by Minekus et al. (2014)); calciumchloride (0.3 M); pepsin solution (2000 U/mL in final chyme); demineralised water and HCl (2M) until the pH was adjusted to 3. The headspaces of the samples were flushed with nitrogen to minimize carotenoid isomerisation and degradation. The chyme was rotated end-over-end (40 rpm) in the dark at 37 °C for 2 h. The small intestinal phase was simulated by adding 20 mL of small intestinal fluids to 20 mL of chyme (ratio 1:1). The intestinal fluids consisted on: simulated intestinal fluid (composition is as described by Minekus et al. (2014)); calciumchloride (0.3 M); demineralised water; bile solution (10 mM in the final digest) and a pancreatic solution (100 U/mL based on trypsin activity in final digest). The pancreatic solution contained pancreatin and lipase, to account for a total lipase activity of 200 U/mL in the final digest. In this study, α-tocopherol (1.4% w/v) and pyrogallol (0.6% w/v) were added to the pancreatic solution to avoid carotenoid isomerisation and degradation (Lemmens, Colle, Van Buggenhout, Van Loey, & Hendrickx, 2011). All intestinal fluids were tempered at 37 °C. The headspaces of the samples were flushed with nitrogen and the samples were rotated end-over-end (40 rpm) in dark at 37 °C. Incubation times were varied (0–120 min) to investigate the time dependency of lipolysis and the incorporation of carotenoids and multiple lipid digestion products into mixed micelles. Lipase was inactivated by applying a heat shock at 85 °C for 10 min and immediately thereafter samples were cooled to 4 °C. The digest was ultracentrifuged (Optima XPN-80 Ultracentrifuge, Beckman Coulter, Fullerton, CA, USA) at 165,000*g* for 68 min at 4 °C in order to harvest the micelles. The aqueous, micellar phase was collected, filtered (Chromafil PET filter, 0.2 μm pore size, 25 mm diameter) and analysed in terms of lipid composition and carotenoid content. The lipase activity used in the present study (200 U/mL digest) was reduced in comparison to the one suggested by Minekus et al. (2014) (2000 U/mL), and was in the range of the lipase units used in other, recent digestion studies (63–550 U/mL digest), such as O'Sullivan et al. (2017) and Mutsokoti et al. (2017). However, the lipase amount used, is still in excess based on the calculated lipase amount needed to cover the complete oil droplet surface area. Based on the calculations of Al-Zuhair, Ramachandran, and Hasan (2008), it can be stated that 1*g* op lipase is needed to cover an area of 270 m^2. Taking into account the oil amount added per digestion tube in this work (500 mg/tube), the oil density (± 920 kg/m^3) and its initial oil droplet size (± 1 μm), it can be calculated that 0.012*g* lipase is needed to cover already all emulsified oil droplets. In our work, an amount of 0.05*g* pure porcine lipase was added per digestion tube to reach a final lipase amount of 200 U/mL digest, allowing full coverage of all (emulsified) oil droplets and resulting even in free lipase (i.e. lipase in excess). Nevertheless, the presented results might not fully represent human digestion, and should therefore not be considered for *in vivo* human digestion predictions. However, they are valuable for increased mechanistic insight in the lipid digestion process.

2.5. Particle size distribution and microstructure

Particle size distribution was measured using a laser diffraction equipment (Beckman Coulter Inc., LS 13 320, Miami, Florida, USA). Samples were poured into a stirring tank filled with demineralised water and pumped (speed 30%) to the measurement cells. The intensity of the diffracted laser light (wavelength main illumination source: 750 nm; wavelengths halogen light for Polarization Intensity Differential Scattering (PIDS): 450 nm; 600 nm; 900 nm) is detected and analysed with the Fraunhofer model. The particle sizes of the initial emulsions, after the gastric phase and the intestinal phase (digestion end point) were evaluated using the D[v;0.5] value, also defined as the median volume-weighted particle size. The D[v;0.5] defines the particle diameter at which 50% of the particles are smaller. The volume-weighted particle size is a more sensitive indicator for instability of emulsions in comparison with surface-weighted particle size as it largely increases with the presence of a limited number of large particles.

In order to detect possible destabilisation phenomena of the initial emulsions during simulated digestion conditions, the microstructure after the gastric phase and after 2 h of intestinal phase was visualised by differential interference contrast microscopy (Olympus BX41, Olympus Corporation, Tokyo, Japan) equipped with a digital camera (Olympus BX51, Olympus Optical Co. Ltd., Tokyo, Japan) at different magnifications.

2.6. Quantitative analysis of multiple lipid digestion products

Extraction of lipids was based on the procedure described previously by Verrijssen et al. (2015) with minor modifications. 1 mL of sample (emulsion, digest or micelles), 2 mL of ethanol, 3 mL of diethylether:heptane (1:1 v/v) and 0.2 mL 2.5 M sulfuric acid were vortexed for 2 min and rotated end-over-end at room temperature for 30 min. To collect the upper, organic layer, the mixture was first centrifuged at 500*g* during 5 min at 20 °C (Sigma 6–16 K centrifuge, swing-out rotor, Germany). Hereafter, the extraction was repeated as described above by adding 1 mL of diethylether:heptane to the lower, inorganic phase. The upper layer was collected twice into a volumetric flask and volume was brought to 5 mL with diethylether:heptane. The collected organic phase was filtered (Chromafil PET filter, 0.2 μm pore size, 25 mm diameter) into a dark vial before analysis. Different lipid species (triacylglycerols, monoacylglycerols and free fatty acids) were quantified using normal phase HPLC with an Evaporative Light Scattering Detector (ELSD) and was adapted from the method described by

Graeve and Janssen (2009). The HPLC system (Agilent Technologies 1200 Series, Diegem, Belgium) was equipped with a silica column (5 μm × 150 mm × 4.6 mm, Silica Spherisorb, Waters, Zellik, Belgium) and an external column oven (Chromaster 5310 column oven, VWR International, Leuven, Belgium) set at 40 °C. The ELSD (Alltech 3300 ELSD Detector, Grace, Lokeren, Belgium) settings were as follows: temperature of the drift tube set at 44 °C, nitrogen gas flow of 1.9 mL/min and gain set on 1. The quaternary gradient was build up in 35 min with variable flow rates in function of elution time. Identification and quantification of the multiple lipid species was done using corresponding calibration curves of pure standards.

In the digest, the amount of released glycerol (GLY) at each time moment in the small intestinal phase was calculated based on the stoichiometric reactions of TAG hydrolysis and the analytical results of monoacylglycerols (MAG) and free fatty acids (FFA) in the digest (diacylglycerols were detected, but below the quantification limit), as has been described before by Salvia-Trujillo et al. (2017) and Verkempinck et al. (2018). Subsequently, mass balances were used to calculate the amount of undigested TAG at each time moment as the amount of undigested TAG is the difference between the initial TAG amount (analytical value) and the sum of all lipid digestion products in the small intestine (MAG + FFA + GLY-H_2O). The TAG digestion is expressed as the percentage of digested TAG in the initial emulsion. The lipid composition of mixed micelles is based on the analytical results of MAG and FFA.

2.7. Carotenoid analysis

Carotenoid extraction was carried out as described by Colle, Lemmens, Van Buggenhout, Van Loey, and Hendrickx (2010). Either emulsion or micelle fraction was mixed with 0.5 g of sodium chloride, 25 mL of extraction solution containing hexane:acetone:ethanol (2:1:1 v/v/v) and butylated hydroxytoluene (0.1% w/v), and 7.5 mL of reagent grade water (18.2 MΩ). The organic phase, containing carotenoids, was collected and filtered (Chromafil PET filter, 0.2 μm pore size, 25 mm diameter). Carotenoids were identified and quantified using reversed phase HPLC and a diode array detector, equipped with an apolar C30 column (3 μm × 150 mm × 4.6 mm, YMC Europe, Dinslaken, Germany) and column temperature was kept constant at 25 °C. A linear gradient was used to elute different carotenoids and a constant flow rate of 1 mL/min was applied. Starting conditions were: 4% of reagent grade water (A), 81% methanol (B) and 15% methyl-tert-butyl-ether (C). Final conditions for carrot-based samples were: 4% A, 41% B and 55% C, build up in 17 min; while the final conditions for tomato-based samples were: 4% A, 16% B and 80% C, build up in 39 min. Carotenoid identification and quantification was performed at the wavelength of maximal absorption: 450 nm for α- and β-carotene and 472 nm for lycopene and isomers. Quantification was done using corresponding calibration curves of pure standards. *In vitro* carotenoid bioaccessibility (BAC) per incubation time is defined as the ratio of the carotenoid amount in the micelle fraction to the initial carotenoid amount in the emulsion, both expressed per g initial emulsion. So, carotenoid BAC is expressed as a percentage value, taking into account variations in carotenoid amounts of the initial emulsion.

