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Carotenoid Bioaccessibility and the Relation to Lipid Digestion: A kinetic study

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Abstract

The micellar incorporation of carotenoids (lycopene, α - and β -carotene) and lipid digestion products (free fatty acids, FFAs, and monoacylglycerides, MAGs) during *in vitro* digestion of oil-in-water emulsions was investigated by a kinetic approach. A fractional conversion model could adequately describe the hydrolysis of triacylglycerides, formation of FFAs and MAGs, and micellar incorporation of carotenoids, FFAs and MAGs. The release of FFAs and MAGs from TAGs proceeded faster than their incorporation into micelles. Rate constants of carotenoid micellar incorporation were inversely proportional to their hydrophobicity and dependent on the isomeric configuration, being the incorporation of the *cis* faster than their all-*trans* isomers. Furthermore, a positive linear relation was found between the micellar incorporation of carotenoids and lipid digestion products. The isomeric form of carotenoids did not affect such relation. The present kinetic approach can be useful to gain mechanistic insight into carotenoid bioaccessibility as affected by various process- and product-related factors.

Keywords: *lycopene, β -carotene, α -carotene, lipid digestion, bioaccessibility, kinetics.*

36 **Highlights**

- 37 • A kinetic approach was applied to carotenoid and lipid micellar incorporation
- 38 • Carotenoid micellar incorporation is inversely proportional to their hydrophobicity
- 39 • *Cis* isomers are selectively incorporated into micelles compared to the all-*trans*
- 40 • Positive correlation exists between carotenoid and (FFA+MAG) micellar incorporation

1. Introduction

Carotenoids are naturally occurring lipophilic pigments responsible for the red, orange, and yellow colours of most fruits and vegetables. They have been extensively reported to exert a large number of health benefits, mainly attributable to their antioxidant and provitamin A activity, as well as to gene function regulation, gap-junction communication, hormone and immune modulation (Rao & Rao, 2007; van het Hof, West, Weststrate, & Hautvast, 2000). For this reason, a diet rich in fruits and vegetables has been associated with reduced risk of several chronic diseases such as cancer and cardiovascular diseases (Gan et al., 2015; He, Nowson, Lucas, & MacGregor, 2007). However, despite their prevalence in fruit and vegetable based products, the fraction of carotenoids that is actually absorbed and serves health related functions (i.e. carotenoid bioavailability) is generally low (Courraud, Berger, Cristol, & Avallone, 2013). As lipophilic compounds, carotenoids are digested and absorbed along with lipids. Therefore, carotenoids need to be released from the food matrix, solubilized into a lipid phase, and incorporated into mixed micelles (i.e. bioaccessibility) in order to be absorbed through the intestine epithelium (Hedrén, Diaz, & Svanberg, 2002).

In recent years, many literature studies have focused on understanding and improving the bioaccessibility and bioavailability of carotenoids. Carotenoid bioaccessibility has been shown to depend on various product and process related factors. For example, their availability for absorption has been shown to depend on the species of carotenoids present in a food system (Granado-Lorencio, Olmedilla-Alonso, Herrero-Barbudo, Pérez-Sacristan, et al., 2007; Ryan, O'Connell, O'Sullivan, Aherne, & O'Brien, 2008; van het Hof et al., 2000). Specifically for β -carotene and lycopene, in several studies the extent of micellar incorporation of the former has been found to be higher than that of the latter (Garrett, Failla, & Sarama, 1999; Granado-Lorencio, et al., 2007; Ryan et al., 2008). Furthermore, the molecular size and shape of carotenoids, which is related to isomeric configuration (Britton, 1995), has been shown to influence their bioaccessibility. In general, the all-*trans* configuration has been postulated to exhibit linearity and rigidity that increases the space occupied by the molecule and, therefore, its tendency to

form aggregates and crystallize within lipid droplets (Köhn et al., 2008). The change to *cis*-configurations implies a modification of the overall shape of the molecule to a bent structure, which likely prevents carotenoid aggregation and crystallisation. As a result, *cis*-isomers of carotenoids have higher solubility in oil (Britton, 1995; Clinton, 2009), and consequently potential higher bioaccessibility than all-*trans* configurations. To this regard, several *in vitro* studies have reported higher bioaccessibility of *cis* isomers of lycopene and β -carotene compared to their corresponding all-*trans* forms (Failla, Chitchumroonchokchai, & Ishida, 2008; Ferruzzi, Lumpkin, Schwartz, & Failla, 2006). In addition, carotenoid transfer to the oil phase during mechanical (Mutsokoti, Panozzo, Musabe, Van Loey, & Hendrickx, 2015) or thermal (Mutsokoti, Panozzo, Van Loey, & Hendrickx, 2016) processing before digestion was suggested to enhance carotenoid bioaccessibility. However, up to date *in vitro* and *in vivo* literature studies have mainly focused on the end point of the digestion process, thus considering the final bioaccessibility value only. To this regard, kinetic studies could be a useful tool to obtain a better understanding not only of the final carotenoid bioaccessibility but also of the mechanisms of carotenoid incorporation into the micelles. The full digestion profile of carotenoids can be revealed by considering different incubation time moments in the simulated intestinal phase, instead of single end-point measurements. Furthermore, no clear relation between carotenoid bioaccessibility and lipid digestion has been established yet. Therefore, systematic investigations aimed at discerning the kinetic behaviour of the micellar incorporation of carotenoids concomitantly with lipid digestion products are needed.

The aim of this study was to gain insight in the kinetics of carotenoid micellar incorporation during digestion as a function of carotenoid species and isomeric configuration and in relation to lipid digestion. Carotenoid of different species, i.e. α -carotene, β -carotene and lycopene, and isomeric form, *cis-trans*, from carrot, tomato, and mango, were selected. Low fat oil-in-water emulsions, containing carotenoids enriched oil (5%, w/w) and L- α -phosphatidylcholine (1% w/w) as emulsifier, were prepared and subjected to a static *in vitro* digestion. The kinetics of lipid hydrolysis and micellar incorporation of lipid

digestion products as well as carotenoids was modelled by a first order fractional conversion model. Finally, the relation between carotenoid and lipid digestion products incorporation into mixed micelles was investigated.

