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Complete List of Authors:	Yuan, Xi; South China Agricultural University, Food Science Liu, Xiaojuan; South China Agricultural University, Food Science McClements, David; University of Massachusetts, Food Science Cao, Yong; South China Agricultural University, Food Science Xiao, Hang; University of Massachusetts Amherst, Food Science

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ARTICLE

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Xi Yuan,[ab] Xiaojuan Liu,*[ab] David Julian McClements,[c] Yong Cao[ab] and Hang Xiao*[c]

Effects of lipid type in excipient emulsions on the bioaccessibility of carotenoids (lutein and β-carotene) in spinach were studied using a simulated gastrointestinal tract (GIT). Results showed that the lipid type only had a minor impact on the physical and structural characteristics of the spinach/emulsion mixtures as they passed through simulated mouth, stomach, and small intestine phases. However, a significant effect was observed on lipid digestion, mixed micelle formation, and carotenoid bioaccessibility. Excipient emulsions containing mainly medium chain triacylglycerols (MCTs) (MCT and coconut oils) had faster initial lipid digestion rates, higher overall digestibilities, smaller mixed micelle sizes, and higher lutein bioaccessibilities than those containing mainly long chain triacylglycerols (LCTs) (corn, olive, and fish oils). Excipient emulsions rich in long chain monounsaturated fatty acids (MUFAs) (corn and olive oils) formed larger mixed micelles and gave higher β-carotene bioaccessibilities than those rich in either medium chain saturated fatty acids (SFAs) (MCT and coconut oils) or long chain polyunsaturated fatty acids (PUFAs) (fish oil). These differences in bioaccessibility were attributed to differences in micelle size and solubilization capacity, as well as carotenoid dimensions. Finally, emulsions containing a mixed oil phase (MCT oil : corn oil = 1 : 1, w/w) appreciably increased both lutein (from 21% to 42%) and β-carotene (from 6.8% to 25%) bioaccessibility from spinach compared to a control (no oil). These results suggest that mixed LCT-MCT oil phases may be useful for the design of excipient emulsions for improving the bioaccessibility of various hydrophobic nutraceuticals.

1. Introduction

Dark green leafy vegetables are the primary carotenoid contributors in the human diet.[1] Epidemiological studies have consistently demonstrated that dietary carotenoids play critical roles in the prevention of chronic diseases, including cancer, hip fractures, cardiovascular disease, and age-related function decline.[2-4] However, because carotenoids are highly hydrophobic, they are unable to simply move from inside plant tissues to the enterocyte absorption site. Therefore, carotenoid bioavailability is both extremely low and variable when they are ingested directly from green leafy vegetables.[5, 6]

Carotenoids present within the food matrix must be released from the plant tissue and solubilized into the gastric lipid phase prior to incorporation into mixed micelles in the duodenum.[7] Only the fraction solubilized within the mixed micelle phase generated in the small intestine is considered to be available for absorption by intestinal epithelial cells, and is typically considered a marker of its bioaccessibility.[8] Previous studies have reported that lipids stimulate bile and chylomicron secretion to facilitate mixed micelle formation.[5] In addition, lipid digestion products increase carotenoid solubilization within mixed micelles by increasing their volume of their hydrophobic domains.[9] Therefore, lipids play a critical role in determining carotenoid bioaccessibility. However, the relatively low levels of naturally occurring lipids present in the majority of fruits and vegetables are insufficient to generate mixed micelles within the human small intestine.[10] There is therefore great interest within the field in understanding how to enhance carotenoid bioavailability from the human diet with the addition of extra lipids.

Previous studies have demonstrated that co-ingestion of plant-based foods with specific types of digestible lipids[11, 12] (sunflower oil, olive oil, palm oil, or rapeseed oil) or fat-rich foods[13,14] (milk or avocado) enhances carotenoid bioavailability. For example, dietary lipids have been demonstrated to increase carotenoid bioaccessibility in mangos[15], spinach[16], and carrots[11]. Based on the impact of food matrix effects on nutraceutical bioavailability, the concept of excipient foods was proposed as a means of increasing nutraceutical bioavailability in the human diet by McClements et al. in 2014.[17] Excipient foods possessing a specific composition and structure are specifically designed to enhance nutraceutical efficacy in fruits and vegetables

[a] Department of Food Science, College of Food Science, South China Agricultural University, GZ 510642, CN. E-mail: liuxj@scau.edu.cn; Tel: +86-20-85286234
[b] Research Centre of Natural Active Compound Engineering Technology of Guangdong Province, GZ 510642, CN.
[c] Department of Food Science University of Massachusetts, Amherst, MA 01003. E-mail: hangxiao@foodsci.umass.edu; Tel: +01-413-5452281

that are co-ingested with them.[17] Numerous studies have demonstrated that excipient emulsions are more effective at enhancing the bioaccessibility of highly lipophilic bioactive agents compared to bulk oils. This effect could be due to the small size of the lipid droplets, resulting in a tendency for them to be digested more rapidly and extensively in comparison to bulk oils, thereby promoting carotenoid micellization.[18]

However, the nature of the lipid phase utilized to form the excipient emulsions has a major impact on carotenoid bioaccessibility, specifically the fatty acid chain length and degree of unsaturation. For example, carotenoid bioaccessibility from peppers[19] and carrots[20] increased appreciably when co-digested with emulsions prepared using long chain fatty acids (LCFAs, > C12) compared to when they were co-digested with emulsions prepared using medium (MCFAs, C6-C12) or short chain fatty acids (SCFAs, < C6). Other studies have demonstrated that emulsions containing long chain triglycerides (LCTs) enhanced total carotenoid bioaccessibility in mangos, but not phenolics, which are less hydrophobic than carotenoids.[21] Huo et al.[22] demonstrated that there was no significant effect of the degree of lipid saturation on the bioaccessibility of dietary carotenoids. However, other studies demonstrated that lipids rich in unsaturated fatty acids (USFAs) (olive oil, soybean oil, and sunflower oil) exhibited an increased ability to enhance carotenoid bioaccessibility in comparison to those rich in saturated fatty acids (SFAs) (peanut oil and coconut oil).[23] No definitive conclusions can currently be made regarding the specific impact of lipid type on the bioaccessibility of different hydrophobic components. It is likely that efficacious excipient emulsions will need to be designed for each specific bioactive agent and food matrix. Consequently, studies are required to fully understand the effects of lipid type on carotenoid bioaccessibility in order to develop excipient emulsions that are more conducive for the promotion of human health.

The main goal of the current research was to study the effect of lipid type (fatty acid chain length and degree of unsaturation) in excipient emulsions on lutein and β-carotene bioaccessibility in samples derived from spinach. In addition, we aimed to design an excipient emulsion that could simultaneously improve the bioaccessibility of these two different types of carotenoid. The results obtained in this study advance our understanding of the impact of lipid type on the bioaccessibility of nutraceuticals of different hydrophobicity. Moreover, these studies will facilitate the rational design of excipient emulsions that enhance the bioaccessibility of various types of hydrophobic health-promoting phytochemicals found in plant-based foods.

2. Materials and methods

2.1. Materials

Fresh spinach, virgin coconut oil, and corn oil were purchased from a local supermarket (Guangzhou, CN). Olive oil was purchased from Exiom Food S.L. (Oviedo, ES), fish oil was purchased from General Nutrition Centres (Pittsburgh, USA), and powdered sodium caseinate (CS, P1204583-100 g) was purchased from Adamas Reagent Co., Ltd. (Shanghai, CN). The following chemicals were purchased from Shanghai YuanYe Biological Technology Co., Ltd. (Shanghai, CN): medium chain triglycerides (MCT) (S25953-500 mL) and bile salt (pig) (S30895-25 g). Mucin from porcine stomach (M2378-100 g), pepsin from porcine gastric mucosa (250 units/mg, P7000-25 g), and lipase from porcine pancreas (type II, L3126-100 g) were purchased from Sigma Chemical Company (St. Louis, MO, USA). All other chemicals used were of analytical grade. Double distilled water obtained from a water purification system was used for the preparation of all aqueous solutions and emulsions.

2.2. Methods

2.2.1. Sample preparation. An aqueous phase was prepared by dissolving 1% sodium caseinate in a buffer solution (5 mM phosphate buffer, pH 7.0). An oil phase was prepared using MCT oil, coconut oil, corn oil, olive oil, or fish oil. A stock emulsion was generated by homogenization of the oil phase (10%, w/w) with the aqueous phase (90%, w/w) using a high-speed blender (10 Kr/min, 2 min) (FJ200-S, Nenggu Mechanical & Electrical Equipment Co., Ltd., Shanghai, CN). Excipient emulsions were then generated by passing the stock emulsion through a high-pressure homogenizer (AH-BASIC, ATS Engineering Inc., Shanghai, CN) with a homogenization pressure of 120 Mp, for a total of 5 passes. All emulsions were diluted to 4% (w/w) oil content with buffer solution (5 mM phosphate buffer, pH 7.0) prior to use. The buffer solution was used as a control (no oil) for comparison purposes.