2.8. Statistical analysis

All statistical analysis were performed using the statistical software SAS (version 9.4, SAS Institute Inc., Cary, NC, USA). Significant differences in particle sizes of all samples and carotenoid content of enriched oil and emulsions were analysed by one way ANOVA and Tukey's Studentised Range Post-hoc test with a 95% level of significance ($P < .05$). Particle size measurements were determined in duplicate for all emulsions. Following our kinetic approach, the digestion of a particular system (e.g. emulsions with particular characteristics) is evaluated not only based on a single time moment, but it is evaluated as a function of small intestinal digestion time. The evaluations of all these time moments (i.e. 6 time moments per system) belong to the characterization of the same system. In the context of data analysis, these consecutive evaluations of the same system, need to be analysed together. In other words, to be able to determine the kinetic parameters of the system, all consecutive evaluations of the same system will be taken into account together and, from a statistical point of view, they can be seen as repetitions. All measurements were performed for both carrot- and tomato-based emulsions, representing two independent sets of experiments which can validate the results obtained.

Experimental data of the lipid digestion kinetic study and carotenoid bioaccessibility kinetics were modelled through nonlinear regression. The empirical, most simple model best describing the experimental data was selected using model discrimination. This resulted in use of the fractional conversion model, described by a first order reaction until attainment of a plateau value (Eq. (1)). Hereby C (%) is the studied parameter at time t in the simulated intestinal phase; C_f (%) is the final, maximum of the studied parameter that can be obtained with the imposed conditions; C_0 (%) is the initial studied parameter at time 0 in the simulated intestinal phase; k (min^{-1}) is the reaction rate constant of the studied process and t (min) is time in the simulated intestinal phase. The initial TAG concentration in the simulated intestinal phase was presumed to be equal to the amount of TAG present in the initial emulsion.

$$C = C_f + (C_0 - C_f)\cdot e^{(-kt)} \quad (1)$$

This model was simplified as digestion at time 0 of the simulated intestinal phase was considered to be negligible (Eq. (2)). Therefore, concentrations of MAG; FFA and GLY, and carotenoid concentration in micelles was presumed to be zero ($C_0 = 0$).

$$C = C_f\cdot(1 - e^{(-kt)}) \quad (2)$$

The standard deviation of the estimated parameters is the result of the insecurity of the consecutive evaluations of the same system and the deviation of these values from the theoretical kinetic model equation structure. Significant differences between these jointly estimated parameters were determined and visualised by calculating the 90% joint confidence regions.

3. Results and discussion

Carotenoid-enriched oil-in-water emulsions (5% w/v) composed with oils differing in unsaturation degree (olive, soybean and linseed oil) were *in vitro* digested. These oils had large differences in concentrations of oleic (C18:1), linoleic (C18:2) and linolenic (C18:3) acid, while having similar concentrations of C16:0, C18:0, C20:0, C20:1, C22:0 and C22:1 (Table A in supplementary material). In this sense, olive oil was rich in oleic acid, soybean oil in linoleic acid and linseed oil in linolenic acid. The simulated digestion procedure consisted of a gastric phase and a small intestinal phase of which the latter was approached in a kinetic way. In this way, the time dependent evolution of the lipid digestion process (Section 3.2.1), as well as the micellarisation of multiple lipid digestion products and carotenoids could be evaluated (Sections 3.2.2 and 3.3.2, respectively). Additionally, physicochemical properties (particle size and microstructure) were monitored as these might have implications on the oil droplet digestion kinetics (Section 3.1).

3.1. Evolution of (micro)structural changes during in vitro digestion

The particle size distributions of initial emulsions, after the simulated gastric phase and at the small intestinal digestion end point were measured in order to detect possible oil droplet destabilisation phenomena occurring during *in vitro* digestion.

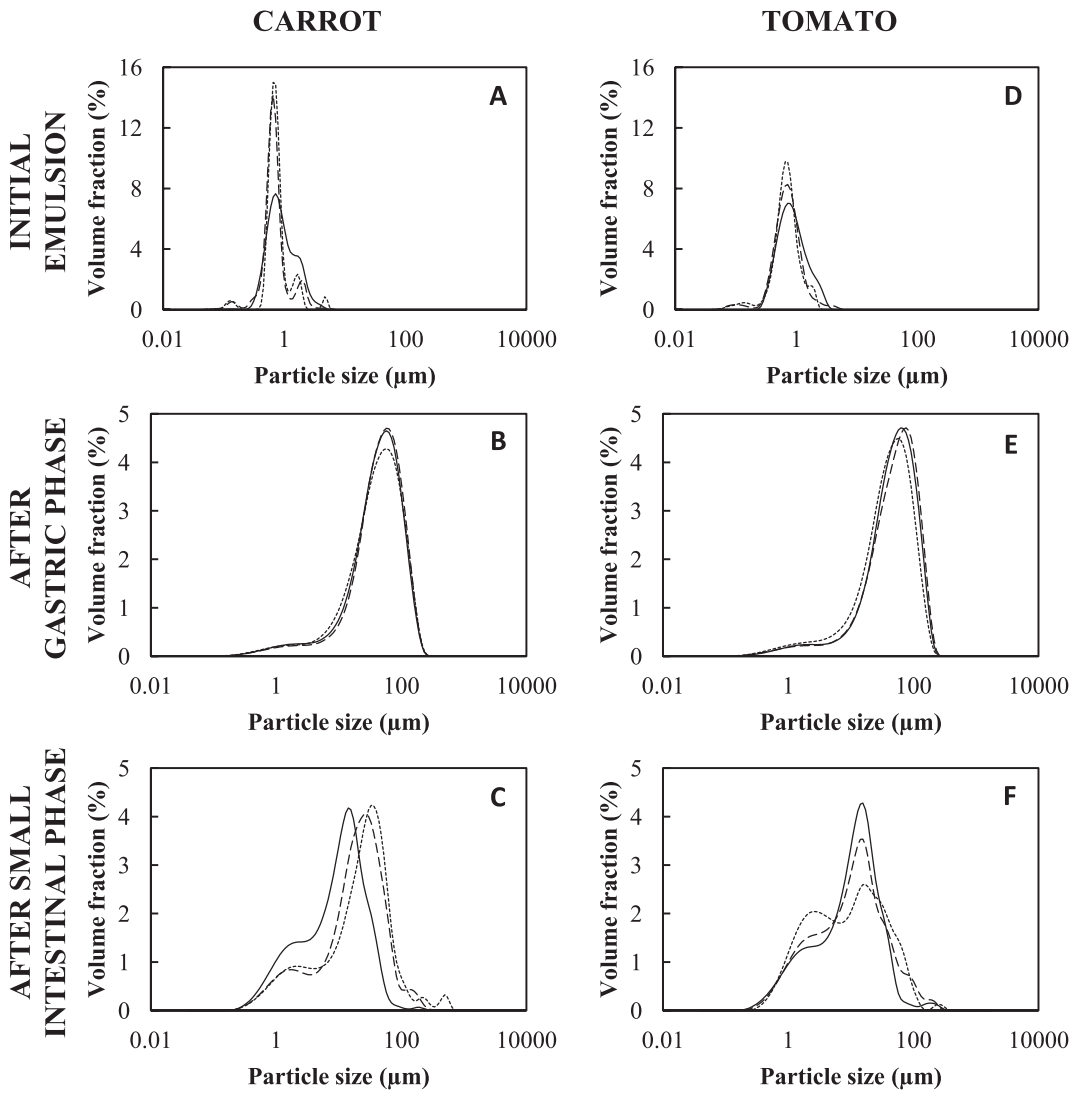

Fig. 1. Particle size distribution of the carrot- and tomato-based emulsions during digestion: (A) initial carrot emulsions; (B) carrot emulsions after gastric phase; (C) carrot emulsions after small intestinal phase; (D) initial tomato emulsions; (E) tomato emulsions after gastric phase and (F) tomato emulsions after small intestinal phase (full line: olive oil emulsions; dashed line: soy oil emulsions; dotted line: linseed oil emulsions).