2. Materials and Methods

2.1 Materials

All chemicals and reagents were of analytical or HPLC-grade. Carotenoid all-*trans* standards (lycopene, β -carotene, α -carotene), lipid standards (oleic acid, triolein, diolein and monoolein), pepsin (≥ 2500 U/mg protein), pancreatin (P1750 4XUSP), porcine bile extract (B8631), lipase (Type II, L3126), α -tocopherol (T3251), pyrogallol (P0381), and L- α -phosphatidylcholine were obtained from Sigma-Aldrich (Borne, Belgium). 5-*Cis* lycopene, 9-*cis*, 13-*cis* and 15-*cis* β -carotene were purchased from CaroteNature (Lupsingen, Switzerland). Olive oil (extra virgin) was kindly donated by Vandemoortele (Ghent, Belgium). Red tomatoes (*Lycopersicon esculentum* cv Prunus) and orange carrots (*Daucus carota* cv Nerac) were obtained fresh from a local supplier in Belgium. The vegetables were washed, peeled (in the case of carrots), cut, vacuum packed into polyethylene bags, frozen in liquid N₂ and stored at -40 °C prior to use. Mango (*Mangifera indica* L, unknown variety) puree was purchased from Spain, received frozen and immediately stored at -40 °C for maximal 1 week until use.

2.2 Preparation of carotenoid-enriched oil

The olive oil was enriched with carotenoids from tomatoes, carrots or mangoes. First, carrot and tomato purees were obtained. Carrots and tomatoes were thawed at 25 °C for 15 min. Tomatoes were blended (Waring Commercial, Torrington, CT, USA) for 1 min and sieved (pore size 1.0 mm) to remove the seeds and the excess skin. Carrot pieces were mixed with deionised water (1:1 w/w) and blended using the same conditions previously described. The obtained purees were mixed with olive oil (20 %, w/w). The mango puree was diluted with deionised water in a ratio 1:1 and mixed with olive oil (10 %, w/w). The puree-oil blends were then separately homogenised (Panda 2K, Gea Niro Soavi, Parma, Italy) at 100 MPa for one cycle. Finally, the carotenoid-enriched oil fraction was separated from the solid matrix by a combination

of freezing (-20 °C, 24 hrs), thawing (25 °C, 30 min), and centrifugation (65000 g, 40 min at 4 °C) (Beckman Optima XPN-100 Ultracentrifuge, Brea, CA, USA). The carotenoid enriched oils were collected separately into brown falcon tubes, flashed with N₂ (headspace), frozen in liquid N₂ and stored at -80 °C until use.

2.3 Preparation of emulsions

Emulsions were prepared by mixing [9500 rpm, 10 min (UltraTurrax, IKA-Werke, Staufen, Germany)] the carotenoid-enriched oil (5 %, w/w) with an aqueous solution of L- α -phosphatidylcholine (1 %, w/w). The mixtures were then immediately homogenized at 100 MPa for one cycle (Stansted Fluid Power, Pressure cell homogenizer, UK). The obtained carotenoid-enriched oil-in-water emulsions, from here on referred to simply as lycopene, β -carotene, and Cis/*Trans* emulsion, were stored at 4 °C and used within four days for the experiments. The emulsions were prepared in duplicate.

2.4 Particle size distribution

The oil droplet size distribution in the emulsions was measured by laser diffraction analysis (Beckman Coulter LS 13 320, Miami, FL, USA) as reported in (Mutsokoti et al., 2015, 2016). The surface-based mean diameter (D_{3,2}) was considered to quantitatively compare the emulsions.

2.5 *In vitro* digestion

Emulsions were independently subjected to *in vitro* digestion according to the method described by Minekus et al. (2014), with some modifications, where the gastric and intestinal digestion were simulated. The composition (% w/w) of the simulated gastric and intestinal fluids, SGF (pH 3 $\pm$ 0.05) and SIF (pH 7 $\pm$ 0.05), respectively, were as follows: SGF, 0.064% KCl, 0.015% KH₂PO₄, 0.263% NaHCO₃, 0.345% NaCl, 0.003% MgCl₂·(H₂O)₆ and 0.006% (NH₄)₂CO₃; SIF, 0.063% KCl, 0.014% KH₂PO₄, 0.893% NaHCO₃, 0.281% NaCl and 0.008% MgCl₂·(H₂O)₆.

To simulate the gastric phase, 10 mL of emulsion was transferred into a 50 mL capacity brown falcon tube. The sample was diluted with 7.5 mL SGF, 1.6 mL of porcine pepsin solution (25000 U/mL, in SGF) and 5 μ L of CaCl₂ (0.3 M). The pH was adjusted to 3 $\pm$ 0.05 by addition of 10 μ L of HCl (2 M). Thereafter

885 μ L deionized water was added to bring the final volume of the chyme to 20 mL, and the headspace of the samples flushed with N₂ for 10 s. The samples were incubated in the dark at 37 °C while shaking end-over-end for 2 hours.

Thereafter, the intestinal phase was simulated by adding 11 mL SIF to the chyme (20 mL). 5 mL of enzymes solution [pancreatin, 800 U/mL⁻¹, based on trypsin activity; lipase, 1600 U/mL⁻¹, α -tocopherol 1.4% (w/w) and pyrogallol 0.6% (w/w), in SIF] was then added to achieve a lipase activity of 200 U/mL⁻¹ in the final digestion mixture. 40 μ L CaCl₂ (0.3 M), 1.46 mL deionized water and 2.5 mL bile solution [160 mM, in SIF] were then added. The pH of all the samples was 7 $\pm$ 0.05. The headspace of the samples was flushed with N₂ for 10 s. The samples were incubated for 7.5, 15, 20, 30, 45, 60, 90 and 120 min at the same conditions as previously.

At the end of each incubation time of the simulated intestinal phase, the digested samples (digests) were subjected to a heat shock (85 °C for 10 min) in a water bath (Haak W15 DC-10, Germany) to inactivate lipase, and immediately cooled in an ice bath. To obtain the micellar phase, the digests were ultracentrifuged at 65000 g for 68 min at 4 °C (Beckman Optima XPN-100 Ultracentrifuge, Brea, CA, USA). The supernatant, corresponding to the micellar phase, was filtered (Chromafil PET filters, 0.2 μ m pore size, 25 mm diameter) and analysed for carotenoid content (C_m). The carotenoid *in vitro* bioaccessibility (BAC), at each incubation time (t, min), of the simulated small intestinal phase was defined as the percentage ratio between the carotenoid content in the micelles (C_m) and in the digest (C_d):

$$BAC_t(\%) = (C_m/C_d) \times 100 \quad (1)$$

Analogously, micellar incorporation of lipid digestion products, Lip (%), was expressed as the concentration of free fatty acids and monoacylglycerides (FFAs+MAGs) in the micellar fraction, $C_{(Lip) m}$, relative to its concentration in the digest, $C_{(Lip) d}$:

$$Lip(\%) = [C_{(Lip) m}/C_{(Lip) d}] \times 100 \quad (2)$$

2.6 Carotenoid analysis

Carotenoids were extracted as previously described Lemmens, Van Buggenhout, Oey, Van Loey, & Hendrickx (2009) with minor modifications. The analysis was performed under subdued light to prevent carotenoid degradation and isomerization. Five grams of sample (emulsion or digest) was mixed with 25 mL of extraction solution [hexane/acetone/ethanol (50:25:25 v/v/v) containing 0.1% of butylated hydroxytoluene (BHT)], and 0.25 g NaCl during 20 min at 4 °C. Reagent grade water (7.5 mL, 18.2 MΩ) was added and stirring was continued at 4 °C for 10 min. The apolar phase, containing the carotenoids, was then separated from the aqueous phase using a separation funnel, collected, filtered (Chromafil PET filters, 0.2 µm pore size – 25 mm diameter) and transferred to an amber HPLC vial.

The identification and quantification of carotenoids were performed using a HPLC-DAD system (Agilent Technologies 1200 Series, Diegem, Belgium) equipped with a C₃₀-column (3µm×150mm×4.6mm, YMC Europe, Diegem, Belgium). The column temperature was 25 °C. A mobile phase of reagent grade water (A), methanol (B) and methyl-t-butyl-ether (C) was used. The gradient elution for α - and β -carotene analysis was as follows: 0 min: 4% (A), 81% (B), 15% (C); 12–17min: 4% (A), 41% (B), 55% (C); 17–24 min: 4% (A), 81% (B), 15% (C). For lycopene analysis, the gradient elution was as follows: 0 min: 4% (A), 81% (B), 15% (C); 3–9 min: 4% (A), 36% (B), 60% (C); 9–24 min: 4% (A), 28% (B), 68% (C); 24–38 min: 4% (A), 16% (B), 80% (C); 38–44 min: 4% (A), 81% (B), 15% (C). The flow rate was 1 mL/min and the gradient was built up in 44 min for all-*trans* and *cis*-lycopene, and 24 min for all-*trans* α -carotene, all-*trans* and *cis* β -carotene analyses. Carotenoid identification and quantification were performed at 472 nm for all-*trans* and *cis*-lycopene, and at 450 nm for all-*trans* α -carotene, all-*trans* and *cis* β -carotene on the basis of retention times and spectral characteristics of pure standards. The carotenoid content was calculated with the use of the corresponding calibration curves except for 9-*cis* and 13-*cis* lycopene where the calibration curves were constructed with all-*trans* lycopene. Pure standard solutions were used to construct the calibration curves, which were linear throughout the whole concentration range ($R^2 \geq 0.99$).

2.7 Lipid analysis

The lipid classes: triacylglycerides (TAGs), diacylglycerides (DAGs), monoacylglycerides (MAGs), and free fatty acids (FFAs) were extracted from the initial emulsions, digests and micellar fractions according to Verrijssen, *et al.* (2015) with some modifications. To 1 mL of sample, ethanol (2 mL), heptane-diethylether (HDEE) (1:1 v/v, 3mL) and sulphuric acid (2.5 M, 0.2 mL) were added and the mixture vortexed for 2 min. The mixture was mixed further (30 min, end-over-end rotation) and then centrifuged at 500 g for 5 min at 20 °C (Sigma 6-16K centrifuge, *swing-out* rotor, Germany). The upper layer was collected and transferred to a volumetric flask containing anhydrous sodium sulphate (100 mg). The remainder was subjected to a second extraction. Thus, 1 mL HDEE was added, the mixture vortexed for 2 min, further mixed (15 min, end-over-end rotation) and then centrifuged as previously described. The upper layer was collected and added to the volumetric flask. The volume of the mixture was then made up to 5 mL with HDEE. Finally, an aliquot was taken from the volumetric flask, filtered (Chromafil PET filters, 0.2 µm pore size, 25 mm diameter), diluted further with HDEE, and transferred to an amber HPLC vial. Separation and detection of the lipid classes was conducted on a HPLC system (Agilent Technologies 1200 Series, Diegem, Belgium) equipped with a column oven (Chromaster 5310, VWR International, Leuven, Belgium) and coupled with Evaporative Light Scattering Detector (Alltech 3300 ELSD, Grace, Lokeren, Belgium). The method was based on Graeve and Janssen (2009) with some modifications. TAGs, DAGs, MAGs, and FFAs, were separated with a silica column (5µm×150mm×4.6mm) (Silica Spherisorb, Waters, Zellik, Belgium) maintained at 40 °C. A mobile phase of isooctane (A), acetone: ethyl acetate (2:1 v/v) containing 70 mmol/L acetic acid (B), propan-2-ol:water (85:15 v/v) containing 7.5 mmol/L acetic acid and 7.5 mmol/L triethylamine (C), and propan-2-ol (D) was used. The gradient elution was as follows: 0 min: 100% A; 1.5-1.6 min: 97% A, 3% B; 1.6-6 min: 94% A, 6% B; 6-8 min: 50% A, 50% B; 8-8.1- min: 47% A, 38% B, 15% C; 8.1-14 min: 47% A, 29% B, 24% C; 14.1-18 min: 40% A, 60% C; 18-23 min: 40% A, 60% C. The elution was carried out at 1 mL/min and immediately followed by a column washing and equilibrating step: 23.1-25 min: 100% B,

25.1-27 min: 100% D; 27-30 min: 100% A; 30-35 min: 100% A. The flow rate for the washing and equilibrating steps was 1.5 mL/min. The detector drift tube temperature was 44 °C while the N₂ gas flow was set at 1.9 L/min. Data acquisition was performed with ChemStation LabView (Agilent Technologies, Diegem, Belgium). Quantification of the TAGs, DAGs, MAGs, and FFAs was done by means of the calibration curves of equivalent standards, namely triolein, diolein, monoolein, and oleic acid, respectively (amount on column 0-2.6 µg of the standard). The amount of the lipid classes were expressed as mg/mL emulsion.

3. Data Analysis

All data are expressed as mean±standard deviation unless otherwise specified. The Tukey test was carried out in order to identify significant differences in carotenoid concentration, mean droplet diameter and lipid concentration among samples (SAS version 9.4, (Carry, NC, USA). The level of significance was set at α level of $P<0.05$.