Roots and stems were removed from the fresh spinach, and the leaves were cut into small pieces (approximately 10 mm length and width). The spinach puree was then generated by homogenizing the spinach pieces using an equal mass of buffer solution (5 mM phosphate buffer, pH 7.0).

2.2.2. Simulated gastrointestinal tract. *Initial system***.** The spinach puree was mixed with an equal mass of excipient emulsions or buffer solution (control) prior to passing it through the GIT model. This was regarded as the initial sample.

The spinach/emulsion or spinach/buffer mixtures were then passed through an *in vitro* simulated gastrointestinal tract (GIT), which included mouth, stomach, and small intestine phases, in an effort to mimic the potential gastrointestinal fate of these mixtures.[24]

Mouth phase. The simulated saliva fluid (SSF) contained 0.03 g/mL mucin and various salts and was generated using previously described methods.[25] The SSF was preheated to 37 ^{o}C prior to use. A 20 mL volume of the initial sample was then placed into a glass beaker and mixed with 20 mL of SSF. The pH of the mixture was then adjusted to 6.8, and the mixture was incubated in a shaking incubator at 37 ^{o}C for 10 min to mimic the conditions in the mouth.

Gastric phase. A 20 mL volume of simulated gastric fluid (SGF), containing 0.0032 g/mL pepsin, 2 mg/mL NaCl, and 0.7% (v/v) HCl was prepared according to a previously described method.[25] A 20 mL-aliquot sample from the mouth phase was mixed with 20 mL of SGF, and the pH was adjusted to 2.5 using NaOH. The final mixture was incubated at 37 ^{o}C for 2 h with continuous agitation at 100 rpm.

Small intestine phase. A 30 mL-aliquot of sample from the gastric phase was added to a 100 mL glass beaker and incubated in a 37 ^{o}C water bath. The pH of the mixture was then adjusted to 7.0. A 1.5

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mL volume of simulated intestinal fluid (150 mM sodium chloride and 10 mM calcium chloride) and 3.5 mL of bile salt solution (187.5 mg/3.5 mL) were added to the glass beaker with constant stirring, and the pH of the resulting mixture was adjusted to pH 7.0 as necessary. Next, a 2.5 mL volume of freshly prepared lipase solution (24 mg/mL) was immediately added to the sample with continuous stirring. A pH-stat method was utilized to monitor the volume of 0.25 M NaOH that was titrated into the mixture to maintain a pH 7.0 throughout the 2 h digestion period. The percentage of free fatty acids (FFA) released was calculated using an equation described previously.[26]

2.2.3. Particle size and charge measurements. The particle diameter, particle size distribution, and ζ-potential of the particles in the samples were monitored immediately following passage through each stage of the GIT model. Spinach fragments were removed by diluting samples with buffer solution prior to analysis. This allowed large plant tissues to settle to the bottom of the samples due to gravity, and the upper emulsion layer was then collected and measured. This enabled measurement of changes in lipid droplet properties without interference from large plant tissue fragments.

Mean particle sizes and particle size distributions of the emulsions were determined using dynamic light scattering (DLS) (BIC 90 Plus particle size analyser, Brookhaven Inc., New York, USA). Measurements were carried out at 25 ^{o}C, using a fixed 90 ° angle, and a wavelength of 658 nm. To avoid multiple scattering effects, the initial, mouth, small intestine and mixed micelle samples were diluted using phosphate buffer (5 mM, pH 7.0) prior to analysis, while the stomach samples were diluted with distilled water adjusted to pH 2.5. The mean particle sizes are reported as surface-weighted mean diameters (d_{32}).

The ζ-potential of the particles in the samples was determined using electrophoresis-light scattering instrument (Zeta Plus, Brookhaven Inc., New York, USA). Prior to analysis, samples were diluted in appropriate solutions to avoid multiple scattering effects: initial, small intestine and mixed micelle phase (phosphate buffer 5 mM at pH 7.0), mouth phase (ASSS at pH 6.8), and stomach (SGJ at pH 2.5).

2.2.4. Microstructure measurements. Microstructures of samples following passage through each stage of the simulated GIT were determined using optical microscopy (CX41, Olympus Corporation, Tokyo, JP) using a 10× objective lens and a 100× oil immersion objective lens. A small portion of sample was collected and placed on a microscope slide, covered with a cover slip, and then imaged immediately.

2.2.5. Bioaccessibility. Carotenoid bioaccessibility was taken to be the fraction solubilized within the mixed micelle phase versus the total concentration in the digesta after the final stage of the simulated GIT digestion.[24] Following passage through the small intestinal stage, raw digesta were collected and centrifuged (D3024R high speed micro-centrifuge, Scilogex Inc., Berlin, USA) at 15000 rpm, for 50 min at 4 ^{o}C. Each sample was then separated into two or three parts: an opaque sediment at the bottom; a micelle phase in the middle; and sometimes an oil phase at the top. After filtration (0.45 μm pore size), the middle phase was assumed to be the "micelle" fraction containing the solubilized (bioaccessible) carotenoids. This is due to the fact that large particles (d > 450 nm) are not expected to pass through the mucus layer and be absorbed by epithelium cells. Carotenoid bioaccessibility was calculated using the following equation, which has been described previously[24, 27]:

$$\text{Bioaccessibility (\%)} = \frac{100 \times C_{\text{Micelle}}}{C_{\text{Raw digesta}}} \quad (1)$$

where, $C_{micelle}$ and $C_{Raw\ digesta}$ represent the carotenoid concentrations in the mixed micelle phase and in the digesta following the simulated intestinal digestion, respectively.

2.2.6. Carotenoid extraction and analysis. Carotenoid extraction was carried out according to the method described by Biehler et al.[28] with minor modifications. Briefly, 5 mL digesta or 10 mL micelle phase was mixed with hexane : acetone (1 : 1, v/v), vigorously shaken, and centrifuged for 2 min at 4000 rpm. The supernatant layer was then collected in a second tube. The above procedure was repeated three times. A saturated sodium chloride solution was then added to the collected organic fractions and shaken vigorously. The supernatant layer was collected, and the hexane was added into the lower phase for another extraction. The above procedure was repeated until the lower phase was colourless. The combined supernatant hexane phases were evaporated at 25 ^{o}C under vacuum, dried in a freeze dryer (FD-1PF, Beijing Detianyou Instrument Co., Ltd., Beijing, CN) for 24 h, and stored at -20 ^{o}C prior to analysis. The final samples were re-dissolved in HPLC grade dichloromethane and filtered through a 0.45 μm polytetrafluoroethylene (PTFE) filter prior to injection into an HPLC system. All procedures were carried out on ice under low light exposure.

The carotenoid concentration was determined using high performance liquid chromatography. The HPLC system was comprised of a column temperature, a binary solvent delivery system (LC-15C), and a diode array detector (SPD-M10A) that performed wavelength scanning from 200 to 800 nm. System control and data analysis were carried using the LC-VP HPLC system and LC-VP software (Shimadzu, Tokyo, JP). A C18 column (250 mm × 4.6 mm i.d., 5 μm, DIKAM, Beijing, CN) was utilized as the stationary phase. The mobile phases were comprised of (A) acetonitrile : methanol (95 : 5, v/v) and (B) acetonitrile : methanol: ethyl acetate (60 : 20 : 20, v/v/v). A linear gradient program was carried out as follows: initial condition of mobile phase A : B was 100 : 0; followed 30 : 70 for 10 min, 100 : 0 for 45 min, and finally back to the initial condition for 15 min to allow for re-equilibration. Triethylamine (5%) was included with the acetonitrile to improve carotenoid recovery from the chromatographic column. The flow rate was set to 1 mL/min and an injection volume of 20 μL was used. The detection wavelength was set to 450 nm. The contents of lutein and β-carotene in the samples were calculated from carotenoid standard curves.

2.3. Statistical analysis

All experiments were carried out at least twice, and all data were reported as the mean ± SD for triplicate analysis (n = 3). Results were subjected to variance analyses using the SPSS software (SPSS Inc., Chicago, IL, USA) and the mean difference was determined

using a post hoc Duncan test with a confidence level of 95% considered as significant.

