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Encapsulation of curcumin within oil-in-water emulsions prepared by premix membrane emulsification: Impact of droplet size and carrier oil type on physicochemical stability and *in vitro* bioaccessibility

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Abstract

Oil-in-water emulsions containing curcumin with different droplet size (small ≈ 0.5 μm, medium ≈ 0.8 μm, large ≈ 3.7 μm and premix ≈ 60 μm) were prepared through premix membrane emulsification using different carrier oils: tributyrin (short chain triglycerides, SCT), medium chain triglycerides (MCT) and corn oil (long chain triglycerides, LCT). An *in vitro* gastrointestinal model was used to evaluate the impact of oil and droplet size on lipid digestion and bioaccessibility. Lipid digestion and bioaccessibility decreased with the increase of droplet size for LCT-based emulsions, whereas there was no significant difference for small, medium and large emulsions in SCT and MCT-based emulsions. In addition, encapsulation efficiency played an important role in determining bioaccessibility. Bioaccessibility in MCT premix was significantly lower than that in other size MCT-based emulsions because of its low encapsulation efficiency. The bioaccessibility decreased in the order MCT > SCT > LCT in each size of emulsions.

Keywords: Curcumin; Emulsions; Digestion; Droplet size; Carrier oil; Bioaccessibility

1. Introduction

Curcumin, a plant-derived polyphenolic compound, is naturally present in the rhizomes of *Curcuma longa* (turmeric). Numerous studies have revealed that curcumin has various beneficial biological and pharmacological activities, such as antioxidant, anti-inflammatory and anticancer activities (Perrone et al., 2015; Jiang et al., 2020). Due to its potential health benefits, incorporation of curcumin into functional foods or dietary supplements is of great interest for both consumers and food industry. However, its low water-solubility makes challenging to incorporate curcumin into commercial foods, and results in low bioavailability within the gastrointestinal tract (GIT). As a result, a number of attempts have been made to improve the aqueous solubility and hence the bioaccessibility of curcumin through encapsulation of curcumin into delivery systems.

Emulsions-based delivery systems are particularly effective for encapsulating and delivering of lipophilic bioactive substances, such as curcumin (Chuacharoen & Sabliov, 2019; Iqbal et al., 2020; Sharma et al., 2021). Oil-in-water emulsions systems (O/W) contain relatively small emulsifier-coated lipid droplets dispersed within water, making them particularly suitable for using in liquid food products. Moreover, powders can be obtained from emulsions by commercial dehydration technologies such as spray drying (Rehman et al., 2021), therefore allowing them to be successfully incorporated into dried foods. The small oil droplets in emulsions can be quickly digested within the human GIT, thereby rapidly generating mixed micelles capable of solubilizing curcumin (Ahmed et al., 2012; Zou et al., 2015; Pinheiro et al., 2016). Then, this fraction of solubilized curcumin can be transported across the epithelial intestinal cells, where it is absorbed, and therefore can deliver its beneficial biological effects. However, it should be noted that the absorption of ingested curcumin by the human body is a complex process that depends on many factors, including the composition of emulsions, the nature of the original food matrix and the type of production process (Ahmed et al., 2012; Salvia-Trujillo et al., 2013; Pinheiro et al., 2016; Zheng et al., 2018). The design and fabrication

of delivery systems is a crucial step, in which the properties of emulsions, such as, droplet size, carrier oil type, and interfacial properties, have great impact on the release and uptake of encapsulated compounds (Salvia-Trujillo et al., 2013; Pinheiro et al., 2016; Shah et al., 2016). Some previous studies have suggested that the rate and extent of lipid digestion and bioaccessibility of encapsulated bioactive increased with the decrease of emulsions droplet size (Salvia-Trujillo et al., 2013; Li et al., 2017), while Ahmed et al. (2012) found that the lipid digestion was independent upon the emulsions initial droplet size. This difference could be explained by the different experimental conditions used: lipid concentrations, emulsifier type, and simulated fluid composition (Ahmed et al., 2012). In order to gain further insight on the influence of the emulsions droplet size, we focused on carrier oil type. Three carrier oils were selected that had different susceptibilities to lipase digestion and different molecular characteristics: tributyrin, medium chain triglycerides (MCT) and corn oil. Tributyrin was used as an example of short chain triglycerides (SCT), and corn oil is an example of long chain triglycerides (LCT).

Membrane emulsification is a promising technique to produce emulsions using mild mechanical forces (Vladisavljevic et al., 2004; Charcosset, 2009). It creates mono-dispersed emulsions with controlled droplet sizes and narrow droplet size distributions in comparison to conventional methods. Direct membrane emulsification and premix membrane emulsification are two types of emulsification process, while premix membrane emulsification shows several advantages compared to direct membrane emulsification: (i) the mean droplet size is smaller; (ii) the dispersed phase flux is higher; (iii) the process parameters are easier to control (Vladisavljevic et al., 2004). Therefore, premix membrane emulsification is particularly attractive to produce emulsions. Premix membrane emulsification with hydrophilic Shirasu Porous Glass (SPG) membranes with different pore size can produce different droplet size O/W emulsions or nanoemulsions (Alliod et al., 2018; Jiang & Charcosset, 2022).

The aim of the present work was to study the influence of emulsion droplet size for three different carrier oils on the lipid digestion, micelle formation and associated curcumin bioaccessibility under *in vitro* gastrointestinal conditions. For this purpose, emulsions with different droplet size for each oil were prepared by premix membrane emulsification using different pore size membranes. Moreover, the behavior during *in vitro* digestion was assessed in terms of droplet size and microstructure.

2. Materials and methods

2.1. Materials

Tween 80, Span 80, curcumin (≥99.5%), curcumin analytical standard (≥98.0%), dichloromethane, chloroform and acetic acid were obtained from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile of HPLC grade was purchased from Carlo Erba. Labrafac^{TM} lipophilic WL 1349 (MCT oil) was obtained by an esterification reaction between glycerol and caprylic/capric acids (C8-C10), and kindly provided by Gattefossé (Saint-Priest, France). Tributyrin (SCT oil) and corn oil (LCT oil), pepsin (from porcine gastric mucosa), pancreatin (from porcine pancreas) and bile extract (porcine) were obtained from Sigma-Aldrich. Ultrapure water was obtained from a Millipore Synergy Unit system. All other chemicals used in this work were of analytical grade and purchased from Sigma-Aldrich.

2.2. Viscosity measurements and curcumin solubility

Shear viscosity measurements were performed using a controlled stress rheometer (MCR 302 Rheometer, Anton Paar Gmbh, Graz, Austria), equipped with a cone-and-plate geometry (12.490 mm cone diameter, 0.978 cone angle, 49 μm gap). All measurements were carried out at shear rate ranging from 0.01 to 100 s^{-1}, with temperature controlled at 25.0 ± 0.1 °C.

The solubility of curcumin in each oil phases (SCT, MCT and LCT) and surfactants (Tween 80 and Span 80) was determined using a HPLC method. An excess amount of curcumin was added to 1 g of oil or surfactant, magnetically stirred at 700 rpm for 20 min at 80°C, and then left

overnight to reach equilibrium at room temperature. The equilibrated samples were centrifuged (centrifuge GT2R, Fisher Scientific) at 8 000 g for 10 min at room temperature. The supernatant was collected and then diluted with absolute ethanol to an appropriate concentration and analyzed with an Agilent 1200 HPLC system (Agilent Technologies Inc., USA) with UV detector (UV lamp and vis lamp), a Phenomenex C18 column (100 mm×4.6 mm, 2.6 μm) and Agilent ChemStation software. The mobile phase was composed of acetonitrile and 0.6% (v%) aqueous acetic acid at ratio of 40/60% (v%), the flow rate was 1 mL/min with a column temperature of 35°C, and an injection volume of 10 μL. Curcumin was detected at a wavelength of 425 nm. The calibration curve was established based on the relationship between the HPLC peak area (y) and curcumin concentration (x), which is $y = 63091.96 x - 14869.14$ ($R^2 = 0.9979$). Quantification of curcumin was performed using the calibration curve.