Emulsions formulated with olive oil (OO) presented a larger particle size compared to emulsions formulated with soybean oil (SO) or linseed oil (LO), regardless whether these oils were enriched with carotenoids from carrot or tomato. In this sense, the D[v;0.5] for emulsions formulated with CEOO was 0.89 µm and 0.86 µm for the ones containing TEOO. By contrast, CESO; CELO; TESO and TELO emulsions had slightly smaller median particle diameters, being 0.70 µm; 0.75 µm; 0.73 µm and 0.72 µm respectively (Table B in supplementary material). Oils with a higher degree of unsaturation, such as SO and LO, may promote the formation of smaller droplets in a emulsion due to the presence of unsaturated linkages increasing interface flexibility (Trotta, Gallarate, Pattarino, & Carlotti, 1999). Some authors (Golding & Wooster, 2010; Salvia-Trujillo et al., 2017) reported that differences in initial emulsion droplet size may have a significant influence during digestion. Based on the results of Salvia-Trujillo et al. (2017), the minor differences observed in the present work between the particle sizes of the initial emulsions are not expected to result in differences in digestibility.

A dramatic increase in particle size of all emulsions was detected after simulation of the gastric conditions (Fig. 1B and E; Table B in supplementary material), with particle sizes of around 40–60 µm. Both flocculation and coalescence phenomena occurred which was visually seen in the microscopic pictures (Fig. A in supplementary data). Sucrose esters, used as emulsifier in this study, have the ability to interact with proteins via hydrophobic as well as hydrophilic bindings. The latter might take place during the gastric phase in which hydrophilic parts of the added enzymes (e.g. pepsin) would be able to interact with the hydrophilic head of the sucrose esters leading to flocculation (Nelen, Bax, & Cooper, 2014). In addition, also coalescence occurred, which can be induced by prior flocculation of oil droplets. Moreover, the sucrose part of the emulsifier can be hydrolysed due to the low pH of the gastric phase. Subsequently, it can be hypothesised that parts of the o/w interface are no longer covered with emulsifier. Therefore, a certain amount of oil can be released into the bulk phase, resulting in larger oil droplets (Verkempinck et al., 2018).

At the end of the intestinal phase, the D[v;0.5] values decreased in all studied emulsions compared to the gastric phase (Table B in supplementary material). A multimodal particle size distribution can be seen in Fig. 1C and F with a main intensity peak around 20 µm and a smaller intensity peak around 2 µm. The decrease of the main intensity peak in comparison to the particle size distribution of the gastric phase, can be due to both lipolysis and breakdown of the flocs formed during the gastric phase. The latter was visually confirmed by microscopic pictures (Fig. A in supplementary data). This main intensity peak also

showed that lipid digestion was incomplete and undigested oil was still present. The presence of a second, smaller intensity peak evidenced formation of colloidal structures from lipid digestion products (e.g. MAG, FFA, bile and calcium). Note that no peak was visible in the micelle size range, typically 4–60 nm (Yonekura & Nagao, 2007). This can be due to the presence of larger particles and the volumetric measuring method of the laser diffraction equipment, which can mask the presence of nanostructures like mixed micelles. In addition, lipid digestion products may also arrange as vesicles or other colloidal structures and subsequently present a larger particle size than individual mixed micelles.

In conclusion, all initial emulsions had a relatively small particle size which increased dramatically during gastric conditions. Subsequently, this probably caused an incomplete lipid digestion at the digestion end point, as large, coalesced oil droplets were observed during particle size measurements and microscopic pictures. Nevertheless, formation of colloidal structures were expected, carrying lipid digestion products and carotenoids.

3.2. Study of kinetics of multiple lipid digestion species

The lipid hydrolysis reaction and micelle formation were studied during the simulated small intestinal phase through a kinetic approach, which allowed elucidating their time dependency and the relationship between the rate and extent of these processes. For all studied emulsion-based systems, TAG concentration decreased as a function of the time, while the MAG, FFA and GLY concentration increased in the digest as a consequence of the lipolysis reaction. Simultaneously, MAG and FFA contributed to the build-up of mixed micelles. Experimental (MAG and FFA) and calculated (TAG and GLY) data were modelled using a fractional conversion model as lipid digestion kinetics showed a linear progress at first (characterised by the *k*-value) until a plateau was reached (characterised by the final concentration C_f). Subsequently, these jointly estimated parameters (k and C_f) were used to determine joint confidence regions (90%) in order to show significant differences between the lipolysis reaction and micelle formation in emulsions with different oils (OO, SO or LO) and enriched with carotenoids from different matrices (carrot or tomato).

3.2.1. Lipolysis reaction kinetics in the small intestinal digest

In general, it could be observed that a steady-state condition in digested TAG percentage was reached in all cases within two hours of digestion (Fig. 2). Emulsions containing OO presented higher *k*-values compared to SO and LO emulsions, regardless if the oils were enriched with carotenoids from carrot or tomato (Table 1). Subsequently, it can be stated that OO emulsions were digested faster than the SO and LO emulsions. For example, after 30 min of digestion in the small intestinal phase, OO emulsions were digested in a higher extent than the SO and LO emulsions (Fig. 2). Moreover, the TAG present in OO emulsions were also digested in a higher extent than the ones in the SO and LO emulsions at the digestion end point. These differences were also confirmed by joint confidence region analysis (90%) whereby the regions of the OO emulsions did not overlap with any of the regions of the LO emulsions (Fig. 3A and D).

The observed differences in TAG hydrolysis might be attributed to the different unsaturation degree of the fatty acyl groups of the oil types: LO and SO being richer in PUFA, while OO is more rich in MUFA. On the one hand, PUFA have a bended structure, so the methylgroup comes closer to the GLY backbone which can cause a steric hinder effect and might protect the ester bond from lipolysis. This behaviour was also observed in the studies of Bottino, Vandenburg, and Reiser (1967), Wanasundara and Shahidi (1998) and Sun et al. (2015) in which (long chain) PUFA were more resistant to lipase hydrolysis in comparison with MUFA. On the other hand, PUFA are considered to be more surface active than MUFA, so they can stay longer at the interface, competing with lipase which eventually can result in a slower lipolysis of oils rich in PUFA (Reis et al., 2008; Li & McClements, 2010).

Nevertheless, the lipid hydrolysis reaction was incomplete for all studied emulsions since at the end of the simulated intestinal phase (120 min) a considerable amount of TAG was still present. Less than half of the initial TAG were digested, being 43.1%; 41.6%; 36.4%; 44.2%; 45.0% and 34.2% for the CEOO; CESO; CELO; TEOO; TESO and TELO emulsions, respectively (Fig. 2). This confirmed the results of particle size by laser diffraction (Section 3.1) at the end of the simulated intestinal phase, which indicated the presence of large oil droplets. The presence of non-digested TAG was also confirmed by optical microscopy (Fig. A supplementary material) as well as visual observations during experiment execution. The incomplete lipid hydrolysis reaction might be related to the dramatic increase in particle size during gastric conditions. In other words, the bulk oil and large oil droplets present at the start of the intestinal phase have a much lower active surface area for lipase to adsorb than small, emulsified oil droplets. Subsequently, not all TAG might be hydrolysed resulting in an incomplete lipid digestion. These observations suggest that intestinal lipases preferably adsorbed at the oil-water interface of small, emulsified oil droplets. The obtained results are in agreement with previously reported information where a much faster lipid hydrolysis of emulsified oils with small emulsion droplet size is described in comparison with larger oil droplet sizes or bulk oils (Zhang et al., 2016; Salvia-Trujillo et al., 2017).