3. Results & Discussion

In this study, we mixed five types of excipient emulsions fabricated from five different oils (MCT oil, coconut oil, corn oil, olive oil, and fish oil) with pureed spinach and then passed them through the simulated GIT. The effect of lipid type on lipid digestion and carotenoid bioaccessibility was then determined. During digestion, changes in the microstructure and physicochemical properties of the mixtures were also monitored to better understand the potential gastrointestinal fate of the spinach/excipient emulsion mixtures. This information was acquired as it may help understand any observed differences in the bioaccessibility of the carotenoids. These oils were chosen due to the fact that they are food-grade lipids with different fatty acids compositions. MCT oil contains all medium chain (C8-C10) saturated fatty acids (SFAs), coconut oil contains primarily medium chain (C8-C12) saturated fatty acids (MCFAs > 60%, SFAs > 90%), corn oil and olive oil contain primarily long chain (C16-C18) monounsaturated fatty acids (MUFAs > 65%), and fish oil contains primarily long chain (C14-C24) polyunsaturated fatty acids (PUFAs > 60%) (Table 1).

3.1. Properties of the original excipient emulsions

The mean particle diameter (d_{32}) was relatively small and fairly similar (from 170 to 220 nm) for all emulsions, regardless of the oil phase used to produce them (Fig. 1A). The polydispersity index (PDI) of all emulsions was < 0.3, indicating a narrow particle size distribution.[29] All of the oil droplets were fairly uniformly distributed throughout the emulsions and no visible phase separation was observed during a one-week storage period at 4°C (data not shown). These results demonstrated that stable excipient emulsions with fairly similar droplets sizes could be produced using the different oils.

Initially, the surface potentials (ζ-potential) (Fig. 1B) of all the excipient emulsions were negative, which was primarily attributed to the sodium caseinate (CS) molecules that coated the droplet surfaces. The pH value of the original emulsions (pH 7) was well above the isoelectric point (pI ≈ 4.5) of sodium caseinate, so the adsorbed proteins would be strongly negatively charged. However, the magnitudes of the electric charges present on the excipient emulsions with different oil phases (ζ = -10.0 to -28.1 mV) were significantly different. A similar finding has been reported previously, and could be due to the absorption of other charged substances, such as free fatty acids, phospholipids, or mineral ions, to the droplet surfaces.[30]

3.2. Impact of lipid type on physical and structural properties of spinach/emulsion mixtures in the simulated GIT

Measurements of particle size, charge, aggregation state, and microstructure of the spinach/emulsion mixtures is important for understanding the impact of lipid type on their gastrointestinal fate. In particular, these factors may influence the transfer of lipophilic phytochemicals from the plant tissues into the lipid droplets, the digestibility of the lipid droplets, and the formation of mixed micelles within the GIT model.[31, 32] Therefore, the effect of lipid type on the physical and structural changes of the spinach/emulsion mixtures prior to (initial) and following passage through each stage of the GIT (mouth, stomach, small intestine) was monitored.

3.2.1. Initial system. The mean particle diameters (d_{32}) (Fig. 2A) of the spinach/emulsion mixtures were significantly higher than those of the original emulsions, with values of 0.69, 0.40, 0.33, 0.31, and 0.52 μm for MCT, coconut oil, corn oil, olive oi and fish oil, respectively. However, all emulsions maintained a monomodal particle distribution (Fig. 3), and the lipid droplets were relatively small and evenly distributed throughout the mixtures (Fig. 4). There are two potential explanations for this phenomenon: (1) some of the smaller droplets within the original excipient emulsions were preferentially trapped within the plant structure; (2) some droplet flocculation or coalescence occurred due to components (such as biopolymers or mineral ions) released from the spinach leaves; (3) some fragments from the spinach leaves contributed to the light scattering signal. In the absence of the excipient emulsions (control), we observed relatively large particles in the initial system (d_{32} ≈ 6 μm) (Fig. 2A). These large particles were likely spinach puree fragments that were not completely removed by gravitational settlement. Following mixing with the excipient emulsions, the mean particle diameter of the spinach/emulsion mixture measured by light scattering was much smaller (d_{32} < 1 μm), due to the contribution of the small lipid droplets from the emulsions to the light scattering signal (Fig. 2A).

Following mixing with pureed spinach, the magnitude of the negative charges on the droplets in the spinach/emulsion mixtures (ζ = -11.2 to -22.4 mV) decreased slightly relative to those measured prior to mixing (ζ = -10.0 to -28.1 mV) (Fig. 2B). This could be due to the presence of charged components (such as biopolymers or mineral ions) that leaked from the spinach tissues and absorbed onto the lipid droplet surfaces or dissolved in the aqueous phase, thereby changing the surface charge or ionic strength of the overall sample. Meanwhile, we note that the decrease in the magnitudes of negative charges on the droplet surfaces is coincident with droplet flocculation. Interestingly, we observed significant differences in ζ-potential among the different samples, with the negative charge increasing in the following order: MCT (ζ = -13.0 to -11.2 mV) < LCT (ζ = -17.6 to -22.4 mV). This effect could be due to the different interfacial charges of the original emulsions with different oils, leading to different absorption of the charged components from the spinach puree to the oil-water interface.

These results suggest that mixing with the pureed spinach only resulted in a slight amount of droplet flocculation in the excipient emulsions and had no major effects on their physical properties. In our previous study, we found that mixing excipient emulsions stabilized by sodium caseinate with orange juice led to protein precipitation and destabilized the emulsions. However, the same type of excipient emulsion appears to be suitable for co-ingestion with spinach and was therefore utilized in the further studies.

3.2.2. Mouth. Following passage through the simulated mouth stage, spinach/emulsion mixtures containing different oil types behaved fairly similarly. The mean particle size of all emulsions increased, with the smallest change being observed in the samples containing corn oil (d_{32} ≈ 1.25 μm) and the largest changes observed in the samples containing fish oil (d_{32} ≈ 2.71 μm) (Fig. 2A). These results suggest that some flocculation of the droplets occurred within the simulated mouth phase, which was confirmed by optical microscopy (Fig. 4). Lipid droplet

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flocculation can be attributed to depletion or bridging effects caused by mucin molecules in the simulated saliva.[33] In the absence of the excipient emulsion, no significant change in the mean particle diameter of the spinach puree was observed prior to and following passage through the simulated mouth phase.

Following incubation in the mouth stage, the magnitude of the negative charge in the mixed systems (ζ = -6.8 to -15.4 mV) was less than that in the initial samples (ζ = -11.2 to -22.4 mV), but still followed a similar trend to that observed in the initial samples i.e., MCTs < LCTs (Fig. 2B). This decrease in surface potential can be attributed to electrostatic screening effects by mineral ions in the simulated saliva, as well as binding effects associated with the adsorption of mucin to the lipid droplet surfaces.[34]

3.2.3. Stomach. Following passage through the simulated stomach phase, a further significant increase in the mean particle diameter was observed in the presence of excipient emulsions (Fig. 2A) (d_{32} ≈ 7 μm). In addition, an appreciable increase in the population of large particles (d_{32} ≈ 10 μm) present in the particle size distributions was observed (Fig. 3), as well as extensive droplet flocculation in the optical microscopy images (Fig. 4). The nature of the lipid used did not have a major impact on the physical and structural properties of the spinach/emulsion mixtures in the simulated stomach stage. The increased aggregation of the lipid droplets in the stomach can be attributed to the following reasons: (1) sodium caseinate was hydrolysed by digestive enzymes (pepsin) in the gastric fluids; (2) the electrostatic repulsion between the protein-coated droplets was reduced; and, (3) mucin from the simulated saliva fluids induced depletion and bridging flocculation.[35]

In the absence of the excipient emulsion, no significant difference in the spinach puree mean particle diameter prior to and following passage through the simulated gastric phase was observed. In addition, no significant difference in the mean particle diameter between the controls (no oil) and spinach/emulsion mixtures was observed following the simulated gastric phase. The relatively large mean particle size of the two mixed systems, as measured by light scattering, could be due to the presence of different kinds of particles, as observed in the optical microscopy images (Fig. 4). In the presence of the excipient emulsion, there was no appreciable presence of plant fragments observed in the microscopy images (Fig. 4). However, an extensive coalescence of the lipid droplets was observed. In the absence of the excipient emulsions, we detected the presence of some plant tissue fragments in the optical microscopy images (Fig. 4). Therefore, we hypothesized that the large particles detected within the spinach/emulsion or the spinach/buffer solution system represent aggregated lipid droplets and fragments of spinach tissues, respectively. This observation indicates that the presence of the excipient emulsion somehow functioned to promote the disruption of the spinach fragments during the simulated stomach phase.