2.3. Preparation of curcumin emulsions

O/W emulsions with different carrier oils and droplet sizes containing curcumin were obtained by premix membrane emulsification. The surfactant systems and surfactant concentration used in this article were the optimal parameters selected from our previous research (Jiang & Charcosset, 2022). The aqueous phase surfactant solution was prepared by dispersing 2% w/w Tween 80 and 0.01% w/w sodium azide (as a preservative) into 85% w/w 5 mM phosphate buffer (pH 7.0) and stirred for at least 10 min to ensure complete dissolution. The oil phase was prepared by adding 0.1% w/w curcumin into 10% w/w SCT, MCT or LCT containing 3% w/w Span 80, then magnetically stirring for 20 min at 80°C to ensure curcumin fully dissolution. Coarse emulsions were obtained by mixing curcumin oil phase and aqueous phase, using a magnetic stirrer (RCT Basic, IKA, Germany) at 700 rpm for 10 min at room temperature. For some experiments, these coarse emulsions were used as premix, for other experiments they were further processed by SPG premix membrane emulsification to produce emulsions. Emulsions were obtained by passing the premix through a SPG tubular membrane (10 mm

diameter, 0.7 mm wall thickness), which was from SPG Technology Co., Ltd. (Japan). The experimental set-up consists of a computer-controlled high-pressure benchtop single cylinder pump BTSP 500-5 (Floxlab, Nanterre, France) and a membrane module (SPG Technology Co. Ltd., Miyazaki, Japan), which was equipped with the SPG tubular membrane. Emulsions with different droplet sizes were produced using 0.4 μm (small emulsions), 0.8 μm (medium emulsions) and 3.1 μm (large emulsions) membranes. All experiments were carried out with 100 mL premix. After preparation, all emulsions were stored at 4 °C.

2.4. Characterization of the curcumin-loaded emulsions

2.4.1. Measurements of droplet size

Droplet size of small and medium emulsions were measured using a Zetasizer Nano ZS (Malvern Instruments, UK). The droplet size was determined using dynamic light scattering (DLS), at a wavelength of 633 nm and scattering angle of 173° at 25°C. All samples were diluted with 5 mM phosphate buffer (pH 7.0) (the dilution factor was adjusted to obtain an attenuation factor between 5 and 7) before analysis to avoid multiple scattering effects. Droplet size of large and premixes emulsions were determined by laser diffraction (LD) particle size analysis with a Mastersizer 3000 (Malvern Instruments, UK). The technique is based on measurement of the angular variation in intensity of light scattered as a laser beam passes through a dispersed particulate sample. The Mie scattering theory was used to analyze angular scattering intensity data for calculating the size of the droplets responsible for creating the scattering pattern. The refractive index used for SCT, MCT and LCT emulsions were 1.435, 1.448 and 1.470, respectively. D [4,3] values representing volume mean diameters were obtained. All droplet size measurements were performed after emulsions were redispersed using a magnetic stirrer at 300 rpm for 1 min at room temperature.

2.4.2. Encapsulation efficiency

The encapsulation efficiency (EE) was determined according to the method described by Tan

et al. (Tan et al., 2016) with slight modification. Briefly, 1 mL curcumin-loaded emulsions and 5 mL dichloromethane were mixed by being centrifuged at 1000 g for 5 min at 25°C (centrifuge GT2R, Fisher Scientific). The subnatant was then collected immediately to determine non-encapsulated curcumin (free curcumin) concentration (μg/mL) according to the standard curve of curcumin solutions using a UV5 spectrophotometer (Mettler Toledo, Columbus Ohio, USA) at 420 nm.

For the quantification of the total amount of curcumin in emulsions, the samples were extracted by ethanol. Briefly, 1 mL emulsion was added to 5 mL ethanol and the mixture was centrifuged at 5000 g for 15 min at 25°C. Then, the supernatant was collected and diluted with ethanol to an appropriate concentration and analyzed by a UV5 spectrophotometer (Mettler Toledo, Columbus Ohio, USA) at 430 nm.

The EE of the curcumin-loaded emulsion was calculated by the following equation (1):

$$\text{EE (\%)} = \frac{Total\ amount\ of\ curcumin - Am\quad of\ free\ curcumin}{Total\ amount\ of\ curcumin} \times 100 \qquad \text{Equation (1)}$$

where the total amount of curcumin is the initial content of curcumin, and the amount of free curcumin is the curcumin amount that was not loaded in the emulsions.

2.5. *In vitro* digestion

Biological fate of the encapsulated curcumin after digestion was evaluated by *in vitro* simulated digestion assay. Due to the short residence time in the mouth, only gastrointestinal digestion was assessed. Before starting of experiment, all solutions were preheated to 37 °C. To mimic gastric digestion, simulated gastric fluid (SGF) was prepared according to the method described by Sarkar et al. (2009) by dissolving of 3.2 g of pepsin, 2 g of NaCl and 7 mL of HCl in 1 L of water and adjusting the pH to 2.0. Gastric digestion was initiated by adding 20 mL of emulsions or free curcumin solution (10 μg/mL) to an equal volume of SGF. The mixture was adjusted to pH 2.0 using 1 M NaOH and incubated at 37 °C for 2 h with continuous stirring at a speed of 100 rpm/min using a magnetic bar to mimic stomach conditions. After incubation for 2 h, SGF

digestion suspensions were subjected to intestinal digestion as described by Ahmed et al. (2012). 30 mL of sample was placed in a conical flask (100 mL) and the pH was adjusted to 7.0 using 1 M NaOH solution, then 1 mL of simulated intestinal fluid (110 mg/mL $CaCl_2$) (SIF) and 4 mL of bile extract (46.87 mg/mL) dissolved in 5 mM phosphate buffer were added. The resulted mixture was then adjusted to pH 7 again. Finally, 2.5 mL of freshly prepared pancreatin solution (24 mg/mL) dissolved in phosphate buffer was added, and an automatic titration unit (877 Titrino plus Metrohm, USA, Inc.) was used to monitor the pH and maintain it at pH 7.00 by titrating 0.5 M NaOH solution into the reaction vessel for 2 h at 37 °C. The volume of NaOH solution required to neutralize the free fatty acids (FFA) released was recorded and used to calculate the amount of FFA produced by lipolysis (assuming 2 FFA produced per 1 triacylglycerol molecule) by the equation (2):

$$\text{FFA (\%)} = 100 \times \frac{V_{NaOH} \times m_{NaOH} \times M_{oil}}{W_{oil} \times 2} \qquad \text{Equation (2)}$$

where FFA (%) is the percentage of released FFA; V_{NaOH} is the volume of NaOH required to titrate the FFA (mL); m_{NaOH} is the molarity of NaOH (mol/L); M_{oil} is the molecular weight of SCT (302 g/mol), MCT (503 g/mol) (Silva et al., 2019) and LCT (865.4 g/mol) (Bardone & Associazione Italiana di Ingegneria Chimica, 2012) (**Table 1**); and W_{oil} is the total weight of oil initially present (g).