3.1 Kinetic model assumptions and equation

To describe the hydrolysis reaction of TAGs by pancreatic lipase two considerations were taken into account. Firstly, it was considered that the reaction generates, in a first step, one FFA and a DAG which is, in a second step, further converted into a second FFA and the sn-2-monoacylglycerols (MAGs) (Giang et al., 2015). Therefore, the evolution of the kinetics of TAG lipolysis could be inferred from the disappearance of TAGs concomitantly with the appearance of FFAs, MAGs and DAGs. Important to note is that DAGs were not detected during the course of the experiments and therefore, in line with Giang *et al.* (2015), this implies that the conversion of DAGs into MAGs and FFAs was not rate limiting under the present experimental conditions. In fact, it is reported that lipase from porcine pancreatin hydrolyses DAGs at least nine fold faster than TAGs (Lykidis, Mougios, & Arzoglou, 1995).

Secondly, it is generally postulated that *in vitro* lipolysis kinetics of emulsified lipids often reach a plateau before reaction is completed as a result of the inhibition of the TAG hydrolysis reaction due to e.g. accumulation of the reaction products at the droplet surface (Grundy, Wilde, Butterworth, Gray, & Ellis,

2015), or droplet coalescence leading to the reduction of the surface area available for the adsorption of the pancreatic lipase (Giang et al., 2015). Moreover, it was assumed that the incorporation of lipid digestion products (FFAs and MAGs) into mixed micelles during the digestion of carotenoid-enriched oil-in-water emulsions is a step that precedes micellar incorporation of carotenoids. Therefore it can be expected that the incomplete *in vitro* TAG hydrolysis might be reflected in the levelling off of micellar formation kinetics and therefore, carotenoid bioaccessibility at longer digestion times in the simulated intestinal phase.

Based on these considerations, a fractional conversion kinetic model (Eq 3), characterised by first order kinetics until a plateau value is reached, was applied to describe the disappearance of TAGs and appearance of FFAs and MAGs during *in vitro* digestion of the tested emulsions:

$$X_t = X_f + (X_0 - X_f) \exp(-kt) \quad (3)$$

where X represents the response property at time t ; X_0 is the initial response property; X_f corresponds to the response property in equilibrium state/at plateau value, and k is the rate constant (min^{-1}), a time independent parameter.

The response property, X , can therefore represent: (i) FFAs (%) and MAGs (%), i.e. the mass percentage of released FFAs and MAGs, respectively, considering that pancreatic lipase produces two FFAs and one sn-2-MAG per TAG molecule, (ii) TAG (%), the hydrolysis of TAGs, calculated as mass percentage of residual TAGs present after each incubation time, t , of the simulated intestinal phase relative to its value at $t=0$, and (iii) micellar incorporation of lipid digestion products (Lip) (%) calculated according to Eq. 2 and (iv) micellar incorporation of carotenoids (BAC) (%). It was assumed that at $t=0$ there can be no FFA nor MAG formation and therefore no micellar formation. Consequently at $t=0$, Lip and BAC=0 implying that the response property $X_0=0$ at 0 min of incubation.

3.2 Parameter estimation and model evaluation

A non-linear regression approach, assuming first order fractional conversion kinetics, was applied in which the k and corresponding X_f values were simultaneously estimated (NLIN procedure, SAS version

9.6, Carry, NC, USA). The goodness of fit between the experimental and predicted data was evaluated by visual inspection of residual plots and by means of the adjusted correlation coefficient (R^2 -adj) (Eq. 4), considering that the higher the R^2 -adj, the better the fit:

$$R^2\text{-adj} = \left[\frac{(m-1) \left(1 - \frac{SSQ_{regression}}{SSQ_{total}} \right)}{(m-j)} \right] \quad (4)$$

where m , j and SSQ represents the number of observations, the number of model parameters and the sum of squares, respectively.

To evaluate significant differences of the jointly estimated parameters (k and X_f) values, 90% joint confidence regions (JCR) were constructed using Eq 5.

$$SSQ \leq SSQ(\theta) \left\{ 1 + \frac{p}{m-p} F(p, m-p, 1-\varphi) \right\} \quad (5)$$

$SSQ(\theta)$ represents the error sum of squares associated with the least squares estimate θ , p the number of simultaneously estimated parameters, m the number of observations, F the upper $1-\varphi$ quantile for an F distribution with p and $m-p$ degrees of freedom (Draper & Smith, 1998).

4 Results and discussion

The oils enriched with lycopene/ β -carotene, β -carotene/ α -carotene, and β -carotene *cis/trans*, from tomato, carrot, and mango, respectively were used to prepared oil-in-water emulsions. The emulsions were individually subjected to an *in vitro* digestion procedure and the lipid digestion as well as the carotenoid bioaccessibility were investigated by a kinetic approach.

4.1 Characterization of initial emulsions

Since lipid droplet size can affect the rate of lipid hydrolysis (Borel et al., 1994) and therefore, carotenoid bioaccessibility (Salvia-Trujillo, Qian, Martín-Belloso, & McClements, 2013), the droplet size distribution of the initial emulsions was measured prior to *in vitro* digestion. The particle size distributions (PSDs), as well as carotenoid concentration (expressed as $\mu\text{g/mL}$ emulsion) of the main isomeric forms identified in the lycopene, β -carotene and *Cis/Trans* emulsions are reported as

supplementary material. The emulsions were characterised by a mono-modal particle size distribution, with similar ($P \geq 0.05$) mean particle diameter ($D_{3,2}$). In particular lycopene, β -carotene and *Cis/Trans* emulsions were characterised by $D_{3,2}$ of 1.36 ± 0.07 , 1.40 ± 0.14 , 1.46 ± 0.16 μm for, respectively. In addition, the PSDs and the average droplet size were similar to those obtained for emulsions prepared from non-enriched oil (Mutsokoti et al., 2015), indicating that the incorporation of carotenoids in the oil did not significantly influence the oil droplet diameter and particle size distribution.

It is important to note that besides the droplet size, other parameters such as the interfacial properties of the lipid droplets can also impact lipolysis (Helbig, Silletti, Timmerman, Hamer, & Gruppen, 2012). In this context, the present emulsions were prepared using the same type and amount of emulsifier, therefore their interfacial properties can be considered similar.