The ζ-potential of the particles in all of the samples were fairly similar and close to zero (ζ = -2.7 to 2.6 mV) following exposure to the simulated gastric fluids (Fig.2B). There are two potential reasons that could explain this phenomenon: (1) the protein (CS) coated on the lipid droplets surface may have been partially digested by proteases in the gastric fluids, or (2) the protein molecules were positively charged at a low pH value below their isoelectric point (pI ≈ 4.5), allowing the anionic molecules (mucin from the simulated mouth stage) to be absorbed to the cationic droplet surfaces, resulting in charge neutralization.

Interestingly, the optical microscopy images (Fig. 4) of the spinach/emulsion mixtures demonstrated that the lipid droplets within the simulated stomach phase were a deeper green than in the mouth stage. This suggests that hydrophobic molecules (such as chlorophyll and carotenoids) are released from plant tissues and transferred into lipid droplets. The carotenoids present in spinach are primarily present in the lipoprotein complexes of photosystems II and I in the inner chloroplast membranes.[36] In the simulated gastric phase, pepsin has been reported to hydrolyze lipoprotein complexes and enhance carotenoid release from chloroplasts.[37] Proteins coated on the lipid droplet surfaces would be partially hydrolyzed by pepsin in the gastric fluids, thereby facilitating the transfer of hydrophobic colorants into the oil phase. In addition, as the ζ-potential of the particles decreased close to zero in the gastric fluids (Fig. 2B), the electrostatic repulsion between them would not have been large enough to overcome the attractive forces between them. This could facilitate the adherence of the oil droplets to the plant tissues, promoting the transfer of carotenoids from plants tissues into the oil droplets.[38] Hence, the stomach stage represents the key process for carotenoid release from plant tissues and transfer to the oil phase. During this process, the oil type would be expected to affect the transfer efficiency of carotenoids from the food matrix into the oil phase. For example, it has been demonstrated that lycopene solubility is higher in MCT than in LCT.[39]

3.2.4. Small intestine. Following exposure to the simulated small intestinal stage, we observed a significant decrease in the mean particle size (d_{32}) of all samples. Using light scattering, we demonstrated that most of these contained only relatively small particles (d_{32} ≤ 1 μm), with the exception of the sample containing fish oil (Fig. 2A). The decrease in the mean particle size was found to be primarily due to the digestion of the lipid droplets by lipase. This effect was confirmed by optical microscopy (Fig. 4), where we observed no lipid droplets following passage of the samples through the small intestinal stage, with the exception of the sample containing fish oil. In addition, the particle size distribution demonstrated that all samples exhibited a bimodal distribution (Fig. 3). The bimodal distribution of these samples could be due to the presence of different types of colloidal particles within the digesta, such as mixed micelles, insoluble calcium soaps, or undigested particles originating from the small intestinal fluids. We note that the mean particle size of the particles within the small intestinal digesta was significantly different, ranging from 540 to 1390 nm (Fig. 2A). This result suggests that the lipid type significantly affected the nature of the colloidal particles generated after the small intestine phase. However, the particle size distribution of the fish oil sample demonstrated that the majority of particles were approximately 4 μm, with a small number of particles around 200 nm (Fig. 3E). The relatively large particles (4 μm) within the fish oil samples could represent non-digested lipid droplets, as confirmed by the pH-stat measurements (described later) and the microscopy images (Fig. 4).

In the absence of the excipient emulsions, the mean particle diameter significantly decreased, as measured by light scattering (Fig. 2A). In addition, only plant tissue fragments could be observed in the optical microscopy images (Fig. 4) following exposure to the small intestinal phase. Taken together, these phenomena suggest that the spinach

fragments were primarily digested by mechanical, chemical, or enzymatic treatments during the simulated GIT.

In addition, all of the samples had higher negative charges following passage through the simulated small intestinal phase (ζ = -8.4 to -22.0 mV) compared to the stomach phase (ζ =-2.7 to 2.6 mV) (Fig. 2B). This relatively high negative charge can be attributed to the various types of anionic colloidal particles present in the simulated small intestine phase, including bile salts, phospholipids, free fatty acids, peptides, and plant tissue fragments. The samples containing excipient emulsions (ζ = -9.5 to -22.0 mV) had a higher negative charge compared to the control samples (ζ = -8.4 mV). This could be due to the anionic free fatty acids released during hydrolysis of the triglycerides in the small intestine.

The nature of the oil phase type had an appreciable effect on the ζ-potential of the mixtures following passage through the simulated small intestine stage (Fig. 2B). LCFAs samples (olive oil and corn oil, ζ = -13.5 and -22.0 mV) had a higher negative charge than MCFAs samples (MCT oil and coconut oil, ζ = -9.5 to -11.2 mV), with the exception of fish oil (ζ = -11.0 mV). This phenomenon could be attributed to LCFAs more readily accumulating at the particle surfaces compared to MCFAs, thereby resulting in a lower negative charge.[19] However, due to the low digestibility of the fish oil, we observed less free fatty acids generated from the sample containing fish oil, resulting in less negative charges absorbed to the droplet surfaces. In addition, the highly bent nature of the PUFA chain may inhibit their adsorption to the droplet surfaces.

Following passage through the entire simulated GIT, our results suggested that the physical and structural properties of the spinach/emulsion mixtures were mainly affected by digestion conditions (enzymes, mucin, bile salts, pH, and salt concentration), rather than by oil type. However, the type of oil in the excipient emulsions did significantly affect lipid digestion and the nature of the mixed micelles formed.

3.3. Impact of oil type on lipid digestion

In this section, we studied the effect of oil type in the excipient emulsions on lipid digestibility using an automatic titration (pH-stat) method.[40] The amount of alkaline solution (0.25 M NaOH) titrated into the samples to maintain a constant pH (7.0) during the small intestine phase was measured. These data were then used to calculate the fraction of free fatty acids released from the lipid phase. Prior to carrying out these calculations, the volume of NaOH consumption in control samples (no oil) was subtracted from the volume in the samples containing lipids to focus on lipid hydrolysis only.

Both the initial digestion rate and the final digestibility of the oils depended on oil type, decreasing in the following order: coconut oil ≈ MCT oil > corn oil ≈ olive oil > fish oil (Fig. 5). This result indicated a faster initial digestion rate and greater final digestibility of lipids containing a high proportion of MCFAs (coconut and MCT oils) compared to lipids containing a high proportion of LCFAs (corn, olive and fish oils). This effect can be attributed to the fact that MCFAs have a higher affinity for water and therefore leave the surface of lipid droplets more readily than LCFAs.[41] LCFAs tend to accumulate at the lipid droplet surfaces, thereby reducing the ability of lipase to get to the undigested triacylglycerols in the interior of the lipid droplets. Consequently, after two hours digestion, the final percentage of FFAs released was around 90% for the MCFA-rich emulsions but only 80% for the LCFA-rich emulsions.

Interestingly, the fish oil emulsions exhibited an extremely low digestion rate and final digestibility (≈ 50%) in comparison to the other types of LCT oil (corn and olive oils, > 80%). This result could be attributed to the relatively high amounts of long chain PUFAs present in fish oil, including DHA and EPA. It has been reported previously that the methyl group is closer to the glycerol backbone due to the bending of the PUFA chains. This could lead to steric hindrance effects and protect the ester bond from lipolysis.[42] In addition, PUFAs have been proposed to possess a higher surface activity than MUFAs, enabling them to stay longer at the interface, thereby competing with lipase, eventually leading to a slower digestion rate.[40] The incomplete digestion of the fish oil after the small intestine phase was also seen in the optical microscopy images (undigested droplets) and appearance of the samples after ultracentrifugation (oil layer at top). This phenomenon has also been reported by other researchers where they have described that triacylglycerols containing PUFAs were hydrolyzed more slowly by porcine pancreatic lipase compared to those containing MUFAs.[43]

In summary, these results suggest that both the chain length and degree of unsaturation of the lipids have a major effect on the digestive profile of the emulsions. These differences could further affect the formation of mixed micelles, and ultimately impact carotenoid bioaccessibility.

3.4. Impact of oil type on *in vitro* carotenoid bioaccessibility

In this section, we quantified the effect of oil type in excipient emulsions on carotenoid bioaccessibility in spinach by measuring the carotenoid concentration in both the mixed micelle fraction and total digesta (the HPLC profile can be seen in supplementary Fig. 1).

Lutein bioaccessibility from the spinach puree depended strongly on the nature of oil phase used in the excipient emulsions: MCT (67.2%), coconut oil (46.5%), corn oil (23.0%), olive oil (14.2%), fish oil (18.7%), and control (20.9%) (Fig. 6A). These results show that MCT-rich excipient emulsions (coconut and MCT oils) appreciably enhanced lutein bioaccessibility compared to the control, but LCT-rich emulsions (corn, olive or fish oils) did not.