2.6. Microstructure analysis

The microstructure changes of curcumin-emulsions that occurred within different stages of GIT model were recorded using confocal scanning laser microscopy (CSLM) with a 63× oil immersion objective lens (Zeiss LSM 880, Oberkochen, Germany). Before CSLM observation, 1 mL of samples were stained with 0.1 mL of Nile Red, a fat-soluble fluorescent dye that was previously dissolved in ethanol (0.1% (w/v)), to highlight the location of the lipid droplets. Then, 0.5 mL of stained sample was placed on a microscope dish (ibidi GmbH, Germany) and covered with a lid with locking feature. Microstructure images were acquired with an excitation

wavelength at 561 nm and emission wavelength at 567-648 nm. In addition, the microstructure of curcumin-emulsions after exposure to intestine fluids were observed using optical microscopy (Leica DM LM, Wetzlar, Germany) with a 10× objective lens. 20 μL of sample was placed on a microscope slide, covered by a coverslip, and then microstructure images were acquired using image analysis software (Leica LAS EZ).

2.7. Curcumin bioaccessibility

The bioaccessibility of curcumin was determined using an *in vitro* lipid digestion assay as described by Ahmed et al. (2012), with slight modification. After the small intestinal stage, final digesta, which went through all the digestion process, was centrifuged at 20 000 g for 30 min at room temperature (Optima^{TM} MAX-XP Ultracentrifuge, Beckman Coulter, USA), which resulted in samples that contained an opaque sediment phase at the bottom, a clear micelle phase in the middle in which curcumin was solubilized, and sometimes an oily or creamed phase at the top. Aliquots (2 ml) of the clear micelle phase were collected, vortexed mixed with 2 mL of chloroform to extract curcumin and then centrifuged at 2000 g for 10 min at 25°C (centrifuge GT2R, Fisher Scientific). The bottom chloroform layer was collected, and the extraction procedure was repeated 3 times with the top layer. The chloroform layers were mixed and analyzed using a UV5 spectrophotometer (Mettler Toledo, Columbus Ohio, USA). Bioaccessibility was calculated using the equation (3) and expressed as available curcumin:

$$\text{Bioaccessibility (\%)} = 100 \times \frac{Curcumin\ in\ micelle}{Total\ curcumin} \qquad \text{Equation (3)}$$

where the total amount of curcumin is the initial content of curcumin, and the amount of curcumin in micelles is the curcumin amount that can be absorbed by epithelial intestinal cells.

2.8. Statistical analysis

All the experiments conducted in this study were based on two original emulsion samples and the digestion experiments of each original emulsion were performed duplicate. All measurements were performed triplicate. The results were expressed as the means ± standard

deviation (SD), and statistical analyses were performed with SPSS17.0. The statistical comparisons were conducted using one-way analysis of variance (ANOVA) followed by least significant difference (LSD) and Duncan test. In all cases, $p < 0.05$ was regarded statistically significant.

3. Results and discussion

3.1. Solubility studies

Curcumin molecule has a high partition coefficient value (i.e. the measure of the extent to which a solute preferentially partitions in octanol over the aqueous phase, log octanol/water = 2.59 ~ 3.2), which makes it practically insoluble in water and highly soluble in lipid (Grynkiewicz & Ślifirski, 2012). The solubility study was carried out for identifying suitable curcumin concentration for preparation of emulsions. Glycerol tributyrate, MCT and corn oil have the molecular weight of 302, 503 and 865 g/mol (**Table 1**) and used as examples of SCT, MCT, and LCT, respectively. The solubility of curcumin in each oil phase and surfactants (Tween 80 and Span 80) is shown in **Table 1**. Solubility in SCT (27.2 ± 5.3 mg/g) was nearly three times as much as that in MCT (10.1 ± 0.7 mg/g) and five times as much as in LCT (5.1 ± 2.4 mg/g). The solubility of curcumin decreased as the molecular weight of the oil molecules increased. This is in agreement with the results reported earlier by Ahmed et al. (2012) and Ma et al. (2017), who explained this effect by different interactions between curcumin and the oil, and the excluded volume effects. More dipole-dipole interactions between polar groups on shorter-chain triglycerides and curcumin molecules occurred owning to the higher amount of polar groups (oxygen) on per unit mass of shorter-chain triglycerides, which results in higher curcumin solubility. On the other hand, a thermodynamically unfavorable exclusion zone exists after curcumin molecule was added to lipid phase. The thickness of the depletion zone increases with increasing molecular weight of the lipid molecules, which leads to lower solubility in LCT than that in SCT.

In addition, Tween 80 and Span 80 are non-ionic surfactants, widely used since they are less irritant and cytotoxic compared to ionic surfactants and less affected by pH and ionic strength that are likely to occur in the gastrointestinal tract. Therefore, Tween 80 and Span 80 were chosen as the surfactants for the preparation of curcumin emulsions. Curcumin solubility in Tween 80 and Span 80 were 32.9 ± 6.4 mg/g and 4.8 ± 1.3 mg/g, respectively. Based on the solubility results, 1 mg/g curcumin in lipid phase was used to prepare all emulsions, so that they can be compared based on similar, fully dissolved curcumin concentration. Other parameters including density, molecular weight and viscosity of three oils and two surfactants are shown in **Table 1.**

3.2. Characterization of curcumin-loaded emulsions

For each oil, emulsions loaded with curcumin with three different droplet size were produced by premix membrane emulsification: small emulsions (prepared with 0.4 μm membrane), medium emulsions (prepared with 0.8 μm membrane), large emulsions (prepared with 3.1 μm membrane). The premix emulsions (coarse emulsions) were also investigated. The emulsions droplet sizes are summarized in **Table 2**. The droplet size are close to the membrane pore size, which is in agreement with our previous study (Alliod et al., 2018). Therefore, premix membrane emulsification can be successfully used for the preparation of emulsions with controlled droplet sizes.

In addition, curcumin-loaded emulsions had a milky yellow appearance after preparation, and the premixes showed deeper yellow color (**Fig. 1**). After 30-days of storage at 4°C, the emulsions containing small droplets of each oil maintained a homogeneous optically opaque yellowish appearance, while medium, large emulsions and premix separated into a yellow creamy layer on the top (MCT and LCT emulsions) or the bottom (SCT emulsions) of the transparent layer because of the gravitational separation (creaming) (**Fig. 1**). However, there were no significant ($p > 0.05$) changes of the droplet size during storage for all emulsions (**Table**

276 **2**). It seems that the results of the stability of SCT emulsions disagree with the findings reported
277 by Ahmed et al.(2012), who found that SCT emulsion stabilized by 1% β-lactoglobulin was
278 highly unstable to droplet growth and creaming. This may be attributed to the different
279 surfactant and surfactant concentration.
280 The encapsulation efficiency of curcumin in emulsions are shown in **Table 2.** Emulsions
281 containing small and medium droplets had high encapsulation efficiency (> 96%), while the
282 encapsulation efficiency of curcumin in large emulsions and premix were lower than that in
283 small and medium emulsions. This result can be explained by the fact that larger droplet
284 emulsions having smaller surface areas need less surfactant, which resulted in more surfactant
285 with curcumin dissolved in water phase. The low encapsulation efficiency in premix explained
286 their deeper yellow color. Therefore, it can be concluded that encapsulation efficiency could be
287 improved by reducing the droplet size of emulsions. On the other hand, the encapsulation
288 efficiency was independent upon the oil type in small and medium emulsions, whereas in
289 premix it increased with the increase of the chain length of oil. It can be suggested that the low
290 encapsulation efficiency in premix of SCT-based emulsions is related to the relatively high
291 water solubility of SCT and therefore SCT molecules may protrude more into the aqueous phase
292 than MCT or LCT molecules because of their short hydrocarbon chains (Ahmed et al., 2012).
293 3.3. Influence of oil type and emulsions droplet size on *in vitro* lipid digestion
294 The impact of the oil type and emulsions droplet size on the rate and extent of lipid digestion
295 in the simulated small intestine was investigated using a pH stat method. The kinetics of lipid
296 digestion was monitored by measuring the amount of the FFAs released over time during the
297 simulated small intestine digestion.
298 For all emulsions, there was a relatively rapid increase in FFAs released during the first few
299 minutes after lipase addition, followed by a more gradual increase at longer times, until a
300 relatively constant final value was reached (**Fig. 2**). For the emulsions containing SCT and MCT,