Differences could be established in the MAG release rate (*k*-values) (Fig. 2 and Table 1), being faster in case of emulsions containing OO in comparison with SO and LO. A similar trend was observed regarding the final MAG concentration reached at the steady-state. From Fig. 3B and E (grey regions), it can be seen that the jointly estimated parameters of MAG release were significant different for the OO and LO emulsions. These findings were also reflected by a slower TAG digestion in o/w emulsions formulated with LO or SO, which also presented a higher concentration of undigested TAG at the end of the small intestinal phase in comparison with OO (Fig. 3A and D).

The FFA release *k*-value of the CEOO emulsion ($0.045 \pm 0.008\ min^{-1}$) was higher compared to the CESO ($0.035 \pm 0.006\ min^{-1}$) and CELO ($0.033 \pm 0.006\ min^{-1}$) emulsion, while the final, plateau concentrations were fairly similar, with values ranging from 14.68 to 15.79 $mg_{FFA}/g_{emulsion}$ (Table 1). This means that the steady-state condition was reached faster for the CEOO emulsion compared to the CESO and CELO emulsions. Similar observations could be drawn from the emulsions composed with tomato-enriched oils. In addition, the FFA concentrations present in the digest (14.68–18.20 $mg/g_{emulsion}$) were high compared to MAG concentrations (2.52–4.49 $mg/g_{emulsion}$). The amount of FFA was 4 to 6 times higher than the amount of MAG, which can be attributed to further conversion of MAG to GLY and FFA (Tokle, Mao, & McClements, 2013). This reaction was probably more pronounced in LO emulsions compared to OO emulsions as the same FFA concentration was reached in the final digest but differences in TAG hydrolysis and MAG formation were observed.

In general, it could be observed that TAG digestion was faster and in a higher extent for OO emulsions in comparison with SO and LO emulsions. In addition, the release of MAG and FFA followed the same trend, showing that oils containing MUFA are more easily digested than oils rich in PUFA. This was attributed to structure differences as well as differences in surface activity of the FFA present in the oils. Additionally, the *k*-values for TAG hydrolysis, MAG and FFA release in the digest were similar for a certain type of emulsion, indicating that the TAG hydrolysis reaction and release of MAG and FFA are not rate limiting steps in the digestion process. Furthermore, these results evidenced that lipid digestion was not influenced by the carotenoid type used for enriching the oils and/or carotenoid concentration.

3.2.2. Mixed micelle composition and formation kinetics

The formation of lipid digestion products as a consequence of lipolysis led to the assembly of mixed micelles in the aqueous phase. The incorporation of MAG and FFA in mixed micelles started immediately in

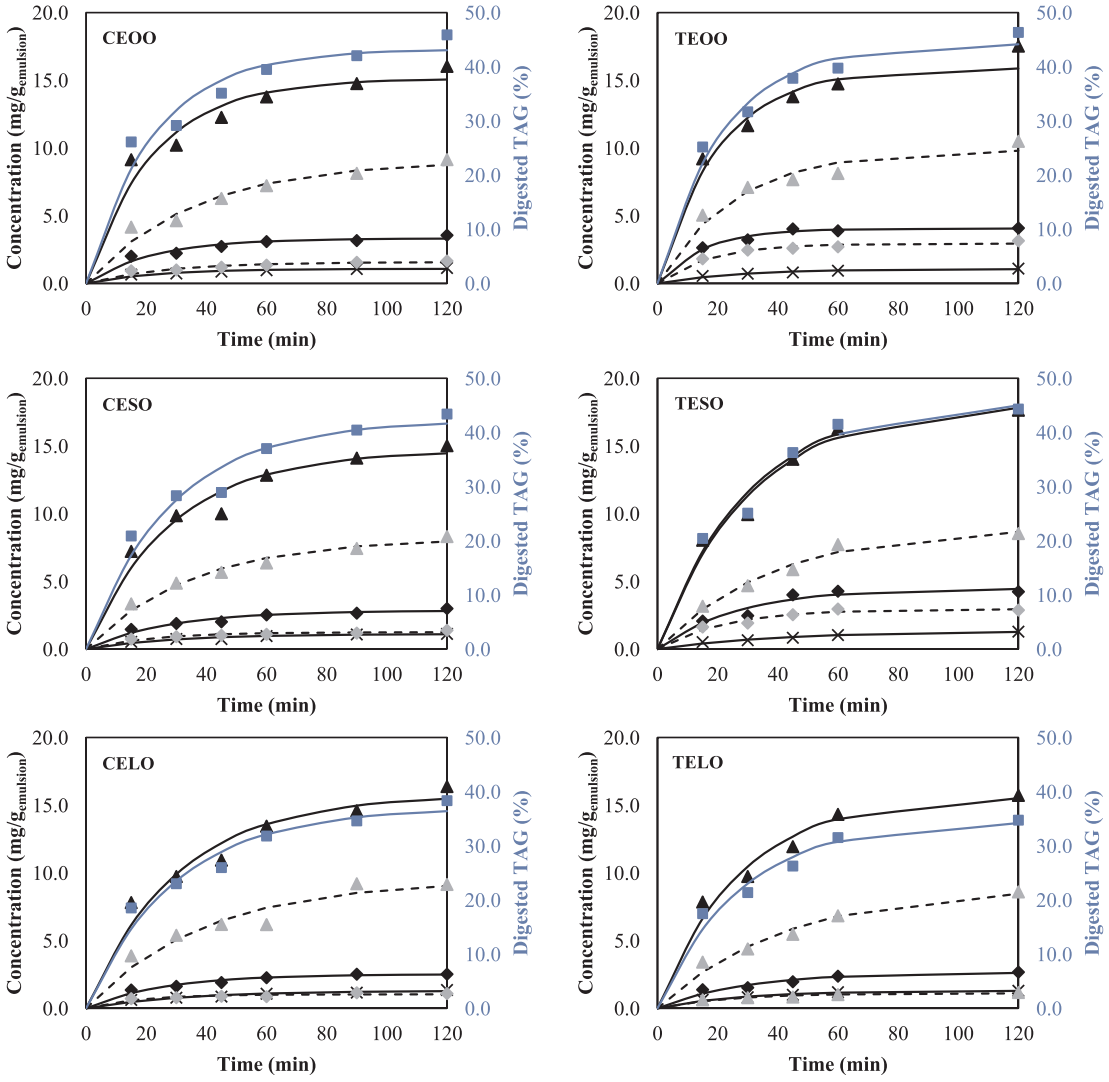

Fig. 2. *In vitro* lipid digestion of carrot- and tomato-based emulsions as function of digestion time in the small intestinal phase ((CEOO) carrot-enriched olive oil emulsion; (CESO) carrot-enriched soy oil emulsion; (CELO) carrot-enriched linseed oil emulsion; (TEOO) tomato-enriched olive oil emulsion; (TESO) tomato-enriched soy oil emulsion; (TELO) tomato-enriched linseed oil emulsion). The symbols indicate the experimental data while the full and dotted lines show the predicted values of the corresponding fractional conversion model (■ TAG_{digest}; ◆ MAG_{digest}; ◆ $MAG_{micelle}$; ▲ FFA_{digest}; ▲ $FFA_{micelle}$; ✖ $glycerol_{digest}$).

the small intestinal phase (Fig. 2). Both the *k*- as well as the MAG_f-values were influenced by the unsaturation degree of the emulsified oil (Table 1). By contrast, no effect of this emulsion characteristic was observed on the estimated parameters for FFA micellarisation. The parameter estimates were also evaluated by JCR analysis, depicted in Fig. 3C and F. From these regions, it can be stated that the MAG micellarisation is significantly different between the OO emulsion and the LO emulsion. In addition, JCR analysis showed that the jointly estimated parameters *k* and C_f were not significantly different for the incorporated FFA in all cases.