In the lycopene emulsion, lycopene (*all-trans* and *cis*- isomers) and *all-trans* β -carotene accounted for about 86% and 14% of the total carotenoid concentration, respectively (Supplementary material, fig. X). The *all-trans* lycopene and β -carotene concentrations, being 5.31 ± 0.22 and 1.89 ± 0.03 $\mu\text{g/mL}$ respectively, were almost twice as high as the values reported by (Palmero et al., 2013) for the same 5% carotenoid-enriched oil-in-water emulsion. This difference can be attributed to the effect of high pressure homogenization in enhancing carotenoid transfer to oil during the preparation of the enriched oil fraction (Mutsokoti et al., 2015). In the β -carotene emulsion, β -carotene (*all-trans* and 13-*cis* β -carotene) was the predominant carotenoid (7.58 ± 0.34 and 1.57 ± 0.13 $\mu\text{g/mL}$, respectively) compared to *all-trans* α -carotene (4.26 ± 0.13 $\mu\text{g/mL}$), while 9-*cis* and 15-*cis* β -carotene were not detected. The total carotenoid concentration was comparable between the *Cis/Trans* and β -carotene emulsions. In the *Cis/Trans* emulsion, the ratio between the sum of *cis* β -carotene isomers and *all-trans* β -carotene was about 0.9:1.

4.2 Kinetics of lipid digestion and carotenoid bioaccessibility

To gain insight in the kinetics of lipid hydrolysis as well as bioaccessibility of *all-trans* and *cis* forms of different carotenoids, a static *in vitro* digestion was applied to the enriched-oil in water emulsions considering different digestion times up to 120 min of the small intestinal phase. The *in vitro* digestion

was simulated with 6 independent replicates for each time moment. At each time moment, the digests and micelles were recovered as previously described and analysed for TAG, DAG, MAG, FFA, and carotenoid content.

4.2.1 Lipid micellar incorporation kinetics

According to the literature, the hydrolytic products of dietary lipids may modify the physicochemical characteristics of micelles and thus affect carotenoid bioaccessibility (Porter & Charman, 2001; Tyssandier, Lyan, & Borel, 2001). Therefore, the mol percentage of each lipid class in the digest as well as the mol percentage of FFAs and MAGs in the micellar fraction during digestion of the lycopene, β -carotene, and *Cis/Trans* emulsions were considered (Fig. 1). During intestinal digestion, the mol% of TAGs in the digest decreased rapidly to about 40-45% in the first 30 min, indicating that most of the TAGs were digested within this time. Thereafter, the decrease of TAGs evolved towards a plateau value of about 14-18% indicating incomplete TAG digestion. As a consequence of TAG hydrolysis, an increase in MAGs and FFAs with digestion time was observed in the digest. By contrast, DAGs were not detected in the digest, indicating their fast conversion into MAGs and FFAs. During digestion, the increase of MAGs reached a plateau in a shorter time than the FFAs (Fig. 1A, B, C). In fact mol% of MAGs increased to 15-20% in the first 30 minutes of digestion and remained fairly unchanged thereafter, while mol% of FFAs reached 65-70% within the same time period. Overall, the decrease in TAGs and the release of FFAs and MAGs in the digests (Fig. 1A, 2B and 2C) during the *in vitro* digestion of lipids exhibited two stages, namely a fast reaction in the first 30 min that markedly slowed down thereafter. This was probably due to a decrease in lipase hydrolysis efficiency as a consequence of the accumulation of lipid digestion products at the interface of the oil droplets and/or coalescence of the oil droplets (Giang et al., 2015). Qualitatively, similar profiles were obtained for all the enriched-oil-in-water emulsions (Fig. 1A, B, C), which are consistent with a typical behaviour of the lipolysis of long chain TAGs (Giang et al., 2016; Zhu, Ye, Verrier, & Singh, 2013).

On the other hand, the micellar incorporation of MAGs and FFAs (Fig. 1a, 2b, and 2c) increased markedly to 60-70% during 1 h digestion. In particular, there seemed to be a preference for the incorporation of FFAs over MAGs during the first 30 min of digestion. Thereafter micellar incorporation of both FFAs and MAGs slowed down and reached a final value of about 80-90% of the FFAs and MAGs released in the digest.

The experimental data were modelled according to the fractional conversion kinetic model reported in Eq. 3. A good correlation ($R^2_{\text{adj}} \geq 0.87$) between experimental and predicted values was found for all the tested emulsions. The estimated kinetic parameters for the changes in the lipid classes in the digest and micellar fraction of all the tested emulsions are reported in Table 1.

Differences among the kinetic parameters relevant to the different lipid classes were evaluated by constructing the corresponding 90% joint confidence regions (JCR) (Supplementary material, fig. Y).

Based on the overlap of the JCRs, similar rate constants (k values, min^{-1}) for the decrease of TAGs and formation of MAGs and FFAs in the digest were obtained among the different emulsions (Table 1 and Supplementary material). This suggests that the rate of hydrolysis of TAGs and formation of FFAs and MAGs were not affected by the presence of different type and amount of carotenoids in the oil phase. Moreover, higher rate constants were obtained for MAGs compared to FFAs formation in the digests even though the extent of formation, represented by the plateau final values (X_f %), was at least 3 times higher for the FFAs. This is consistent with the hypothesis that DAGs immediately convert into a MAG and a FFA, and therefore were not detected.

With regard to the micellar phase, slightly higher k values were obtained for the incorporation of FFAs compared to MAGs, that can be ascribed to the difference in the solubility of the two lipid classes in the aqueous phase of the digest. The estimated mol% value at equilibrium (X_f %) for the micellar incorporation of both FFAs and MAGs was between 80 and 90%. These results suggest on the one hand that the incorporation of the lipid hydrolysis products into mixed micelles was almost complete, on the other hand that despite the incorporation of FFAs and MAGs occurred with different kinetics, the extent

of the incorporation of these two lipid classes into mixed micelles after 120 min of simulated small intestinal digestion was similar (Table 1 and Supplementary material, Fig. Y). Therefore, also the incorporation of FFAs and MAGs into mixed micelles was not influenced by the lipophilic compounds (i.e. carotenoids) initially present in the lipid phase (and eventually into the mixed micelles). Finally, higher rate constants were found for the formation of MAGs and FFAs in the digests compared to their incorporation into the micellar fraction in all the samples (Table 1). In other words, this suggests that under the present experimental conditions lipid hydrolysis proceeded faster than the formation of mixed micelles.