The bioaccessibility of β-carotene also depended on the oil type used for the formation of the excipient emulsions: corn oil (28.8%) > olive oil (23.0%) > MCT oil (14.1%) = coconut oil (14.0%) = control (6.8%) > fish oil (not detected) (Fig. 6A). These results suggest that excipient emulsions mainly containing long chain MUFAs (corn or olive oils) exhibited an increased ability to enhance β-carotene bioaccessibility than those mainly containing medium chain SFAs (MCT or coconut oil) or long chain PUFAs (fish oil). In addition, emulsions produced by lipids that were primarily comprised of medium chain SFAs and long chain PUFAs were not found to enhance β-carotene bioaccessibility from spinach.

These results suggest a significant impact of oil type (chain length and the degree of unsaturation) on the ability of excipient emulsions to enhance carotenoid bioaccessibility. In addition, carotenoid bioaccessibility from spinach/emulsion mixtures seems to be highly affected by the polarity of the carotenoids. Therefore,

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the correlation between carotenoid bioaccessibility, lipid composition, and carotenoid polarity was investigated further.

3.4.1. Impact of the chain length of the fatty acids on carotenoids bioaccessibility. The nature of the mixed micelles formed in the small intestine has been demonstrated to be one of the most important factors affecting carotenoid bioaccessibility.[24] Therefore, we measured the dimensions of the mixed micelles in the different samples following simulated GIT digestion. The mixed micelles formed from MCFA-rich lipid digestion products (MCT and coconut oils) had diameters around 127 to 217 nm. On the other hand, the mixed micelles formed from the LCFA-rich lipid digestion products (corn, olive and fish oils) had diameters around 239 to 461 nm (Fig. 7). These results suggest that MCFAs form smaller mixed micelles than LCFAs, as would be expected. In addition, the excipient emulsions produced from MCFA-rich lipids had a higher lutein bioaccessibility than those produced from LCFA-rich lipids (Fig. 6A). Interestingly, there therefore appears to be an inverse relationship between lutein bioaccessibility and mixed micelle size. Nevertheless, it should be noted that many of the mixed micelles in the digesta may have been too small to characterize using the light scattering method used.

The most probably reason to explain this effect is that lutein, which possess two polar groups, is more likely located at the micelle surface.[44] Because there is a fixed amount of mixed micelles present, a reduction in their dimensions will increase their specific surface area. Therefore, the smaller micelle size may result in an increased amount of lutein incorporated into the mixed micelles, thereby facilitating lutein micellization. On the contrary, the hydrophobic domains in the mixed micelles formed by MCT are not large enough to incorporate the longer, nonpolar β-carotene molecules, and result in a relatively low bioaccessibility of this type of carotenoid. This result is in agreement with a previous study that demonstrated the poor ability of MCT to enhance the bioaccessibility of highly hydrophobic components.[19] However, one of the most interesting findings of the current study was that MCT was efficacious at enhancing lutein bioaccessibility, which is more polar than β-carotene.

β-carotene, which is long hydrophobic molecule, was reported to be more likely to be located deep inside the hydrophobic interiors of lipid droplets and mixed micelles.[44] As discussed earlier, the mixed micelles formed from LCT digestion products (corn, olive, and fish oils) were larger than those formed from MCT digestion products (MCT oil and coconut oil) (Fig. 7). The larger hydrophobic regions in the bigger mixed micelles may accommodate longer nonpolar molecules, which is beneficial for the micellization of β-carotene. In addition, the mixed micelles formed by LCFAs possessed a more hydrophobic core, which exhibited a better solubilization for hydrophobic carotenoids compared to those formed by MCFAs.[45] This finding is in good agreement with previous studies that have demonstrated LCT were more effective than MCT at enhancing β-carotene bioaccessibility when co-digested with mangoes[21] or carrots[20].

We note that the mixed micelles mentioned here do not actually only contain micelles, but also contain vesicles (d > 100 nm), which also have hydrophobic domains for the solubilization of lipophilic bioactives.[46] Therefore , the mean particle size (d_{32}) of the mixed micelles we measured was larger than the micelle size which has been reported in previous publications to be within the range of 4 to 60 nm.[47] In addition, Phan et al.[48] utilized a combination of small-angle x-ray scattering (SAXS) and neutron scattering to study the effect of lipid type on the colloidal structures of mixed micelles. This study demonstrated that mixed micelle size tends to increase with increasing fatty acid chain length, which is in good agreement with our results.

However, the excipient emulsions formed from fish oil (rich in long chain PUFAs) behaved differently from those formed from corn and olive oils (rich in long chain MUFAs), as well as exhibiting a much lower β-carotene bioaccessibility. We hypothesize that differences in the unsaturation degree of the fatty acids could represent another key factor that plays a role in carotenoid bioaccessibility.

3.4.2. Impact of the unsaturation degree of the fatty acids on carotenoids bioaccessibility. All five types of lipids were classified according to their degree of unsaturation. Results demonstrated that lutein bioaccessibility from spinach was higher when co-digested with the excipient emulsions containing lipids rich (> 90%) in SFAs (MCT and coconut oils) compared to lipids rich (> 80%) in USFAs (corn, olive and fish oils) (Fig. 6A). This effect could also be related to the mixed micelle size generated in the small intestine. Gleize et al.[49] demonstrated that the size of the mixed micelles produced in *in vitro* digestion of lipids rich in SFAs were smaller than those produced from lipids rich in USFAs, which is consistent with our results (Fig. 7). As discussed earlier, the smaller size of the mixed micelles may function to positively impact lutein bioaccessibility, while negatively impacting β-carotene bioaccessibility.

As for β-carotene, the excipient emulsions generated by lipids that primarily (> 65%) contained MUFAs (corn and olive oils) exhibited an increased ability at enhancing the bioaccessibility of β-carotene compared to lipids primarily containing SFAs (MCT and coconut oils) and PUFAs (fish oil) (Fig. 6A). These results suggested a positive correlation between the size of the mixed micelle (MUFAs > PUFAs > SFAs) and the bioaccessibility of β-carotene (MUFAs > PUFAs > SFAs). These results lend further support to the above notion that larger hydrophobic regions accommodate longer nonpolar molecules, and is more beneficial for the micellization of β-carotene. In addition, the MUFAs present in corn and olive oils could be more lipophilic in comparison to the PUFAs from fish oil, leading to a micelle phase with increased solubility for highly lipophilic carotenoids such as β-carotene.[42] In addition, the considerable amount of PUFAs (C20:5 and C22:6) within the fish oil led to a lower lipid digestion extent compared to MUFAs (corn and olive oils) (Fig. 5), resulting in the generation of less lipid digestion products for micelle formation. Consequently, the mixed micelles formed in the samples containing fish oil could be insufficient to incorporate the long nonpolar β-carotene molecules. Nagao et al.[12] also demonstrated that MUFAs are more effective at enhancing β-carotene bioaccessibility than PUFAs. This was shown to be due to the fact that PUFAs are more prone to promoting carotenoid oxidation compared to MUFAs.

Interestingly, when the spinach was co-digested with emulsions containing fish oil, we observed no significant effect on lutein bioaccessibility, but did observe a significant decrease in β-carotene bioaccessibility compared to the control (no oil) (Fig. 6A). This effect could be related to the hydrophobicity of carotenoids. As reported previously, lutein is preferentially localized at the oil/aqueous phase interface and can be transferred from plant tissues to the micelle phase without the requirement of oil droplets as an intermediate reservoir.[50] However, β-carotene needs to be first solubilized into the oil phase, which then has to be digested, before the carotenoids are transferred to the mixed micelles.[44] Therefore, the low digestion extent of fish oil could lead to a higher-level of entrapment of β-carotene in the oil phase compared to lutein, and result in less β-carotene being transferred from the oil phase to the mixed micelles. This result clearly suggests that carotenoid hydrophobicity affects their transfer from the oil phase into the mixed micelles.