Similar *k*-values were observed for the incorporation of MAG and FFA into the micellar fraction compared to the ones estimated for the MAG and FFA release in the digest (Table 1). This suggests that when TAG were hydrolysed, a fraction of the formed MAG and FFA was simultaneously incorporated into mixed micelles. These results are in line to the mechanisms described for micelle formation from lipid digestion products. It is known that bile salts can displace lipid digestion products from the droplet interface promoting lipase adsorption at oil droplet surface (Mun, Decker, & McClements, 2007; Singh et al., 2009). In addition, bile salts form mixed micelles together with these lipid digestion products, mainly MAG and FFA (Bauer, Jakob, & Mosenthin, 2005). Nonetheless, the amount of MAG and FFA in the micelle fraction was in all cases lower than the amount in the digest, indicating that not all generated MAG and FFA contributed to micelle formation. Namely, a constant fraction of MAG and FFA released in the digest contributed to mixed micelle formation for all emulsions, ranging between 45 and 70% depending on the oil type. In this regard, and similarly to the results described for lipid digestion (Section 3.2.1), the FFA concentration in the micelle fraction was significantly higher than the one of MAG (Figs. 2 and 3).

In context of mixed micelle formation, only the MAG kinetic characteristics were influenced by the unsaturation degree of the emulsified oil. These differences in mixed micelle composition among the emulsions can have a major impact on the carotenoid bioaccessibility kinetics and will be discussed in Section 3.3.2.

3.3. Study of carotenoid bioaccessibility kinetics as influenced by lipid digestion kinetics

In order to further investigate the mechanism by which carotenoids are released from the core of the oil droplets and further transferred to the mixed micelles, the kinetics of carotenoids BAC were determined. Carotenoid BAC was calculated as the ratio of the amount of micellarised carotenoids to the amount of carotenoids present in the initial

Table 1
Estimated kinetic parameters (± standard deviation) of the different carrot- and tomato-based emulsions modelled by a fractional conversion model through a nonlinear regression procedure. C_f is the final lipid concentration estimated by the model (expressed in % in case of TAG and mg/g emulsion all other cases) and k is the incorporation rate constant of the lipids into micelles (min^{-1}).

	TAG_{digest}	MAG_{digest}	FFA_{digest}	GLY_{digest}	$MAG_{micelle}$	$FFA_{micelle}$
			CEOO			
k ± stdev	0.045 ± 0.008	0.045 ± 0.008	0.045 ± 0.008	0.044 ± 0.008	0.038 ± 0.008	0.028 ± 0.005
C_f ± stdev	43.27 ± 2.21	3.33 ± 0.17	15.14 ± 0.78	1.08 ± 0.06	1.59 ± 0.11	9.10 ± 0.62
R^2_{adj}	0.808	0.799	0.809	0.809	0.730	0.837
			CESO			
k ± stdev	0.035 ± 0.006	0.036 ± 0.006	0.035 ± 0.006	0.035 ± 0.006	0.046 ± 0.009	0.029 ± 0.003
C_f ± stdev	42.25 ± 2.39	2.85 ± 0.16	14.68 ± 0.83	1.11 ± 0.06	1.25 ± 0.07	8.21 ± 0.37
R^2_{adj}	0.832	0.820	0.831	0.823	0.763	0.926
			CELO			
k ± stdev	0.034 ± 0.006	0.039 ± 0.006	0.033 ± 0.006	0.031 ± 0.006	0.057 ± 0.015	0.026 ± 0.006
C_f ± stdev	37.08 ± 2.13	2.52 ± 0.11	15.79 ± 0.94	1.30 ± 0.09	1.04 ± 0.07	9.47 ± 0.91
R^2_{adj}	0.840	0.870	0.831	0.799	0.600	0.722
			TEOO			
k ± stdev	0.046 ± 0.007	0.065 ± 0.008	0.043 ± 0.006	0.039 ± 0.006	0.059 ± 0.009	0.039 ± 0.008
C_f ± stdev	44.35 ± 1.96	4.06 ± 0.13	16.77 ± 0.83	1.07 ± 0.06	2.94 ± 0.12	9.89 ± 0.67
R^2_{adj}	0.927	0.950	0.914	0.914	0.926	0.855
			TESO			
k ± stdev	0.033 ± 0.005	0.038 ± 0.009	0.032 ± 0.005	0.024 ± 0.004	0.045 ± 0.008	0.026 ± 0.003
C_f ± stdev	45.85 ± 2.72	4.49 ± 0.37	18.20 ± 1.03	1.35 ± 0.09	2.96 ± 0.17	9.05 ± 0.51
R^2_{adj}	0.908	0.801	0.919	0.922	0.878	0.941
			TELO			
k ± stdev	0.036 ± 0.006	0.035 ± 0.007	0.037 ± 0.005	0.036 ± 0.004	0.042 ± 0.009	0.024 ± 0.004
C_f ± stdev	34.65 ± 1.95	2.65 ± 0.20	15.72 ± 0.84	1.31 ± 0.06	1.12 ± 0.08	9.01 ± 0.64
R^2_{adj}	0.909	0.840	0.918	0.940	0.834	0.921

emulsion. Subsequently, the experimental values were modelled using a fractional conversion model to (i) gain insight in the time dependency of the carotenoid micellarisation depending on the oil type, (ii) investigate the influence of the carotenoid type and source on carotenoid BAC and (iii) to evaluate the relationship between lipolysis reaction and carotenoid BAC. Carotenoid micellarisation followed an initial linear increase, characterised by the incorporation rate constant k, and finally reached a plateau value, defined as the final carotenoid bioaccessibility value BAC_f (Fig. 4). Carotenoid BAC could be simultaneously described by these two parameters (k and BAC_f) and therefore 90% JCR were calculated and visualised (Fig. 5) to determine whether the unsaturation degree of the oil used in emulsion formulation had a significant effect on carotenoid BAC. Differences in carotenoid BAC might be related to the affinity of each carotenoid type to the free acyl composition of each oil type, as it is discussed in the following sections.

3.3.1. Initial carotenoid concentration in enriched oils and emulsions

The carotenoid concentration ($\mu g/g_{oil}$) present in the enriched oils and different types of emulsions ($\mu g/g_{emulsion}$) is shown in Table C (supplementary material). In carrot-enriched oils, the amount of α-carotene (123.60–200.24 $\mu g/g_{oil}$) was lower than the amount of β-carotene (305.62–452.49 $\mu g/g_{oil}$) with a ratio of about 1:2. This is consistent with the carotenoid concentration present in raw orange carrots as reported previously (Maiani et al., 2009). The carotenoid amount in CEOO was significantly higher than the amount in CESO and CELO which can be due to (i) the experimental variability during oil enrichment or (ii) higher solubility of α- and β-carotene in OO due to its higher hydrophobicity. Tomato-enriched oils contained mainly *trans* lycopene (88.96–93.97 $\mu g/g_{oil}$) and lower concentrations of *cis* lycopene (26.67–33.75 $\mu g/g_{oil}$) and β-carotene (12.83–13.49 $\mu g/g_{oil}$). Lycopene isomerisation may have occurred during the preparation of tomato puree due to the temperature increase when using a high pressure homogenisation step (Colle et al., 2010; Mutsokoti et al., 2015). Subsequently, enriched oils were used to formulate o/w emulsions and its initial carotenoid amount was used to calculate the carotenoid BAC. All carrot- or tomato-based emulsions contained a comparable initial concentration of each individual carotenoid regardless the type of oil, prior being submitted to *in vitro* digestion conditions (Table C in supplementary material).