301 there was no significant ($p > 0.05$) decrease in the amount of FFAs released when the droplet
302 size increased from small to large (**Fig. 2A and B and Table 3**). Even though the surface area
303 of the lipid droplets exposed to the digestive enzymes decreased with the increase of emulsions
304 droplet size in the beginning, the released short or medium-chain FFAs were readily dispersible
305 in aqueous phase and therefore there was always enough surface in contact with enzymes.
306 However, the amount of FFAs released from the premixes was significant ($p < 0.05$) higher than
307 that in large emulsions. This phenomenon may be attributed to the fact that some oil remained
308 in the set-up during the membrane emulsification process leading to the actual oil concentration
309 in emulsions lower than 10% w/w, and therefore the total weight of oil initially present in the
310 simulated intestine phase was lower in small, medium and large emulsions than that in premix
311 emulsions. Nevertheless, both the initial rate and final extent of lipid digestion in emulsions
312 decreased with the increase of droplet size for the emulsions containing LCT (**Fig. 2C**). This
313 effect can be attributed to the changes in the surface area of lipid droplets exposed to the
314 enzymes, and the released long-chain FFAs tend to accumulate at droplet surfaces leading to a
315 slow-down or inhibition of the lipid digestion. This result is in agreement with the results of
316 previous studies in which LCT based emulsions digestion was investigated (Salvia-Trujillo et
317 al., 2013; Zhang et al., 2016; Li et al., 2017).
318 The carrier oil type clearly had a major impact on the lipid digestion (**Fig. 2 and Table 3**). Both
319 the initial rate and final extent of lipid digestion were lower for LCT emulsions than those SCT
320 and MCT emulsions. There are a number of physiochemical explanations that would explain
321 this phenomenon. The water solubility of oil may affect the digestion (Ahmed et al., 2012).
322 LCT has a relatively low water solubility and therefore the digestion occurred in the aqueous
323 phase less than for MCT or SCT emulsions. Moreover, long-chain FFAs have a lower water
324 dispersibility than short and medium-chain FFAs (Yang & McClements, 2013). Consequently,
325 the long-chain FFAs produced during LCT digestion tend to accumulate at the oil-water

interface, thereby inhibiting further pancreatin action. Normally, there is a physicochemical mechanism to remove the long-chain FFAs from the oil droplet surfaces to keep the pancreatin continue to work, such as bile salt solubilisation or calcium precipitation. Efficient digestion of LCT requires enough bile salts and calcium present in the aqueous phase to ensure that the long-chain FFAs are effectively removed from the droplet surfaces. Thus, it can be suggested that the amount of bile salts or calcium present in the reaction vessel was not enough to remove all of the long-chain FFAs produced by digestion.

3.4. Influence of digestion on emulsions droplet size and microstructure

The mean droplet size of curcumin emulsions was determined after stomach digestion. For MCT and LCT emulsions with small, medium and large droplet size, the mean droplet size remained relatively constant after exposure to the stomach phases (**Table 2**). The same result was observed for SCT emulsions with small and medium droplet size. However, for SCT large emulsions, the mean droplet size significantly ($p<0.05$) increased after exposure to the stomach phases (**Table 2**). This may due to the Ostwald ripening effect that happens in relatively large droplet size emulsions at a relatively high temperature (37°C) (Hategekimana et al., 2015) and the complex environment of stomach. Ostwald ripening is the phenomena in which smaller particles in solution dissolve and deposit on larger particles in order to reach a more thermodynamically stable state. On the other hand, the mean droplet size decreased after exposure to the stomach phases for SCT, MCT and LCT premix emulsions. This effect is due to the instability of premix having too large droplet size, and the large droplet size was reduced by the 2 h continuous agitation during stomach digestion. Ostwald ripening did not have a great effect on droplet size changes of SCT premix emulsions.

In order to get further understanding in microstructure change of emulsions, CSLM images of the curcumin emulsions with different droplet size and oil at each stage of simulated digestion were taken (**Fig. 3**). For small and medium emulsions, it was difficult to distinguish the

individual droplets due to the narrow resolution limit of the microscope. In confocal observation, the droplet size increased after exposure to the stomach phase for large size SCT emulsions **(Fig. 3A)**, which proved Ostwald ripening happened. For large size MCT and LCT emulsions, no change was observed after they passed through stomach stage **(Fig. 3B and 3C)**, which indicated that the droplets were relatively stable to coalescence under these conditions. This effect can be attributed to the fact that the droplets were coated by non-ionic surfactants Tween 80 and Span 80 providing sufficient steric repulsion between oil droplets. Consequently, changes in pH, ionic strength, and pepsin activity did not have a strong influence on their coalescence stability. For all premix emulsions, a reduced droplet size was observed, which corresponds to the results of droplet size change.

There was an appreciable change in the confocal microscopy images of all emulsions after they moved from the stomach to the small intestine, which depended on carrier oil type (**Fig. 3**). These changes can be explained by a number of physicochemical events occurring in the small intestine phase: (i) digestion of lipid droplets; (ii) aggregation of lipid droplets; (iii) formation of mixed micelles, vesicles, and other colloidal structures; (iv) formation of insoluble sediments (Ozturk et al., 2015; Zou et al., 2015); (v) the solubilization of undigested lipids within the digested micellar phase. For emulsions containing SCT and MCT **(Fig. 3A and 3B)**, no oil droplet was found after intestine digestion, suggesting almost all oil droplets were digested by pancreatin. However, we observed some oil droplets remaining in all LCT based emulsions **(Fig. 3C)**, corresponding to the results of lipid digestion. The droplet size of each sample after intestine digestion was not measured, because of the presence of insoluble sediments, nondigested fat droplets as well as a complex mixture of different types of colloidal particles, such as micelles, vesicles, bilayers, and liquid crystals.

3.5. Influence of oil type and emulsion droplet size on *in vitro* bioaccessibility

The influence of carrier oil type and initial droplet size of the emulsions on the curcumin

bioaccessibility was studied after full digestion in the simulated GIT system. After the digesta was subjected to centrifugation, two or three distinct layers: a sediment phase at the bottom and a micelle phase above it, and sometimes an oily phase at the top (LCT), were observed. Visually, the micelle phases were optically transparent with yellow color **(Fig. S1)**, in which accessible curcumin was solubilized.