These findings are in agreement with previously published work showing that lutein is more bioaccessible in the presence of lipids rich in SFAs (coconut, peanut or butter oils) than in the presence of the lipids rich in PUFAs (rapeseed or sunflower oils)[22, 49, 51], while β-carotene is more bioaccessible in the presence of lipids rich in USFAs (olive, soybean, and sunflower oils) than SFAs (coconut and butter oils)[12, 23, 52]. In addition, human trials have also reported higher β-carotene absorption when supplemented with lipids rich in MUFAs (canola oil) than lipids rich in SFAs (butter).[53]

It should be noted that there do exist some contradictory results in other publications. For example, Nidhi et al.[54] reported that olive oil (rich in MUFAs) exhibited a higher micellization efficiency for lutein compared to coconut oil (rich in SFAs). Research carried out by Hu et al.[55] demonstrated that the postprandial appearance of β-carotene in the triacylglycerol-rich lipoprotein fraction in women was increased when co-digested with lipids rich in SFAs (beef-tallow) compared to lipids rich in PUFAs (sunflower oil). These differences observed among these studies could be attributed to the fact that there remain numerous other factors that affect the efficiency of the transfer of carotenoids into the oil and mixed micelle phases, including the nature of the food matrix and the overall oil content. For example, it has been reported previously that saturated and unsaturated fats differentially affect the micellization of the same carotenoids at different oil content[56] or in different food matrixes[57]. All of these factors should be taken into consideration when evaluating the effect of oil type within the excipient emulsions on carotenoid bioaccessibility.

In summary, these results suggest a direct relationship among the polarity of the carotenoids, lipid type, and the bioaccessibility of carotenoids from spinach. Excipient emulsions containing certain types of lipids are more effective at increasing lutein or β-carotene bioaccessibility from spinach than others. However, excipient emulsions produced using a single type of oil does not seem effective at simultaneously enhancing the bioaccessibility of both lutein and β-carotene. Therefore, we hypothesized that excipient emulsions could be produced using a mixed oil phase that would be effective at increasing the bioaccessibility of both types of carotenoids.

3.5. Impact of mixed oil phase on carotenoids bioaccessibility

To simultaneously improve the bioaccessibility of both types of carotenoids found in spinach, we created a mixed oil phase using the best oil type for each carotenoid identified in the previous studies. MCT was selected for its ability to increase lutein bioaccessibility, while corn oil was selected for its ability to increase β-carotene bioaccessibility. Excipient emulsions were then formed using an oil phase that contained a 1 : 1 (w/w) mixture of these two oils. The emulsion was then co-digested with the spinach using the simulated GIT model, and carotenoid bioaccessibility was studied. The results showed that the excipient emulsion containing the mixed oil phase simultaneously significantly increased the bioaccessibility of lutein (from 20.9% to 41.9%) and β-carotene (from 6.8% to 25.0%) from spinach compared to the control (no oil) (Fig. 6B).

MCT is typically used as a flavour carrier or to produce beverages that provide instant energy to the human body.[38, 58] However, it's use for human consumption is limited by its lack of essential fatty acids and poor ability to enhance the bioaccessibility of highly hydrophobic components. Our results show that using a mixture of MCT and LCT to form excipient emulsion is beneficial for the simultaneous enhancement of the bioaccessibility of two different hydrophobic nutraceuticals (lutein and β-carotene) found in an important plant-based food (spinach). These results could provide a promising mechanism for the application of MCT in the food industry, and for the enhancement of the bioavailability of different lipophilic components.

In addition, previous studies have demonstrated that mixtures of medium and long chain triacylglycerols (MLCTs) can function to reduce body weight gain and body fat deposition following a long duration of consumption (6 weeks).[59] Moreover, utilization of the MCT and LCT mixture as the oil phase provides greater possibilities for the preparation of excipient emulsions using low-energy methods that require no specialized equipment and is simpler to implement in comparison to the high-pressure method. For instance, it has been demonstrated that it is not possible to form stable nanoemulsions using LCT alone with low-energy methods.[60] However, they can be generated by using mixtures of LCT and MCT as the oil phase.[61] In summary, mixed oil phases can be used to design excipient foods that optimize the bioaccessibility of compounds with different polarities and also have numerous other potential health benefits.

4. Conclusions

Excipient emulsions produced using a lipid phase primarily containing medium chain SFAs exhibited the highest lutein bioaccessibility, while those produced using long chain MUFAs exhibited the highest β-carotene bioaccessibility. These results suggest that the lipid type used to produce excipient emulsions, as well as the polarity of the carotenoids, have a major impact on bioaccessibility. Specifically, the dimensions and solubilization capacity of the mixed micelles formed after digestion were found to be dependent on the nature of the lipids used (chain length and degree of unsaturation). Meanwhile, the solubility of carotenoids within mixed micelles depended on carotenoid hydrophobicity.

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These two effects were found to jointly determine carotenoid bioaccessibility. Finally, we note that a mixed oil phase containing long chain MUFAs (corn oil) and medium chain SFAs (MCT oil) can be utilized to design novel excipient emulsions that enhance the bioaccessibility of different kinds of hydrophobic nutraceuticals from plant-based foods.

Overall, this study has provided the field with valuable information regarding the design of efficacious excipient foods to enhance the bioaccessibility of hydrophobic nutraceuticals with different polarities from natural sources. However, in future studies, it will be necessary to apply more powerful techniques to further study the relationship between the fatty acid composition of digestible lipids, the nature of the mixed micelles formed, and the bioaccessibility of the bioactive components. In addition, the findings of the current study should be further validated using in vivo studies.

Conflicts of interest

There are no conflicts to declare.

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Table 1 Fatty acid composition of selected oils. Medium chain fatty acids (MCFAs) represent fatty acids with C6-C12 carbon chain length; The long chain fatty acids (LCFAs) represent fatty acids with C14-C24 carbon chain length; SFAs represents the saturated fatty acids; MUFAs represents the monounsaturated fatty acids; PUFAs represents polyunsaturated fatty acids.

Fatty acid	MCT oil[62]	Coconut oil[63]	Corn oil[64]	Olive oil[64]	Fish oil[65]
C8:0	74.4	5.8			
C10:0	25.6	4.8			
C12:0		49.1	0.0	0.0	
C14:0		21.8	0.0	0.0	3.2
C16:0		8.4	6.5	11.6	7.8
C16:1			0.6	1.0	3.9
C16:2					0.7
C18:0		2.8	1.4	3.1	2.6
C18:1			65.6	75.0	6.1
C18:2		6.1	25.2	7.8	0.8
C18:3		1.2	0.1	0.6	0.5
C18:4					1.9
C20:0			0.1	0.3	0.6
C20:1			0.1	0.0	2.0
C20:2					0.3
C20:3					<0.2
C22:0			0.0	0.1	0.4
C20:4					1.5
C20:5					27.0
C21:5					1.5
C22:1			0.1	0.0	2.5
C22:5					4.8
C22:6					24.0
C24:0			0.1	0.5	
C24:1					<0.2
MCFAs	100	59.7	0	0	0
LCFAs	0	40.3	100	100	100
SFAs	100	92.7	8.1	15.6	16.5
MUFAs	0	0	66.4	76	14.7
PUFAs	0	7.3	25.3	8.4	61.3

Figure captions

Fig. 1 (A) Surface-weighted mean particle diameter (d_{32}) and polydispersity index (PDI) of the excipient emulsions generated by different oil phases: MCT oil, coconut oil, corn oil, olive oil, and fish oil; (B) ζ-potential of the excipient emulsions generated by different oil phases: MCT oil, coconut oil, corn oil, olive oil and fish oil. Samples designated with different capital letters (A, B, C, D) were significantly different (Duncan, $p < 0.05$).

Fig. 2 Effect of lipid type on mean particle diameter (d_{32}) (A) and electrical characteristics (ζ-potential) (B) of the lipid droplets isolated from spinach pieces following exposure to different GIT regions. Samples designated with different capital letters (A, B, C, D) were significantly different (Duncan, $p < 0.05$) when compared between different GIT regions (the same sample). Samples designated with different lower-case letters (a, b, c) were significantly different (Duncan, $p < 0.05$) when compared between the different samples (the same GIT region).

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Fig. 3 Particle size distribution of spinach/emulsion mixtures with different lipid types following exposure to different GIT regions: (A) MCT oil, (B) coconut oil, (C) corn oil, (D) olive oil, (E) fish oil; (F) particle size distribution of spinach/buffer solution mixtures following exposure to different GIT regions.

Fig. 4 Optical microscopy images of spinach/buffer solution mixtures or spinach/emulsion mixtures with different oil following exposure to different regions of a simulated GIT.

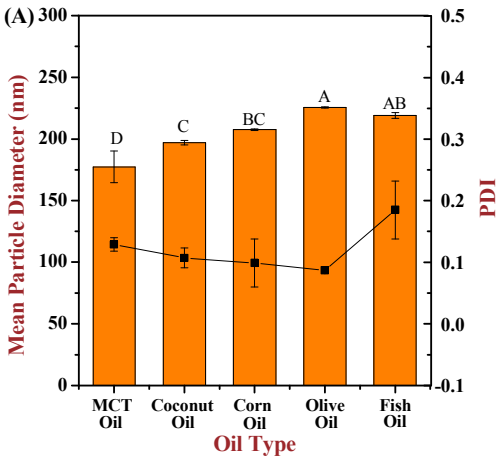
Fig. 5 Percentage of free fatty acids (FFAs) released from spinach/emulsion mixtures with different lipid types over time during the simulated small intestine stage as measured using a pH-stat method.