3.3.2. Carotenoid bioaccessibility kinetics

Carotenoid micellarisation linearly increased as function of digestion time until a plateau value was reached (Fig. 4). A similar micellarisation kinetic pattern per oil type was observed for the incorporation of α- and β-carotene in carrot-based emulsions on the one hand, as for β-carotene, *cis* lycopene, *trans* lycopene in tomato-based emulsions on the other hand (Fig. 4). Similar to lipid digestion and micelle formation, the kinetics of carotenoid BAC were influenced by the unsaturation degree of the different oils. In this sense, carotenoid incorporation into mixed micelles was faster for OO emulsions compared to SO and LO emulsions (Table 2). However, this statement is not valid in case of *cis* lycopene in the tomato-based emulsion. It can be hypothesised that the bended *cis* isomers are more easily incorporated into mixed micelles in comparison to their more rigid, *trans* counterparts. In addition, the highest BAC_f-values were reached in case of the OO emulsions and only minor differences were observed between the SO and LO emulsions. For instance, the final β-carotene BAC in CEOO emulsion was around 13%, whereas it was around 8–10% for CESO and CELO emulsions (Table 2 and Fig. 4B). Similar observations could be made for carotenoid BAC in tomato emulsions, namely the final *trans* lycopene BAC in TEOO emulsion was around 19%, while it was only around 9.5% for TESO and TELO emulsions (Table 2 and Fig. 4E). Differences in the kinetics of carotenoid BAC during small intestinal phase between different oils were evaluated by joint confidence region analysis (90%) (Fig. 5). In all cases, the jointly estimated parameters were significantly different between the OO emulsions and the SO or LO emulsions and confirmed the observations drawn from estimated parameters k- and C_f-values.

Furthermore, differences in BAC_f-values were observed between the carotenoid types used for oil enrichment, regardless the oil unsaturation degree. In all carrot-based emulsions, α-carotene BAC was slightly higher than β-carotene BAC (Fig. 4A and B) which can be related to the carotenoid polarity as α-carotene is somewhat more hydrophilic than β-carotene. In case of tomato-based emulsions, *trans* lycopene BAC at the end of the intestinal phase was lower than the one of β-carotene (Fig. 4C–E). Namely, for TEOO emulsion, *trans* lycopene BAC was

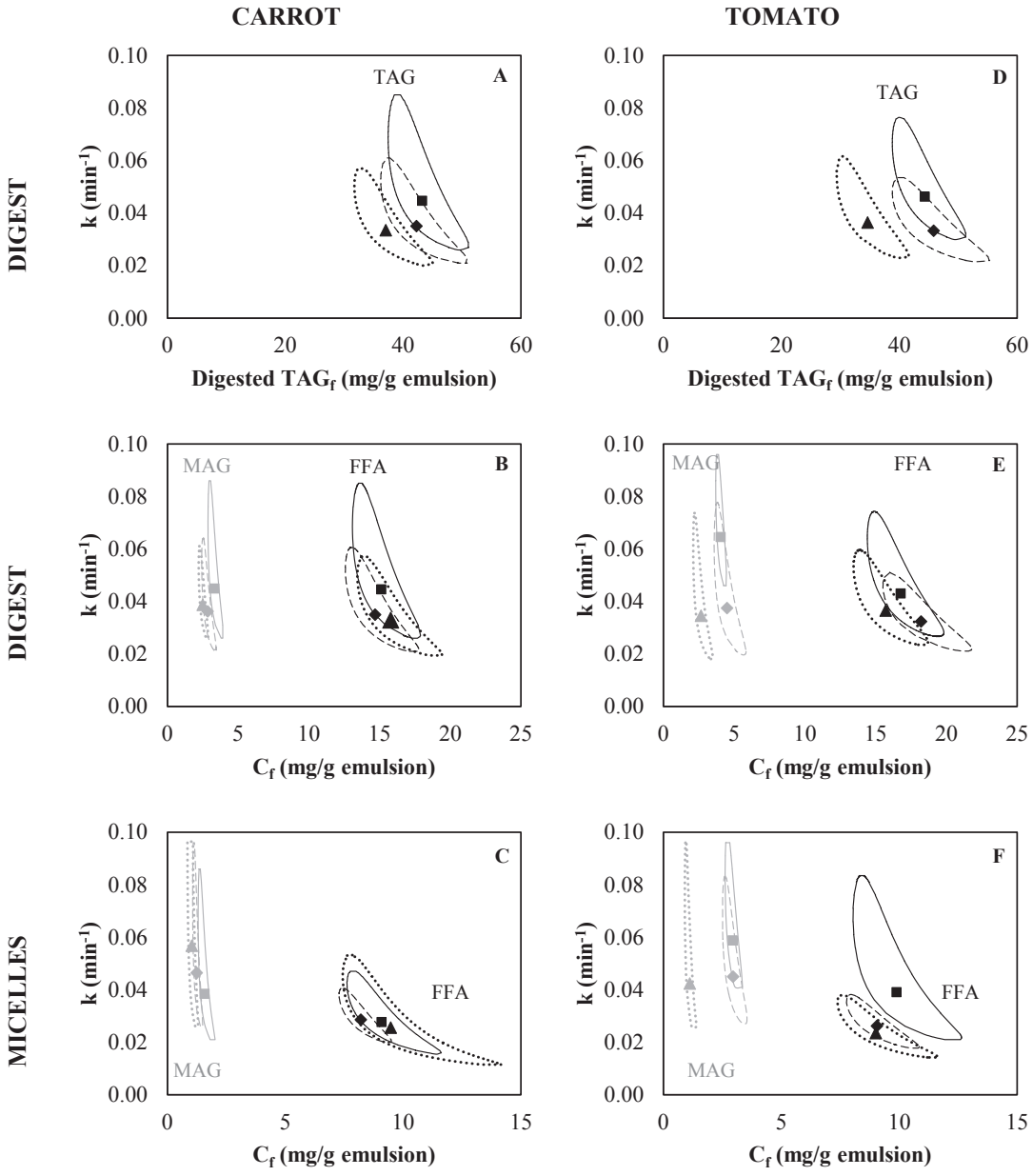

Fig. 3. Joint confidence regions (90%) based on the jointly estimated parameters (Table 1) for *in vitro* small intestinal lipid digestion: (A) digested TAG for carrot emulsions; (B) MAG (grey) and FFA (black) in the digest for carrot emulsions; (C) MAG (grey) and FFA (black) in the micelles for carrot emulsions; (D) digested TAG for tomato emulsions; (E) MAG (grey) and FFA (black) in the digest for tomato emulsions; (F) MAG (grey) and FFA (black) in the micelles for tomato emulsions. The lines represent the joint confidence region, while the symbols represent the estimated value of the corresponding fractional conversion model (full line: olive oil emulsions; dashed line: soy oil emulsions; dotted line: linseed oil emulsions).

around 19% whereas it was 24% for β-carotene. This could also be related to differences in structure of the carotenoid species. β-carotene has cyclic end groups, while *trans* lycopene has a long, linear structure (Britton, 1995). Therefore, it can be hypothesised that due its rigid, linear structure, *trans* lycopene remained in a higher extent in the non-digested oil droplet core in comparison to β-carotene, which may be released and incorporated faster into mixed micelles as these are formed. In agreement to our results, Mutsokoti et al. (2017) showed that the carotenoid structure was one of the main factors influencing the carotenoid transfer from the oil into the mixed micelles. Moreover, *cis* lycopene presented higher *k*- and BAC_f-values in this study. These behaviour might be explained by differences in carotenoid polarity. *Cis* isomers have a bended structure which makes them slightly more polar than their respective *trans* form. Consequently, these carotenoids might be more easily incorporated into the micellar fraction. Previously reported findings, based on a single digestion point, are in accordance with the results of the present kinetic work, where *cis* lycopene was described being more bioaccessible than *trans* lycopene at the end of digestion (Stahl & Sies, 1992; Unlu et al., 2007; Failla, Chitchumroonchokchai, & Ishida, 2008).