The curcumin bioaccessibility was 2.6 ± 0.8% for free curcumin solution, which shows the poor bioavailability of curcumin. As expected, all emulsions greatly increased the bioavailability of curcumin compared to curcumin dispersed within solution. For the emulsions containing MCT, there was no significant ($p > 0.05$) difference among small, medium and large emulsions, while curcumin bioaccessibility in premix was significantly ($p < 0.05$) lower than that in small, medium and large emulsions. The decrease of curcumin bioaccessibility in premix may attributed to the fact that curcumin had a low encapsulation efficiency, therefore there were no enough mixed micelles present to solubilize free curcumin in the beginning of digestion leading to free curcumin precipitated from solution. For MCT-based emulsions, encapsulation efficiency had a great impact on curcumin bioaccessibility. Moreover, we found that curcumin bioaccessibility in each sample was lower than the corresponding total amount of FFAs produced at the end of the lipid digestion process, suggesting that some curcumin molecules released from lipid were precipitated. The optical microscopy images confirmed that there were orange colored curcumin crystals within digesta **(Fig. 3D)**. This has also been observed by other authors (Salvia-Trujillo et al., 2013; Silva et al., 2019; Li et al., 2021). For the emulsions containing LCT, curcumin bioaccessibility was found to decrease with increasing initial droplet size, falling from 57.5 ± 0.3% for the small emulsions to 42.4 ± 0.6% for the premix emulsions. In addition, we found that the curcumin bioaccessibility increased as the total amount of FFAs increased from the comparison of curcumin bioaccessibility with the total amount of FFAs released at the end of the lipid digestion process. Moreover, the curcumin bioaccessibility in

401 medium, large and premix emulsions was higher the total amount of FFAs produced. This result
402 can be attributed to the fact that long chain FFAs form mixed micelles more easily than medium
403 chain FFAs resulting in larger curcumin solubilization capacity (Shah et al., 2016), therefore
404 some of free curcumin was also solubilized in micelles phase. The optical microscopy images
405 show that there was undigested oil present after intestinal digestion **(Fig. 3D)**, which
406 corresponds to confocal microscopy results. For the emulsions containing SCT, curcumin
407 bioaccessibility in premix was significantly ($p < 0.05$) lower than that in small, medium and
408 large emulsions. On the one hand, curcumin encapsulation efficiency in SCT premix was
409 significantly ($p < 0.05$) lower than that in small, medium and large emulsions (**Table 2**), which
410 led to a relatively low curcumin bioaccessibility. On the other hand, the lipid digestion rate in
411 SCT premix emulsions was higher than that in MCT and LCT premix emulsions, and almost
412 the same with that in SCT small, medium and large emulsions. Free curcumin can solubilize in
413 mixed micelles in the beginning of digestion, thereby increasing the amount of curcumin
414 incorporated into the micelle phase. Even though SCT emulsions had a faster digestion rate
415 compared to MCT and LCT emulsions, there were orange colored curcumin crystals within
416 digesta due to the limited solubilization capacity of micelles formed by short chain FFAs (**Fig.**
417 **3D**). However, our results disagree with the results reported by Ahmed (Ahmed et al., 2012),
418 who found that curcumin had a very low bioaccessibility (≈1%) when it was encapsulated in an
419 SCT emulsion. The reason for this difference is that the different surfactants were used, which
420 affects the formation of mixed micelles or/and the solubility and transport of curcumin.
421 The bioaccessibility was dependent on the carrier oil type. For small, medium and large
422 emulsions, curcumin bioaccessibility was higher in MCT emulsions than that in the SCT and
423 LCT emulsions (**Table 3**). There are a number of possible explanations for this phenomenon.
424 Firstly, some of curcumin remained inside the undigested LCT oil droplets because LCT-based
425 emulsions were not completely digested within the small intestine (**Fig. 2C and Fig. 3C**), and

therefore some of curcumin may not have been released into the mixed micelle phase. Secondly, the non-polar regimes in the mixed micelles formed by medium chain FFAs may have been larger than for short chain FFAs, and thus medium chain FFAs formed mixed micelles that have larger solubilization capacity for curcumin. For premix emulsions, the curcumin low encapsulation efficiency had a significant impact on the curcumin bioaccessibility. Additionally, lipid digestion rate and the difficulty of mixed micelle formation affected curcumin solubilization, which is related to curcumin bioaccessibility.

In summary, our results suggest that MCT is the most effective lipid for obtaining a relatively high bioaccessibility of curcumin, and there were no big differences in bioaccessibility for small, medium and large droplet size emulsions.

4. Conclusions

The purpose of this study was to determine the influence of carrier oil type and initial emulsions droplet size on the curcumin bioaccessibility within a simulated gastrointestinal tract. We have shown that curcumin can be encapsulated effectively within O/W emulsions by premix membrane emulsification using different carrier lipids coated by non-ionic surfactants. The curcumin encapsulation efficiency decreased with the increased of emulsions droplet size for the three oil based emulsions: small ≈ medium > large > premix. In addition, we found that the lipid digestion depended on carrier oil type. Both the rate and extent of lipid digestion were higher for SCT and MCT emulsions than LCT emulsions. The rate and extent of LCT digestion decreased as the emulsions droplet size increased, whereas there were no significant ($p > 0.05$) differences in lipid digestion in small, medium and large emulsions for SCT and MCT. Curcumin bioaccessibility depended on the carrier oil type, which determined the properties of FFAs released and the mixed micelle formed, and then the solubilization capacity of curcumin. The bioaccessibility of curcumin was higher in MCT emulsions than that in LCT and SCT emulsions, due to the completely digestion of MCT within the small intestine and the higher

solubilization capacity for curcumin of mixed micelles formed by medium chain FFAs compared to short chain FFAs. The curcumin bioaccessibility in LCT based emulsions decreased as the emulsions droplet size increased because of the different extent of lipid digestion in different droplet size emulsions, whereas there were no significant ($p > 0.05$) differences in bioaccessibility for emulsions with small, medium and large droplet size for SCT and MCT. On the other hand, we found that curcumin encapsulation efficiency played an important role in determining the bioaccessibility because the free curcumin could precipitate from the solution due to the lack of mixed micelles in the beginning of digestion. In SCT and MCT premix emulsions, curcumin bioaccessibility was significantly ($p < 0.05$) lower than that in small, medium and large emulsions.

In summary, MCT emulsions were found to be the most suitable for increasing the bioaccessibility of curcumin. These results have important implications for the design and fabrication of emulsions-based delivery systems for curcumin and other lipophilic functional ingredients. Nevertheless, it will be important to carry out *in vivo* models (animals or humans) to explore the effect of carrier oil type and droplet size of emulsions on bioaccessibility within the complex environment of real gastrointestinal tracts.

Conflicts of interest

The authors declare that they have no conflict of interest.

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References

Ahmed, K., Li, Y., McClements, D. J., & Xiao, H. (2012). Nanoemulsion- and emulsion-based delivery systems for curcumin: Encapsulation and release properties. *Food Chemistry*, *132*(2), 799–807. https://doi.org/10.1016/j.foodchem.2011.11.039

Alliod, O., Valour, J.-P., Urbaniak, S., Fessi, H., Dupin, D., & Charcosset, C. (2018). Preparation of oil-in-water nanoemulsions at large-scale using premix membrane emulsification and Shirasu Porous Glass (SPG) membranes. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *557*, 76–84. https://doi.org/10.1016/j.colsurfa.2018.04.045

Bardone, E., & Associazione Italiana di Ingegneria Chimica (Eds.). (2012). *IBIC 2012, 3rd International Conference on Industrial Biotechnology: 24 - 27 June 2012, Palermo, Italy* (1. ed). AIDIC.