Fig. 6 (A) Carotenoid bioaccessibility from samples initially containing spinach puree and either buffer solutions or excipient emulsions with different lipid types: MCT oil, coconut oil, corn oil, olive oil, fish oil; (B) effect of the mixed oil phase (MCT oil : corn oil= 1 : 1, w/w) in excipient emulsion on carotenoid bioaccessibility from spinach. Samples designated with different capital letters (A, B, C) were significantly different (Duncan, $p < 0.05$) when compared with the same carotenoid.

Fig. 7 The mean particle size (d_{32}) of the middle "micelle" phase collected after passing through simulated GIT digestion and centrifugation of the samples initially containing spinach puree and either buffer solutions or excipient emulsions with different lipid types: MCT oil, coconut oil, corn oil, olive oil, fish oil. Samples designated with different capital letters (A, B, C) were significantly different (Duncan, $p < 0.05$).

Fig. 8 The potential mechanisms of the different effects of lipid type in excipient emulsions on the bioaccessibility of lutein and β-carotene from spinach.

Fig. 1



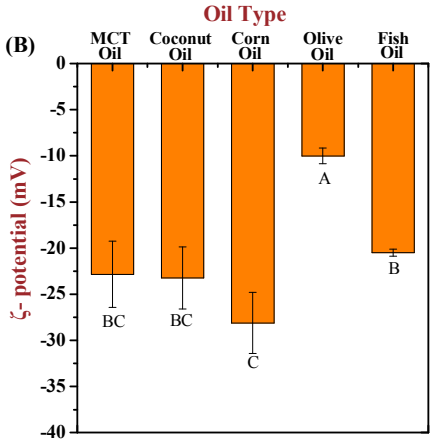
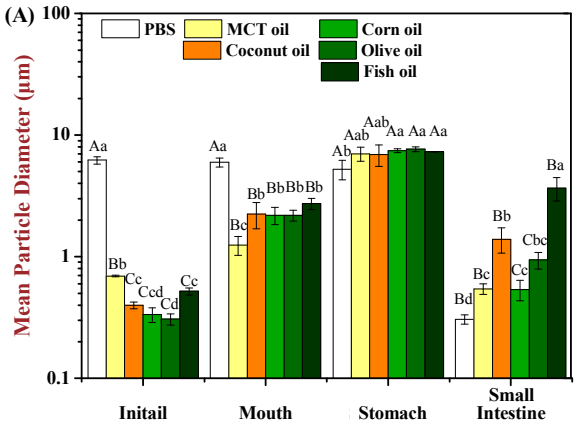