4.2.2 Carotenoid micellar incorporation kinetics

Prior to the actual kinetic study the stability of carotenoids during the static *in vitro* digestion was verified (data not shown). Carotenoid retention (i.e. the percentage ratio between carotenoid concentration in the digest and in the starting emulsion) was found to be higher than 85% at the end of the small intestinal digestion (120 min). Moreover, no significant differences ($P>0.05$) in carotenoid retention were found between the different digestion time moments. This observation is in agreement with previous investigations (Garrett et al., 1999; Granado-Lorencio, et al., 2007). Nevertheless, even higher carotenoid retention (around 100%) have been reported during *in vitro* digestion of, for example, carrot juice (Courraud et al., 2013). Furthermore, in line with Courraud et al. (2013), based on the ratio of the concentration of the individual isomers (C_{isomer}) to the total (all-*trans*+*cis*) concentration as a function of digestion time in the small intestinal phase (data not shown), no significant isomerisation and degradation occurred during the *in vitro* digestion.

The results of the bioaccessibility of all-*trans* α -carotene, β -carotene and lycopene, as well as *cis* forms of lycopene and β -carotene as a function of digestion time in the simulated intestinal phase are illustrated in Fig. 2.

Overall, an increase in carotenoid bioaccessibility between 0 and 45 min of digestion was observed. Thereafter, the bioaccessibility values tended towards a plateau reaching different final values depending

on the carotenoid type. In particular, carotenoid bioaccessibility did not increase significantly after 1 h of digestion.

Considering carotenoids with markedly different shape and present within the same matrix, i.e. all-*trans* β -carotene and all-*trans* lycopene from the lycopene emulsion (Fig. 2A), a significantly higher bioaccessibility was found for all-*trans* β -carotene compared to all-*trans* lycopene at all digestion times. Literature evidences have already reported that the *in vitro* bioaccessibility of lycopene is generally lower compared to β -carotene (Palmero, Lemmens, Hendrickx, & Van Loey, 2014; Tyssandier et al., 2001; van het Hof et al., 2000), even when the two carotenoids are considered within the same matrix (Garrett et al., 1999; Palmero et al., 2014; Ryan et al., 2008). However, it should be pointed out that in previous investigations only end conditions of the simulated small intestine digestion were considered.

By considering different incubation time moments of the simulated intestinal phase, the present results clearly show that the differences observed between the bioaccessibility of all-*trans* lycopene and all-*trans* β -carotene is reflected in their kinetic behaviour during incorporation into mixed micelles (Fig. 2A). Furthermore, the micellar incorporation kinetics appear to be slower for all-*trans* lycopene compared to all-*trans* β -carotene. The difference in all-*trans* lycopene and all-*trans* β -carotene micellar incorporation kinetics might be, on the one hand, determined by their hydrophobicity, which in turn influences the extent of interaction/association with lipid digestion products during the micellarisation process. On the other hand, the overall molecular geometry, i.e. the size and shape of the molecule, might also be playing a role in influencing the extent to which the carotenoids can fit within the mixed micelles. In particular, the type of the end groups on the carotenoid polyene chain are known to influence the way they fit into cellular and subcellular structures (Britton, 1995). More specifically, the presence of cyclic end groups (e.g. α - and β -carotene) makes the molecule shorter compared to the acyclic lycopene (Britton, 1995; Rodriguez-Amaya, 2015). In this regard, the higher bioaccessibility of α - and β -carotene might be a consequence of their different fitting within the micelles. Furthermore, the overall geometry of the carotenoids determines their hydrophobicity. Lycopene, having a linear more rigid structure is more

lipophilic than β -carotene (Britton, 1995) and therefore, can exhibit a higher resistance (reflected in the slower kinetics, Fig. 2A) for the transfer from the oil to the micellar phase. Carotenoids having more similar hydrophobicity, namely all-*trans* α -carotene and all-*trans* β -carotene from the β -carotene emulsion (Fig 2C), were characterised by more similar micellar incorporation kinetics, particularly in the first 45 min of digestion time. In addition, all-*trans* α - and β -carotene reached different bioaccessibility (30 ± 2.6 and 36 ± 4.2 , respectively) at the end of 2 h digestion in the small intestine phase. This observation is in line with previous end-point (2 h simulated intestine digestion) *in vitro* bioaccessibility investigations showing a higher bioaccessibility of β -carotene compared to α -carotene when both carotenoids are present in the same matrix (Huo, Ferruzzi, Schwartz, & Failla, 2007; Reboul et al., 2006). Therefore, the slight difference in molecular structure between α - and β -carotene, due to the position of one of the double bonds in one of the rings (Britton, 1995) seems to be an important factor determining their incorporation into micelles during digestion.

Similarly to the all-*trans* carotenoids, the time course of the bioaccessibility of lycopene *cis* isomers (Fig. 2B), 13-*cis* β -carotene (Fig. 2C), and the *cis* isomers of β -carotene from the *Cis/Trans* emulsion (Fig. 2D) were also characterized by an initial increase in bioaccessibility which tended towards a plateau value at longer digestion times. However, in the case of the *cis* isomers, not only was the plateau reached sooner (~30 min), but also the bioaccessibility values obtained were significantly higher compared to those of the corresponding all-*trans* forms. Finally, regardless of the initial concentration, the presence of other all-*trans* carotenoids (α -carotene or lycopene), as well as the presence of *cis* isomers in the emulsion, the kinetics of micellar incorporation of all-*trans* β -carotene was similar in all the three tested emulsions, particularly during the first 45 min of digestion. Considering all the tested emulsions, it is noteworthy that the large part of the carotenoid incorporation process took place within ~ 45 min of in the simulated small intestinal digestion. This seems logic since it coincides with, on the one side, the first stage of the TAG hydrolysis, characterised by a fast initial reaction rate, and on the other side, the incorporation of about 60% of lipid digestion products (FFAs and MAGs) (Fig. 1). This observation suggests that the second

stage of the TAG hydrolysis reaction, which was characterised by a slow reaction until a plateau was reached (Fig 1), might be the most significant event limiting the transfer of carotenoids from the oil droplets to the mixed micelles during digestion.

Also in the case of carotenoid bioaccessibility, the fractional conversion kinetic model (Eq. 3) was used to fit the experimental data. For all the carotenoids, a good correlation ($R^2_{adj} \geq 0.88$) between experimental and predicted values was found. The estimated kinetic parameters are listed in Table 2.