Charcosset, C. (2009). Preparation of emulsions and particles by membrane emulsification for the food processing industry. *Journal of Food Engineering*, *92*(3), 241–249. https://doi.org/10.1016/j.jfoodeng.2008.11.017

Chuacharoen, T., & Sabliov, C. M. (2019). Comparative effects of curcumin when delivered in a nanoemulsion or nanoparticle form for food applications: Study on stability and lipid oxidation inhibition. *LWT*, *113*, 108319. https://doi.org/10.1016/j.lwt.2019.108319

Grynkiewicz, G., & Ślifirski, P. (2012). Curcumin and curcuminoids in quest for medicinal status. *Acta Biochimica Polonica*, *59*(2). https://doi.org/10.18388/abp.2012_2139

Hategekimana, J., Chamba, Moses. V. M., Shoemaker, C. F., Majeed, H., & Zhong, F. (2015). Vitamin E nanoemulsions by emulsion phase inversion: Effect of environmental stress and long-term storage on stability and degradation in different carrier oil types. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *483*, 70–80. https://doi.org/10.1016/j.colsurfa.2015.03.020

Iqbal, R., Mehmood, Z., Baig, A., & Khalid, N. (2020). Formulation and characterization of food grade O/W nanoemulsions encapsulating quercetin and curcumin: Insights on enhancing solubility characteristics. *Food and Bioproducts Processing*, *123*, 304–311. https://doi.org/10.1016/j.fbp.2020.07.013

Jiang, T., & Charcosset, C. (2022). Premix membrane emulsification for the preparation of curcumin-loaded nanoemulsions. *Journal of Food Engineering*, *316*, 110836. https://doi.org/10.1016/j.jfoodeng.2021.110836

Jiang, T., Liao, W., & Charcosset, C. (2020). Recent advances in encapsulation of curcumin in nanoemulsions: A review of encapsulation technologies, bioaccessibility and applications. *Food Research International*, *132*, 109035. https://doi.org/10.1016/j.foodres.2020.109035

Li, Q., Li, T., Liu, C., Dai, T., Zhang, R., Zhang, Z., & McClements, D. J. (2017). Enhancement of carotenoid bioaccessibility from tomatoes using excipient emulsions: Influence of particle size. *Food Biophysics*, *12*(2), 172–185. https://doi.org/10.1007/s11483-017-

9474-7

Li, Q., Shi, J., Du, X., McClements, D. J., Chen, X., Duan, M., Liu, L., Li, J., Shao, Y., & Cheng, Y. (2021). Polysaccharide conjugates from Chin brick tea (Camellia sinensis) improve the physicochemical stability and bioaccessibility of β-carotene in oil-in-water nanoemulsions. *Food Chemistry*, *357*, 129714. https://doi.org/10.1016/j.foodchem.2021.129714

Ma, P., Zeng, Q., Tai, K., He, X., Yao, Y., Hong, X., & Yuan, F. (2017). Preparation of curcumin-loaded emulsion using high pressure homogenization: Impact of oil phase and concentration on physicochemical stability. *LWT*, *84*, 34–46. https://doi.org/10.1016/j.lwt.2017.04.074

Ozturk, B., Argin, S., Ozilgen, M., & McClements, D. J. (2015). Nanoemulsion delivery systems for oil-soluble vitamins: Influence of carrier oil type on lipid digestion and vitamin D3 bioaccessibility. *Food Chemistry*, *187*, 499–506. https://doi.org/10.1016/j.foodchem.2015.04.065

Perrone, D., Ardito, F., Giannatempo, G., Dioguardi, M., Troiano, G., Lo Russo, L., De Lillo, A., Laino, L., & Lo Muzio, L. (2015). Biological and therapeutic activities, and anticancer properties of curcumin. *Experimental and Therapeutic Medicine*, *10*(5), 1615–1623. https://doi.org/10.3892/etm.2015.2749

Pinheiro, A. C., Coimbra, M. A., & Vicente, A. A. (2016). In vitro behaviour of curcumin nanoemulsions stabilized by biopolymer emulsifiers – Effect of interfacial composition. *Food Hydrocolloids*, *52*, 460–467. https://doi.org/10.1016/j.foodhyd.2015.07.025

Rehman, A., Tong, Q., Jafari, S. M., Korma, S. A., Khan, I. M., Mohsin, A., Manzoor, M. F., Ashraf, W., Mushtaq, B. S., Zainab, S., & Cacciotti, I. (2021). Spray dried nanoemulsions loaded with curcumin, resveratrol, and borage seed oil: The role of two different modified starches as encapsulating materials. *International Journal of Biological Macromolecules*, *186*, 820–828. https://doi.org/10.1016/j.ijbiomac.2021.07.076

Salvia-Trujillo, L., Qian, C., Martín-Belloso, O., & McClements, D. J. (2013). Influence of particle size on lipid digestion and β-carotene bioaccessibility in emulsions and nanoemulsions. *Food Chemistry*, *141*(2), 1472–1480. https://doi.org/10.1016/j.foodchem.2013.03.050

Sarkar, A., Goh, K. K. T., Singh, R. P., & Singh, H. (2009). Behaviour of an oil-in-water emulsion stabilized by β-lactoglobulin in an in vitro gastric model. *Food Hydrocolloids*, *23*(6), 1563–1569. https://doi.org/10.1016/j.foodhyd.2008.10.014

Shah, B. R., Zhang, C., Li, Y., & Li, B. (2016). Bioaccessibility and antioxidant activity of curcumin after encapsulated by nano and Pickering emulsion based on chitosan-tripolyphosphate nanoparticles. *Food Research International*, *89*, 399–407. https://doi.org/10.1016/j.foodres.2016.08.022

Sharma, N., Kaur, G., & Khatkar, S. K. (2021). Optimization of emulsification conditions for designing ultrasound assisted curcumin loaded nanoemulsion: Characterization,

antioxidant assay and release kinetics. *LWT*, *141*, 110962. https://doi.org/10.1016/j.lwt.2021.110962

Silva, H. D., Beldíková, E., Poejo, J., Abrunhosa, L., Serra, A. T., Duarte, C. M. M., Brányik, T., Cerqueira, M. A., Pinheiro, A. C., & Vicente, A. A. (2019). Evaluating the effect of chitosan layer on bioaccessibility and cellular uptake of curcumin nanoemulsions. *Journal of Food Engineering*, *243*, 89–100. https://doi.org/10.1016/j.jfoodeng.2018.09.007

Tan, C., Xie, J., Zhang, X., Cai, J., & Xia, S. (2016). Polysaccharide-based nanoparticles by chitosan and gum arabic polyelectrolyte complexation as carriers for curcumin. *Food Hydrocolloids*, *57*, 236–245. https://doi.org/10.1016/j.foodhyd.2016.01.021

Vladisavljevic, G., Shimizu, M., & Nakashima, T. (2004). Preparation of monodisperse multiple emulsions at high production rates by multi-stage premix membrane emulsification. *Journal of Membrane Science*, *244*(1–2), 97–106. https://doi.org/10.1016/j.memsci.2004.07.008

Yang, Y., & McClements, D. J. (2013). Vitamin E bioaccessibility: Influence of carrier oil type on digestion and release of emulsified α-tocopherol acetate. *Food Chemistry*, *141*(1), 473–481. https://doi.org/10.1016/j.foodchem.2013.03.033

Zhang, R., Zhang, Z., Zou, L., Xiao, H., Zhang, G., Decker, E. A., & McClements, D. J. (2016). Enhancement of carotenoid bioaccessibility from carrots using excipient emulsions: Influence of particle size of digestible lipid droplets. *Food & Function*, *7*(1), 93–103. https://doi.org/10.1039/C5FO01172H

Zheng, B., Peng, S., Zhang, X., & McClements, D. J. (2018). Impact of Delivery System Type on Curcumin Bioaccessibility: Comparison of Curcumin-Loaded Nanoemulsions with Commercial Curcumin Supplements. *Journal of Agricultural and Food Chemistry*, *66*(41), 10816–10826. https://doi.org/10.1021/acs.jafc.8b03174

Zou, L., Zheng, B., Liu, W., Liu, C., Xiao, H., & McClements, D. J. (2015). Enhancing nutraceutical bioavailability using excipient emulsions: Influence of lipid droplet size on solubility and bioaccessibility of powdered curcumin. *Journal of Functional Foods*, *15*, 72–83. https://doi.org/10.1016/j.jff.2015.02.044

584 **Table 1**
585 Properties of different oils and surfactants, and solubility of curcumin in these oils and surfactants.