Generally, final carotenoid BAC values were ranging from 8% to 27%. These percentages are similar to carotenoid BAC values of vegetable purees enriched with oil (Colle et al., 2010; Palmero et al., 2013). Nevertheless, the obtained carotenoid BAC is rather low compared to other researches investigating carotenoid BAC of enriched, emulsified oils (40–80%) (Verrijssen et al., 2015; Zhang et al., 2016; Salvia-Trujillo et al., 2017). This can be attributed to the low TAG lipolysis as around 50–70% remained undigested. Consequently, a significant amount of carotenoids remained in the undigested oil and was unable to be micellarised. However, still clear, quantitative effect of the oil

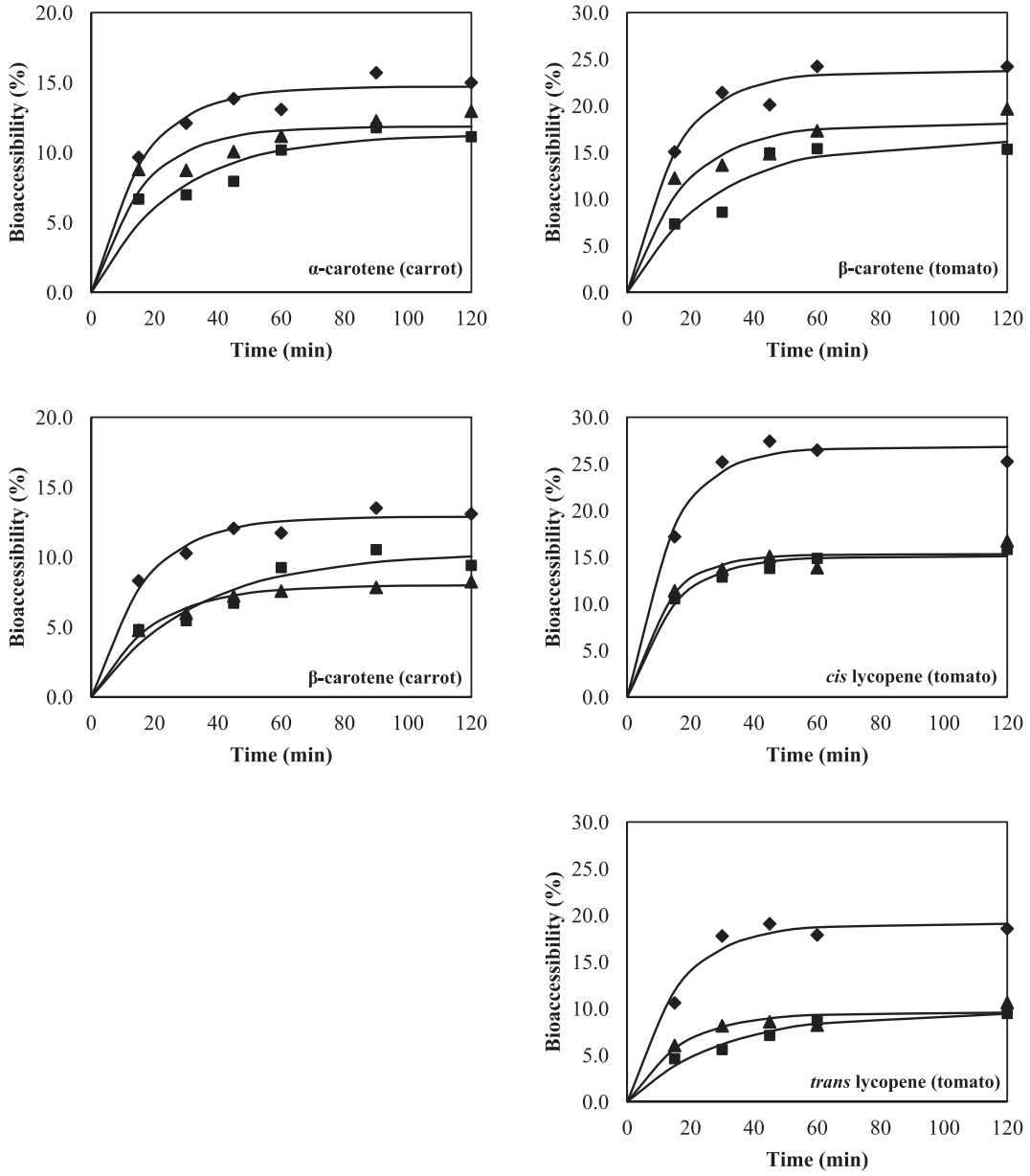

Fig. 4. Carotenoid *in vitro* bioaccessibility of (A) α-carotene and (B) β-carotene in carrot-based emulsions and of β-carotene (C), *cis* lycopene (D) and *trans* lycopene (E) in tomato-based emulsions. The symbols indicate experimental data while the lines show the predicted values of the corresponding fractional conversion model (◆ CEOO and TEOO; ▲ CESO and TESO; ■ CELO and TELO).

unsaturation degree on lipolysis and subsequently carotenoid bioaccessibility could be observed, taking into account the process of experimental *in vitro* digestion, modelling and insecurity of the estimated parameters (C_f and k). Therefore, results in the present work evidence the strong relationship between lipid digestion and carotenoids micellarisation.

3.3.3. Relation between the kinetics of in vitro lipid digestion and carotenoid bioaccessibility

The interrelation between the incorporation of MAG, FFA and carotenoids into the micellar fraction was evaluated based on the estimated parameters k and C_f (Fig. 6).

Regarding the k-values (Fig. 6A), a clear effect was observed of the emulsified oil type on these incorporation rate constants. More specifically, OO emulsions presented higher k-values of both MAG and FFA as well as carotenoid micellarisation in comparison with SO and LO emulsions. This shows that the lipid digestion products from emulsions containing OO, being the systems which were digested the fastest (i.e. Section 3.2.1), were incorporated much faster compared with the lipid digestion products of the SO and LO emulsions. Moreover, this fast incorporation of MAG and FFA is linked with a fast incorporation of carotenoids into mixed micelles. Similar trends were observed regarding the C_f-values of MAG, FFA and carotenoid micellarisation (Fig. 6B). LO and SO emulsions presented lower final concentrations of MAG, FFA and carotenoids in the micellar fraction in comparison to OO emulsions. To conclude, it can be stated there is a direct link between the kinetics of MAG and FFA as well as carotenoid micellarisation, evidencing that the incorporation of these components into the micellar fraction go hand in hand.

4. Conclusions

The present study showed that the oil unsaturation degree had a significant influence on both lipid digestion and carotenoid