Generally, the rate constants, k (min^{-1}), representing the efficiency of carotenoid incorporation into mixed micelles, were found to be higher for the *cis*- compared to the all-*trans* carotenoids in all the three emulsions (Table 2). In addition, the final carotenoid bioaccessibility corresponding to the plateau values (BAC_f) indicated an inverse relationship between carotenoid hydrophobicity and their transfer efficiency into micelles (k values). In particular, in the lycopene emulsion, the k values were at least 3 times higher for *cis* than all-*trans* lycopene. Similarly, in the β -carotene emulsion, the k value for the micellar incorporation of 13-*cis* β -carotene was 4 times higher than the value obtained for all-*trans* β -carotene. From the *Cis/Trans* emulsion, the k values for β -carotene *cis* isomers were at least twice as high as the value obtained for all-*trans* β -carotene. This finding suggests that the slower micellar incorporation kinetics of the all-*trans* isomers (Fig. 2) is reflected in the lower bioaccessibility when compared with the *cis* isomers. This supports the hypothesis that compared to the *trans* forms, the *cis* carotenoid isomers have apparently shorter chain lengths due to the structural bend (Clinton, 2009; Failla et al., 2008). Consequently, *cis* isomers are characterized by lower hydrophobicity, steric hindrance, and thus resistance for the transfer to the micellar phase, ultimately resulting in faster carotenoid micellar incorporation kinetics.

Considering all-*trans* β -carotene and all-*trans* lycopene from the lycopene emulsion, the k value for the former was at least 1.6 times higher than value obtained for the latter (Table 2). From the β -carotene emulsion, a slightly higher k value was obtained for all-*trans* α -carotene compared to all-*trans* β -carotene. With regard to the final bioaccessibility (BAC_f), higher values were obtained for all-*trans* β -carotene

compared to all *all-trans* lycopene in the lycopene emulsion, *all-trans* β -carotene compared to *all-trans* α -carotene, and *cis* isomers of lycopene and β -carotene compared to the corresponding *all trans* forms.

Furthermore, even though *all-trans* β -carotene was present in significantly lower concentrations in the lycopene compared to the β -carotene or *Cis/Trans* emulsions (Supplementary material, Fig X), similar k values were obtained in all the present emulsions (Table 2). A similar rate constant for the micellar incorporation of *all-trans* β -carotene ($k \approx 0.04 \text{ min}^{-1}$) among the tested emulsions suggests that the initial concentration and the concomitant presence of other carotenoid species (type and/or isomers) had limited or negligible effect on β -carotene micellar incorporation.

To compare the simultaneously estimated parameters obtained for the different carotenoids in the different emulsions, 90% JCR were constructed (Fig. 3).

With regard to carotenoid hydrophobicity, results of the kinetic study revealed that the kinetics of micellar incorporation of *all-trans* β -carotene were significantly different to that of *all-trans* lycopene (Fig. 3A), and *all-trans* α -carotene (Fig. 3B). This clearly shows that kinetics of micellar incorporation were influenced by carotenoid molecular structure.

In addition, the kinetics of micellar incorporation of *all trans* forms were significantly different to the corresponding *cis* isomers. In particular, the combination of the k and BAC_f parameters were significantly different (no overlap of JCR) between 9-*cis* and 15-*cis* β -carotene (Fig 3C), as well as between 5-*cis* and 13-*cis* lycopene (Fig 3A), suggesting that micellar incorporation kinetics depended on the position of the *cis* double bond within the molecule. According to Huo et al. (2007) and Tan, Zhang, Abbas, Feng, & Zhang (2014) the ability of mixed micelles to incorporate lipophilic compounds depends on how the latter can be incorporated into their structure. Moreover, the sterical fit of the incorporated molecules into the micellar structure is crucial in determining the extent of their incorporation therein (Levin & Mokady, 1995; Tan et al., 2014). In this regard, Tyssandier et al. (2001) explained that due to the presence of the beta-ionone rings in the structure, α -carotene and β -carotene molecules can orient perpendicularly to the

phosphatidylcholine bilayer plane enhancing their migration into micelles. This can explain why higher rate constants were obtained for β -carotene compared to lycopene.

4.3 Relation between carotenoid bioaccessibility and lipid digestion

The relation between carotenoid bioaccessibility (BAC) and lipid digestion was investigated by linear regression analysis (Fig. 4) of the BAC values as a function of the corresponding micellar incorporation of lipid digestion products (FFA+MAG) (Lip, %), for each incubation time moment of the simulated intestinal phase. For the sake of clarity, *cis* lycopene isomers from the Lycopene emulsion as well as 13-*cis* and 15-*cis* β -carotene from the β -carotene and *Cis/Trans* emulsions are not shown. Generally, a positive correlation between carotenoid BAC and Lip was found, showing that carotenoid bioaccessibility increased with increasing percentage micellar incorporation of FFAs and MAGs. This observation is in line with Rao, Decker, Xiao, & McClements (2013), where a linear relation between amount of released FFAs and β -carotene bioaccessibility was found. The authors attributed this effect to an increase in the amount of mixed micelles formed that are capable of incorporating β -carotene. The results from the present study extend this effect further at the level of carotenoid hydrophobicity and isomeric configuration. Firstly, a higher slope value was found for all-*trans* β -carotene (0.42 ± 0.02) than for lycopene (0.29 ± 0.02), when both carotenoids were considered in the same emulsion (Fig 4). This suggests that the higher the carotenoid hydrophobicity, the lower the dependency on lipid digestion products for its incorporation into mixed micelles and the lower the efficiency of micellar incorporation (Fig 4). Secondly, similar slope values were obtained among all-*trans* and *cis* forms of lycopene and β -carotene, suggesting that the different *cis* isomers and their all-*trans* counterparts exhibited a similar dependency on lipid digestion products (FFA+MAG) for their bioaccessibility. This suggests that, rather than the extent of lipid digestion, the differences in molecular structure between *cis* and *trans* isomers (which are directly linked to their differences in solubility) would be the dominant factor influencing the extent of their micellar incorporation. Nevertheless, the present correlation analysis is based on just one single factor,

being Lip, influencing carotenoid bioaccessibility, while other factors related to the physicochemical properties of the micelles can also play a role (Goncalves et al., 2013; Rao et al., 2013).