Oil or Surfactant	Density (g/m^3)	Molecular weight (g/mol)	Viscosity (mPa·s)	Curcumin solubility (mg/g)
Glyceryl tributyrate (SCT)	1.032	302	10.3 ± 0.5	27.2 ± 5.3
MCT	0.93-0.96	503[a]	26.5 ± 0.5	10.1 ± 0.7
Corn oil (LCT)	0.91-0.93	865[b]	55.9 ± 0.4	5.1 ± 2.4
Tween 80	1.06	1310	481.3 ± 3.7	32.9 ± 6.4
Span 80	1	428.6	1418.7 ± 17.0	4.8 ± 1.3

586 [a]503: this data is from (Silva et al., 2019). [b]865: this data is from (Bardone & Associazione Italiana di
587 Ingegneria Chimica, 2012).
588
589

Table 2

Droplet size stability and encapsulation efficiency of curcumin-loaded emulsions containing different oil phase and obtained using different pore size membrane.

Carrier oil	Membrane pore size (μm)	Droplet size (μm)			Encapsulation efficiency (%)
		0 d	Stomach	30 d	
SCT	0.4 (Small)	0.46 ± 0.01	0.45 ± 0.01	0.47 ± 0.01	98.5 ± 0.4[a]
	0.8 (Medium)	0.80 ± 0.01	0.82 ± 0.04	0.76 ± 0.02	98.2 ± 0.4[a]
	3.1 (Large)	3.58 ± 0.22	5.85 ± 0.39*	3.44 ± 0.05	87.2 ± 2.4[b]
	Premix	62.7 ± 2.5	42.4 ± 1.29*	62.5 ± 0.26	41.2 ± 2.2[c]
MCT	0.4 (Small)	0.50 ± 0.02	0.49 ± 0.02	0.49 ± 0.02	98.0 ± 0.2[a]
	0.8 (Medium)	0.85 ± 0.01	0.83 ± 0.02	0.78 ± 0.02	97.4 ± 0.4[a]
	3.1 (Large)	3.79 ± 0.09	3.71 ± 0.01	3.91 ± 0.14	88.8 ± 1.1[b]
	Premix	58.2 ± 1.8	49.4 ± 2.8*	56.7 ± 0.12	53.0 ± 6.0[c]
LCT	0.4 (Small)	0.48 ± 0.01	0.47 ± 0.01	0.49 ± 0.01	97.4 ± 0.4[a]
	0.8 (Medium)	0.79 ± 0.04	0.80 ± 0.02	0.82 ± 0.01	96.8 ± 0.3[a]
	3.1 (Large)	3.76 ± 0.03	3.83 ± 0.01	3.72 ± 0.01	92.8 ± 1.9[a]
	Premix	63.2 ± 1.3	53.4 ± 0.79*	62.1 ± 0.55	70.0 ± 10.2[b]

The values with different superscript letters in a set of data are significantly different ($p<0.05$). *$p < 0.05$ vs emulsion droplet size at 0 d. Values with the same superscript letter are not significantly different ($p > 0.05$).

Table 3

Influence of carrier oil type and emulsions droplet size on the bioaccessibility of curcumin and the total amount of FFA released after 120 min of digestion.

Droplet size	SCT		MCT		LCT	
	FFA (%)	Bioaccessibility (%)	FFA (%)	Bioaccessibility (%)	FFA (%)	Bioaccessibility (%)
Small	81.9 ± 1.3^{b}	59.3 ± 1.5^{bc}	85.9 ± 2.1^{ab}	69.8 ± 2.4^{a}	63.0 ± 1.9^{a}	57.5 ± 0.3^{a}
Medium	85.6 ± 4.8^{b}	61.4 ± 5.1^{ab}	85.7 ± 2.9^{ab}	70.2 ± 2.4^{a}	46.2 ± 2.7^{b}	50.6 ± 1.2^{b}
Large	83.8 ± 4.2^{b}	63.2 ± 3.1^{a}	83.4 ± 1.8^{b}	71.8 ± 1.7^{a}	39.3 ± 0.6^{c}	45.6 ± 0.4^{c}
Premix	104.4 ± 4.5^{a}	57.4 ± 4.2^{c}	89.1 ± 1.5^{a}	58.1 ± 3.2^{b}	34.6 ± 3.0^{d}	42.4 ± 0.6^{d}

The same set of data with different superscript letters are significantly different ($p < 0.05$). Values with the same superscript letter are not significantly different ($p > 0.05$).

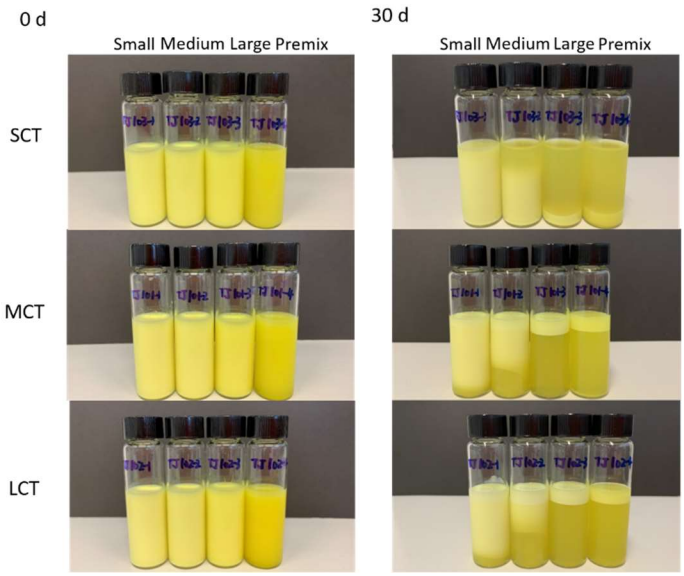


Fig. 1. Photographs of curcumin-loaded SCT, MCT and LCT emulsions with different droplet size (Small, Medium, Large, and Premix, left to right) in the beginning (0 days) and in the end (30 days) of storage (4 °C).