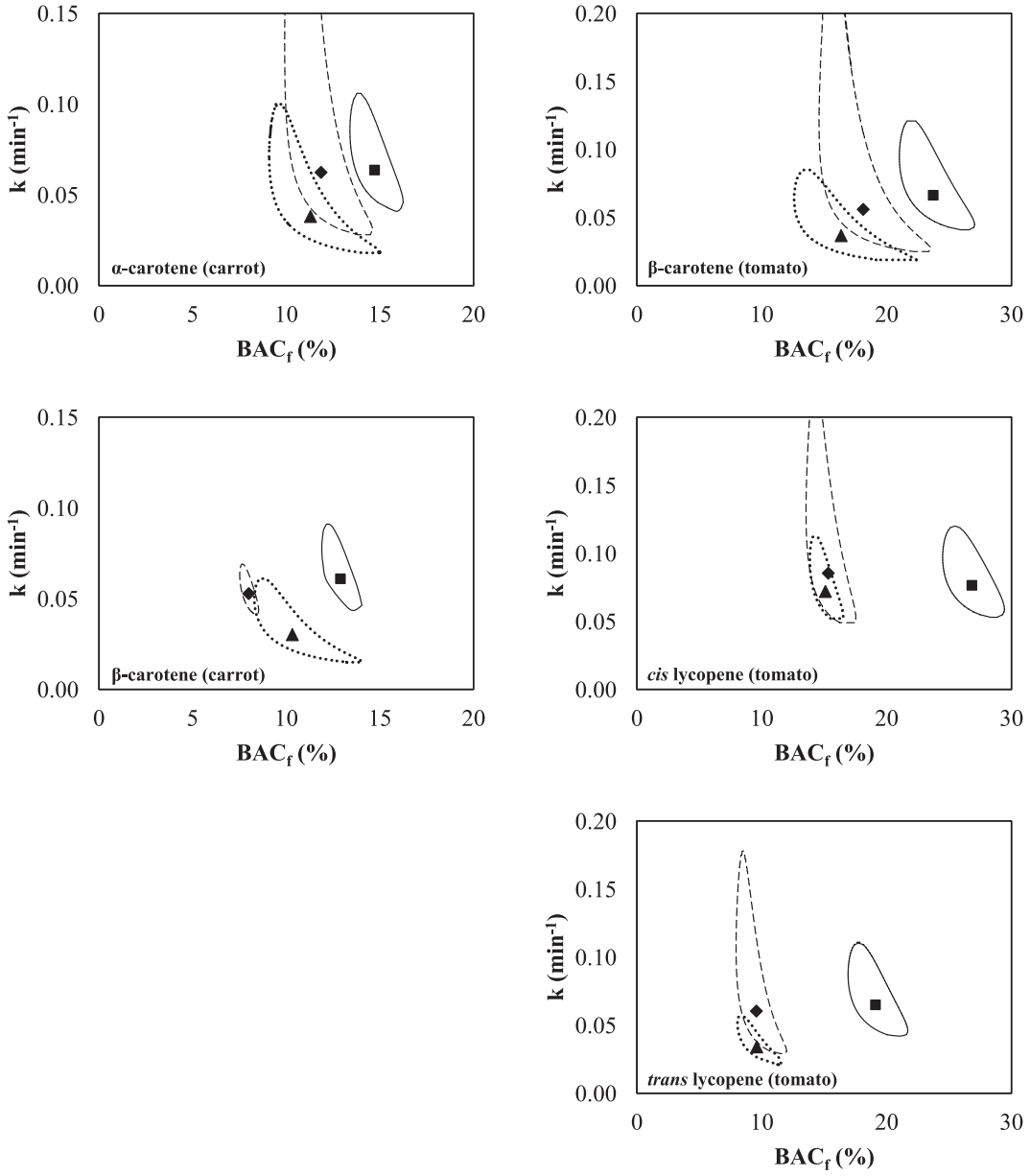

Fig. 5. Joint confidence regions (90%) based on the jointly estimated parameters (Table 2) for carotenoid bioaccessibility for α-carotene in carrot emulsions; β-carotene in carrot emulsions; β-carotene in tomato emulsions; *cis* lycopene in tomato emulsions and *trans* lycopene in tomato emulsions. The lines represent the joint confidence region, while the symbols represent the estimated value of the corresponding fractional conversion model (full line: olive oil emulsions; dashed line: soy oil emulsions; dotted line: linseed oil emulsions).

Table 2
Estimated kinetic parameters (± standard deviation) of the different carrot and tomato based emulsions modelled by a fractional conversion model through a nonlinear regression procedure. BAC_f is the final bioaccessibility estimated by the model (%) and k is the incorporation rate constant of carotenoids into micelles (min^{-1}).

	α-carotene	β-carotene	β-carotene	*cis* lycopene	*trans* lycopene
	CEOO			TEOO	
k ± stdev	0.064 ± 0.010	0.061 ± 0.008	0.066 ± 0.011	0.077 ± 0.011	0.065 ± 0.012
BAC_f ± stdev	14.71 ± 0.49	12.89 ± 0.37	23.73 ± 0.99	26.82 ± 0.87	19.10 ± 0.84
R^2_{adj}	0.901	0.931	0.907	0.937	0.902
	CESO			TESO	
k ± stdev	0.062 ± 0.016	0.053 ± 0.005	0.056 ± 0.014	0.085 ± 0.018	0.060 ± 0.015
BAC_f ± stdev	11.85 ± 0.71	7.99 ± 0.18	18.12 ± 1.27	15.32 ± 0.64	9.57 ± 0.60
R^2_{adj}	0.697	0.963	0.777	0.886	0.809
	CELO			TELO	
k ± stdev	0.038 ± 0.010	0.030 ± 0.007	0.037 ± 0.010	0.072 ± 0.009	0.034 ± 0.006
BAC_f ± stdev	11.29 ± 0.89	10.32 ± 0.90	16.35 ± 1.59	15.09 ± 0.43	9.59 ± 0.59
R^2_{adj}	0.695	0.742	0.740	0.953	0.900

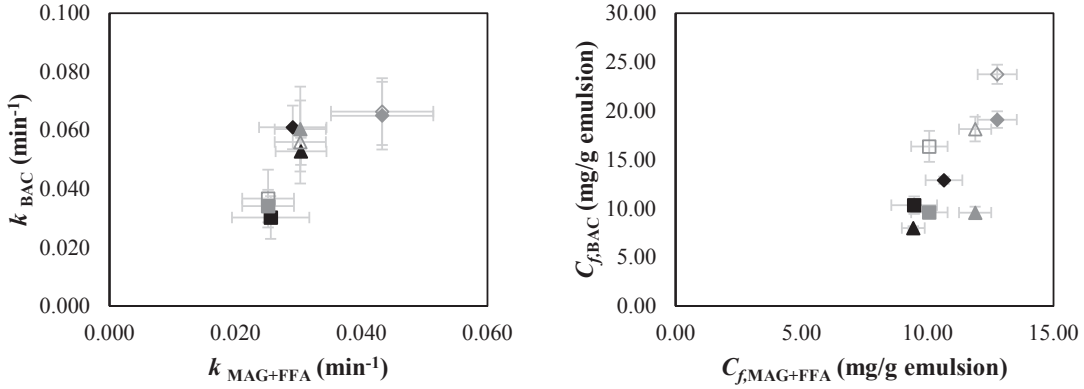

Fig. 6. Relation between the (A) *k*-values (min^{-1}) and (B) C_f-values of MAG and FFA micellarisation, and carotenoid incorporation into mixed micelles (◆ OO emulsions; ▲ SO emulsions; ■ LO emulsions; black symbol: β-carotene from carrot-enriched oil; grey, filled symbol: *trans* lycopene from tomato-enriched oil and grey, open symbol: β-carotene from tomato-enriched oil).

bioaccessibility kinetics. There was an indication that the instability during the gastric phase led to large particle sizes which in turn resulted in an incomplete lipid digestion at the end of the simulated intestinal phase. In addition, olive oil emulsions, rich in MUFA, were digested faster and in a higher extent compared to soybean and linseed oil emulsions, which were rich in PUFA. It can be hypothesised that PUFA have a more bended structure, protecting the glycerol backbone from lipase hydrolysis and eventually led to a slower lipolysis reaction. Furthermore, the lipolysis reaction kinetics were mainly influenced by the emulsified oil type, while the kinetics of mixed micelle formation were also partially influenced by the solubilised carotenoid type. It could be hypothesised that lipid species rich in MUFA (present in olive oil) might lead to mixed micelle formation with greater capacity of solubilising hydrophobic carotenoids, as MUFA might be slightly more hydrophobic in comparison to PUFA present in soybean or linseed oil. Finally, a direct correlation was observed between lipid digestion and carotenoid bioaccessibility (kinetics) as olive oil emulsions presented higher bioaccessibility values than emulsions containing soybean or linseed oil in both carrot- and tomato-based emulsions. Moreover, results of the present work highlight the possibility of structuring o/w emulsions with targeted characteristics in order to modulate lipid hydrolysis rate and extent during digestion.

Acknowledgment

This research was financially supported by the Institute for the Promotion of Innovation through Science and Technology in Flanders (IWT-Vlaanderen). S.H.E. Verkempinck is a Doctoral Researcher funded by IWT-Vlaanderen (Grant no. 141469). L. Salvia-Trujillo is a Postdoctoral Researcher funded by the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 654924.

Declaration of interests

The authors of the present work declare no conflict of interests.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jff.2017.12.030.

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