5 Conclusions

The micellar incorporation of carotenoids and lipid digestion products from low fat carotenoid enriched oil-in-water emulsions during *in vitro* digestion was studied by a kinetic approach. To the best of our knowledge, this is the first study to investigate the kinetic behaviour of the micellar incorporation of carotenoids (lycopene, β - and α -carotene) and lipid digestion products (FFAs+MAGs), simultaneously. The fractional conversion kinetic model enabled reliable prediction of micellar incorporation of different carotenoids and lipid digestion products in low fat emulsions containing 5% carotenoid enriched oil and stabilised by phosphatidylcholine. The kinetic parameters obtained revealed that the release of FFAs and MAGs proceeded faster than their incorporation into micelles. Moreover, release in the digest and incorporation into micelles of FFAs and MAGs were not influenced by the type and amount of carotenoids initially present in the emulsions. On the other hand, the incorporation of carotenoids into micelles depended on the micellar incorporation of lipid digestion products (FFA+MAGs) and this dependency varied as a function of carotenoid type. However, the dependency of carotenoid micellar incorporation on lipid digestion products (FFAs+MAGs) was found to be similar between the different *cis* isomers and their all-*trans* counterparts. All in all, the results demonstrated the need for kinetic studies to reveal differences in the micellar incorporation of lipophilic compounds instead of endpoint studies.

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Captions to figures

Fig. 1: Time course of mol% of released free fatty acids (FFAs) and monoglycerides (MAGs) as well as residual/undigested mol% triacylglycerols (TAGs) in the digest (A, B and C), and mol% of free fatty acids (FFAs) and the monoglycerides (MAGs) in the micellar fraction (a, b, c) during *in vitro* digestion. (A, a) Lycopene emulsion; (B, b) β -carotene emulsion; (C, c) *Cis/Trans* emulsion. Error bars indicate standard deviations. Experimental data points are indicated by the symbols whereas the lines represent values predicted by the kinetic model.

Fig. 2 Time course of carotenoid bioaccessibility of: (A) all-trans carotenoids from the Lycopene emulsion; (B) lycopene cis isomers from the lycopene emulsion (C) 13-cis β -, all-trans α - and all-trans β -carotene from the β -carotene emulsion; (D) All-trans and cis β -carotene from the *Cis/Trans* emulsion during static *in vitro* digestion. Experimental data and predicted values are represented by the symbols and the lines, respectively. Error bars represent standard deviations.

Fig. 3 Joint confidence regions (JCR) (90%) for carotenoid micellar incorporation during *in vitro* digestion of low fat emulsions: (A) Lycopene, (B), β -carotene and (C) *Cis/Trans* emulsion. The specific kinetic parameter estimates (Table 2) are indicated by the symbols enclosed within the JCR (lines).

Fig. 4 Relation between carotenoid bioaccessibility and the micellar incorporation of (FFAs+MAGs) during digestion. (A) β -carotene emulsion, (B) Lycopene emulsion and (C) *Cis/Trans* emulsion. The corresponding linear regression equations and the R^2 are also indicated. For clarity, lycopene *cis* isomers, 13-*cis* β -carotene from the β -carotene emulsion and 15-*cis* β -carotene from the *Cis/Trans* emulsion are not shown.

Captions to figures

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Fig. 2

Fig. 3

Fig. 4

Table 1. Overview of the kinetic parameters ($\pm$ standard error) for the modelling of lipid classes in the digest and the micellar fraction by a fractional conversion model during digestion of emulsions

Emulsion	Lipid Class	Digest		Micelles	
		k (min^{-1})	$X_f(\%)$	k (min^{-1})	$X_f(\%)$
Lycopene	TAG	0.084 $\pm$ 0.016	21.3 $\pm$ 4.6		
	MAG	0.166 $\pm$ 0.051	18.9 $\pm$ 1.0	0.024 $\pm$ 0.004	90.9 $\pm$ 8.2
	FFA	0.052 $\pm$ 0.004	75.7 $\pm$ 2.2	0.039 $\pm$ 0.008	80.3 $\pm$ 6.6
β -carotene	TAG	0.083 $\pm$ 0.012	31.3 $\pm$ 2.8		
	MAG	0.115 $\pm$ 0.021	18.4 $\pm$ 0.7	0.023 $\pm$ 0.004	96.9 $\pm$ 8.7
	FFA	0.048 $\pm$ 0.005	77.2 $\pm$ 2.6	0.042 $\pm$ 0.007	84.0 $\pm$ 5.3
<i>Cis/Trans</i>	TAG	0.075 $\pm$ 0.005	30.0 $\pm$ 3.4		
	MAG	0.078 $\pm$ 0.014	21.4 $\pm$ 1.1	0.021 $\pm$ 0.003	92.9 $\pm$ 6.2
	FFA	0.053 $\pm$ 0.007	70.8 $\pm$ 2.9	0.036 $\pm$ 0.007	83.2 $\pm$ 8.1

X_f represents the estimated plateau values

k rate constant for (i) the hydrolysis of TAG in digest, (ii) formation of MAG or FFA in digest, (iii) incorporation of FFA or MAG in micelles

Table 2. Kinetic parameters ($\pm$ standard error) for modelling of the bioaccessibility of all-*trans* and *cis* carotenoids during static *in vitro* digestion of low fat emulsions.

Emulsion	Carotenoid	k (min^{-1})	BAC_f (%)
Lycopene	all- <i>trans</i> Lycopene	0.026 ± 0.003	21.8 ± 1.3
	5- <i>cis</i> Lycopene	0.159 ± 0.023	19.7 ± 0.4
	9- <i>cis</i> Lycopene	0.105 ± 0.021	23.1 ± 0.0
	13- <i>cis</i> Lycopene	0.079 ± 0.014	25.0 ± 1.2
	all- <i>trans</i> β -carotene	0.044 ± 0.004	31.4 ± 1.1
β -carotene	all- <i>trans</i> β -carotene	0.043 ± 0.004	33.0 ± 1.4
	13- <i>cis</i> β -carotene	0.188 ± 0.025	35.8 ± 0.8
	all- <i>trans</i> α -carotene	0.053 ± 0.006	29.9 ± 1.9
<i>Cis/Trans</i>	all- <i>trans</i> β -carotene	0.038 ± 0.004	41.0 ± 2.7
	9- <i>cis</i> β -carotene	0.060 ± 0.007	48.1 ± 1.8
	13- <i>cis</i> β -carotene	0.076 ± 0.013	47.2 ± 2.2
	15- <i>cis</i> β -carotene	0.072 ± 0.009	52.2 ± 1.7

k represents the efficiency of carotenoid micellar incorporation

BAC_f represents the plateau value (X_f) as stated in the kinetic model Eq. 3

Highlights

- A kinetic approach was applied to carotenoid and lipid micellar incorporation
- Carotenoid micellar incorporation is inversely proportional to their hydrophobicity
- *Cis* isomers are selectively incorporated into micelles compared to the all-*trans*
- Positive correlation exists between carotenoid and (FFA+MAG) micellar incorporation