Fig. 2



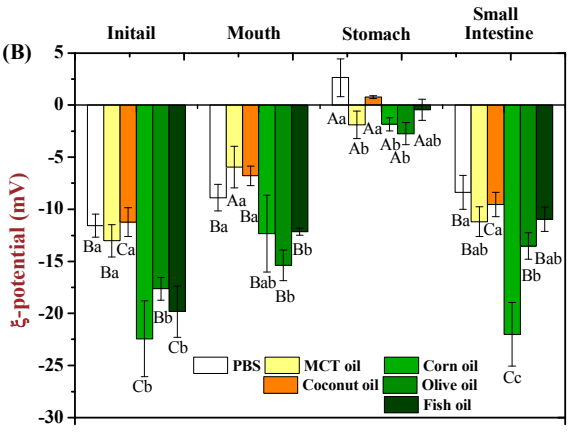


Fig. 3

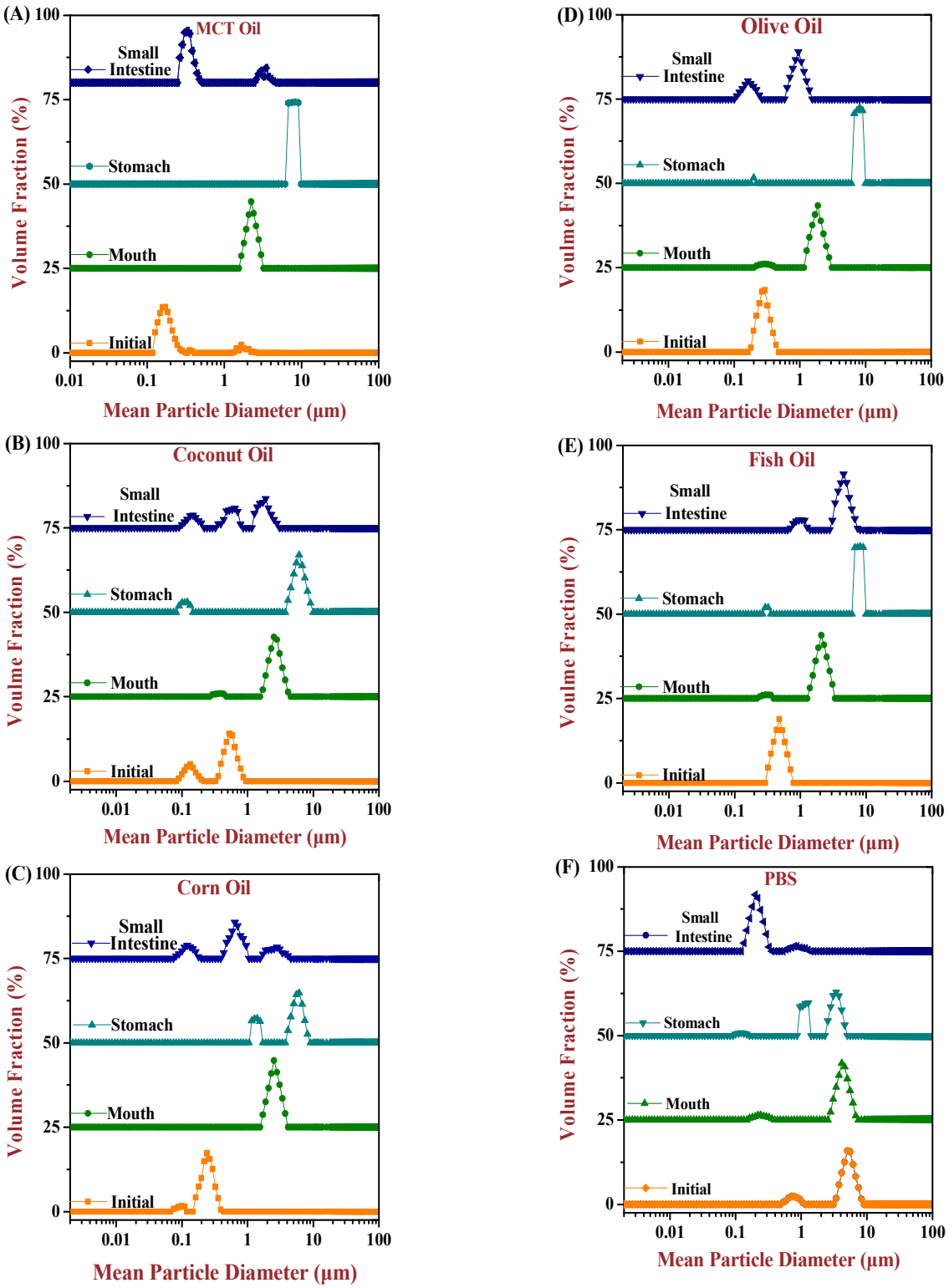


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Fig. 4

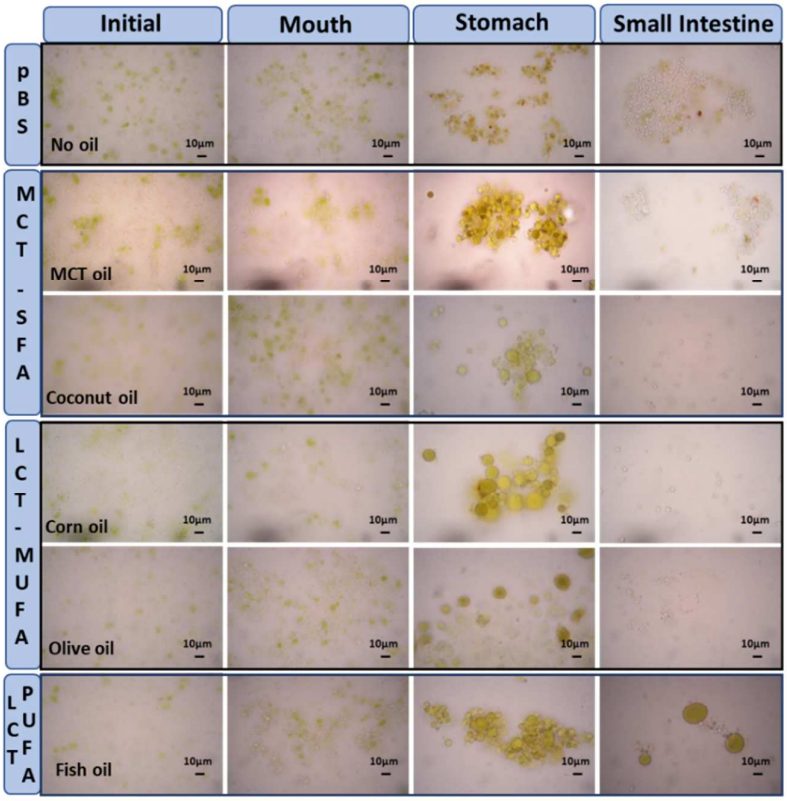

Initial
Mouth
Stomach
Small Intestine
PBS
No oil
MCT-SFA
MCT oil
Coconut oil
LCT-MUFA
Corn oil
Olive oil
LCT PUFA
Fish oil
10μm

Fig.5

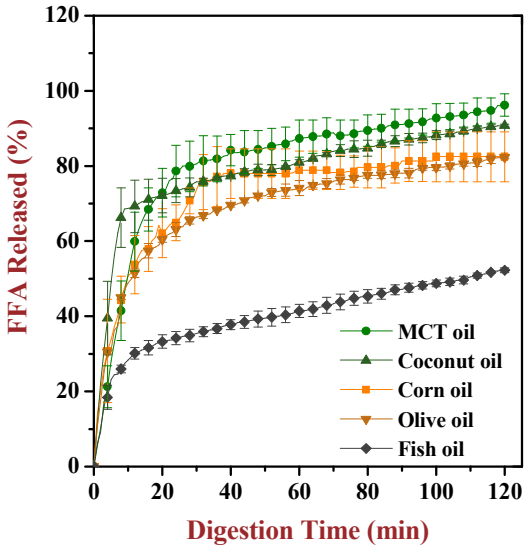
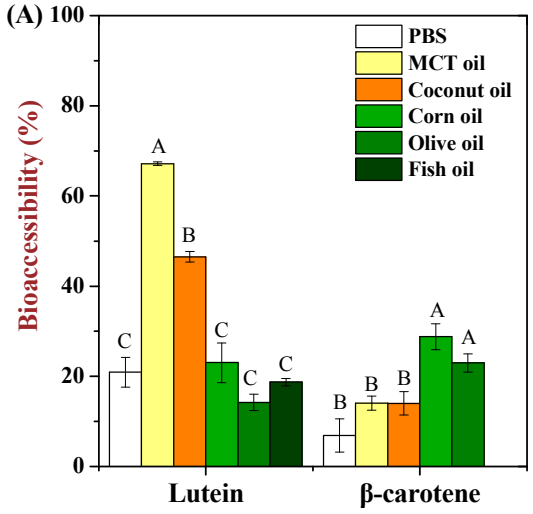

FFA Released (%)
Digestion Time (min)
0
20
40
60
80
100
120
MCT oil
Coconut oil
Corn oil
Olive oil
Fish oil

Fig. 6



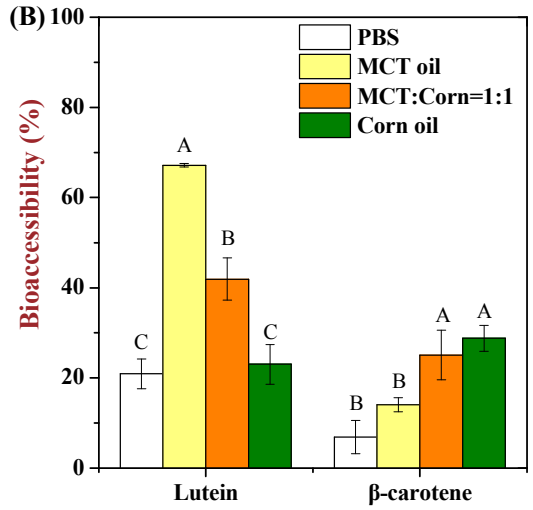


Fig. 7

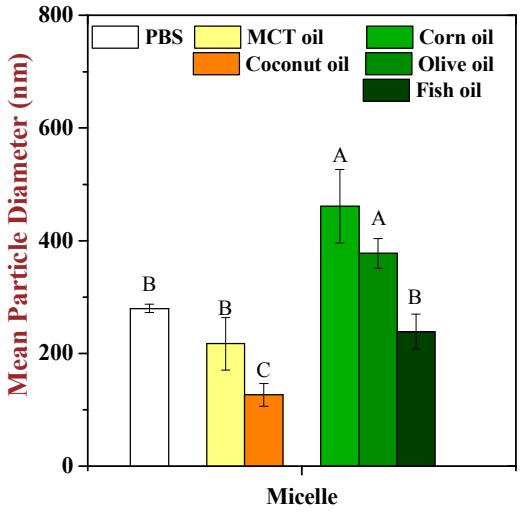


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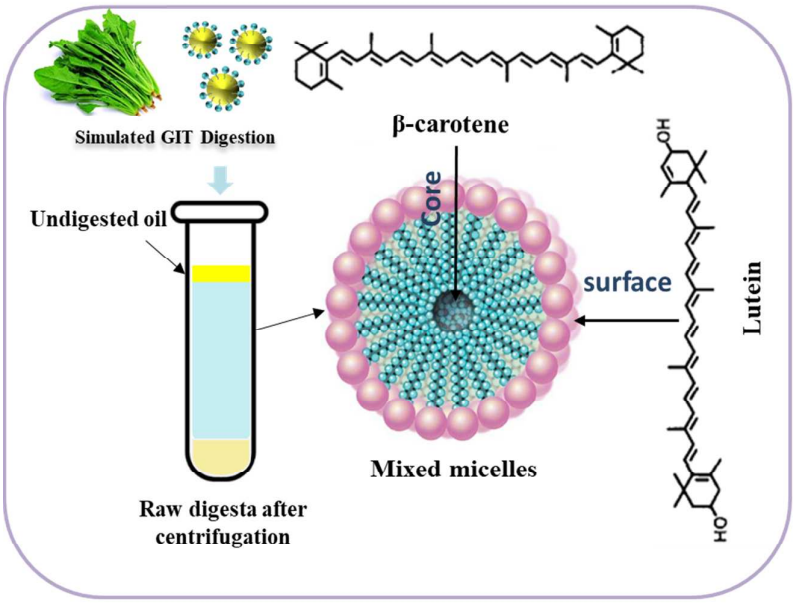
Fig. 8



Lipid rich in

MC-SFA:

- **Smaller size of the mixed micelles**

More beneficial for lutein

Lipid rich in

LC-MUFA

- **Formed a more hydrophobic core**
- **Larger size of the mixed micelles**

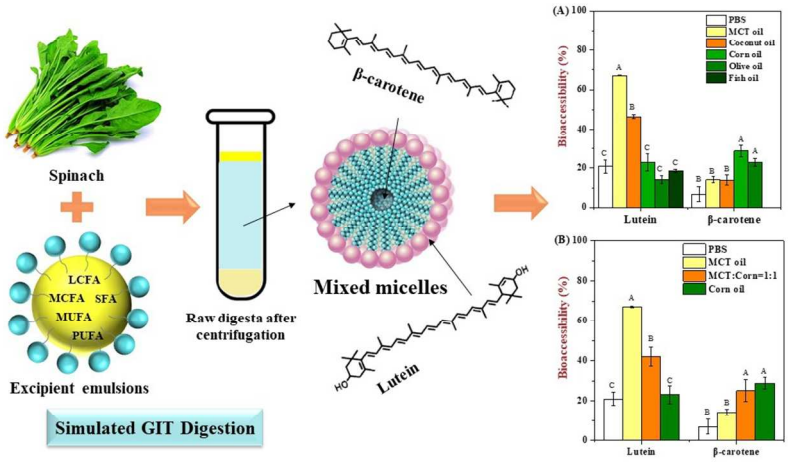
More beneficial for β-carotene

Lipid rich in

LC-PUFA

- **Low digestibility**
- **More prone to promoting carotenoids oxidation**

Low bioaccessbility of β-carotene



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