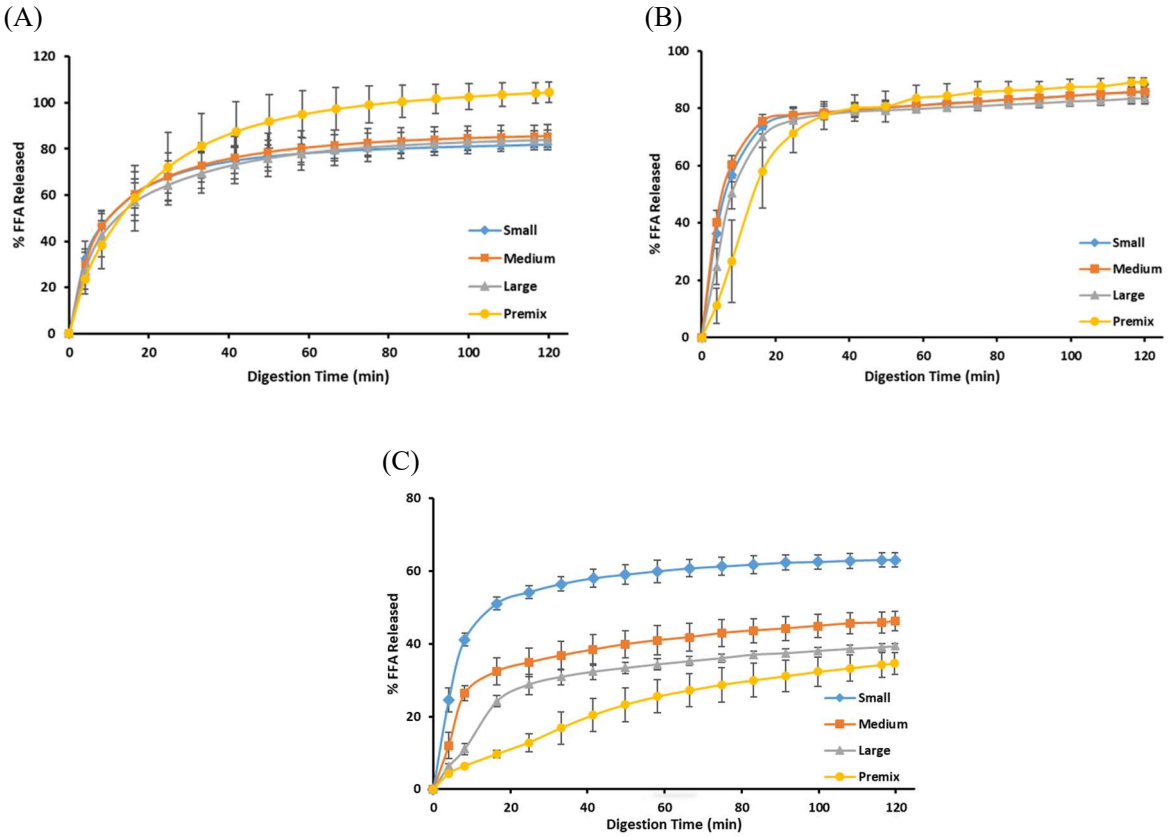
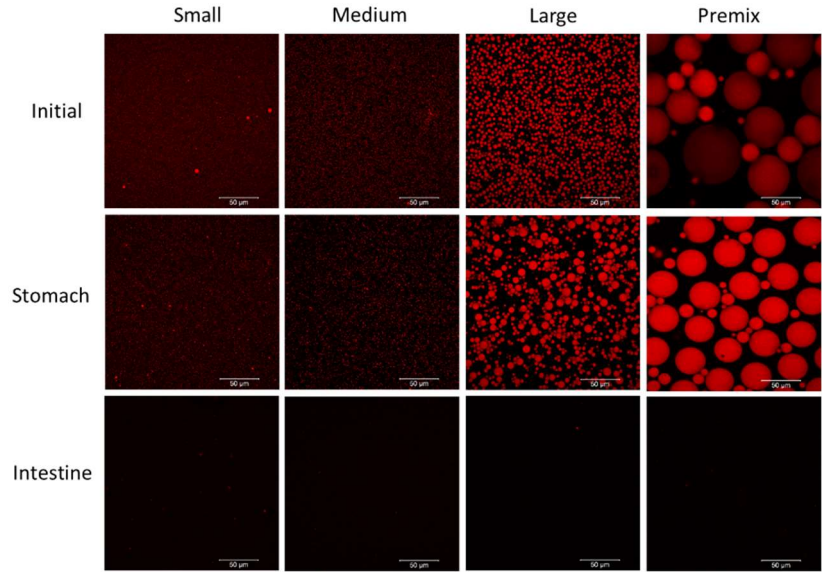
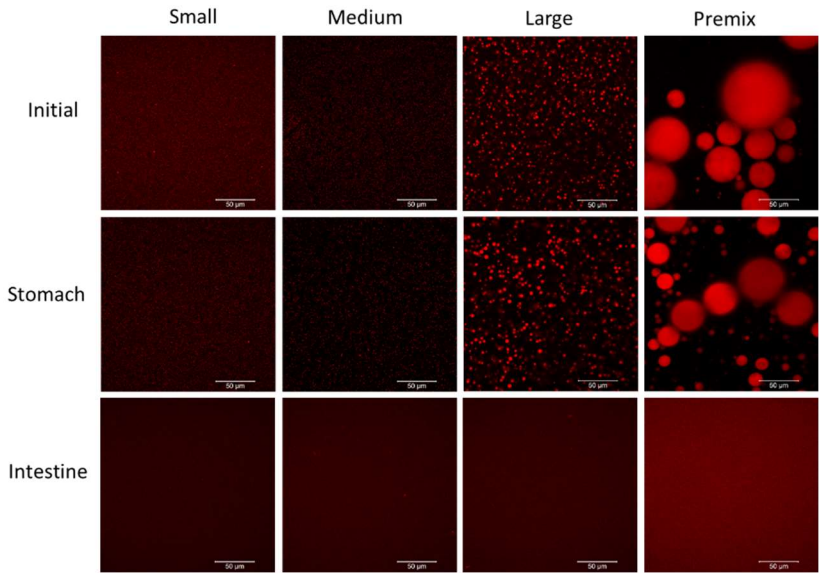


Fig. 2. Influence of emulsion droplet size (Small, Medium, Large, and Premix) on the FFAs release in SCT (A), MCT (B) and LCT (C) emulsions using pH-stat titration method. The scale bars represent a length of 50 μm, and the red regions represent lipids.

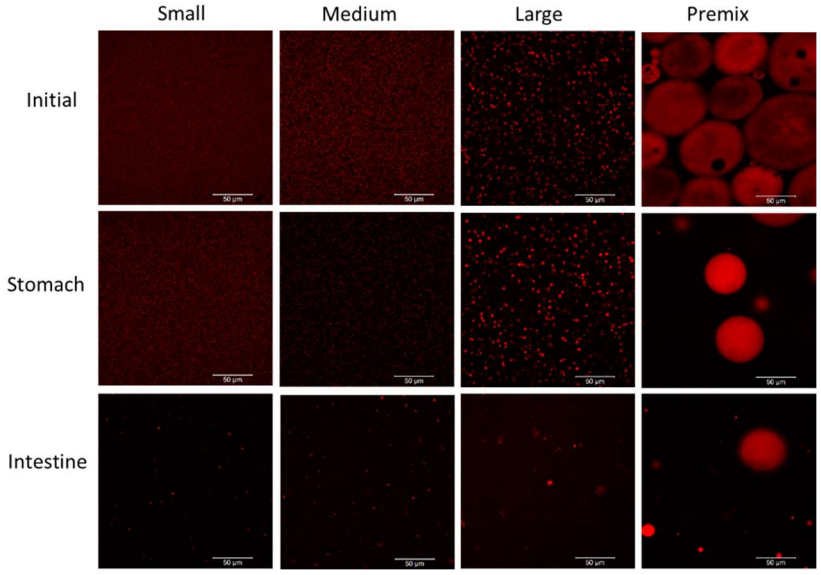
(A)


Small
Medium
Large
Premix
Initial
Stomach
Intestine

(B)


Small
Medium
Large
Premix
Initial
Stomach
Intestine

(C)


Small
Medium
Large
Premix
Initial
Stomach
Intestine

(D)

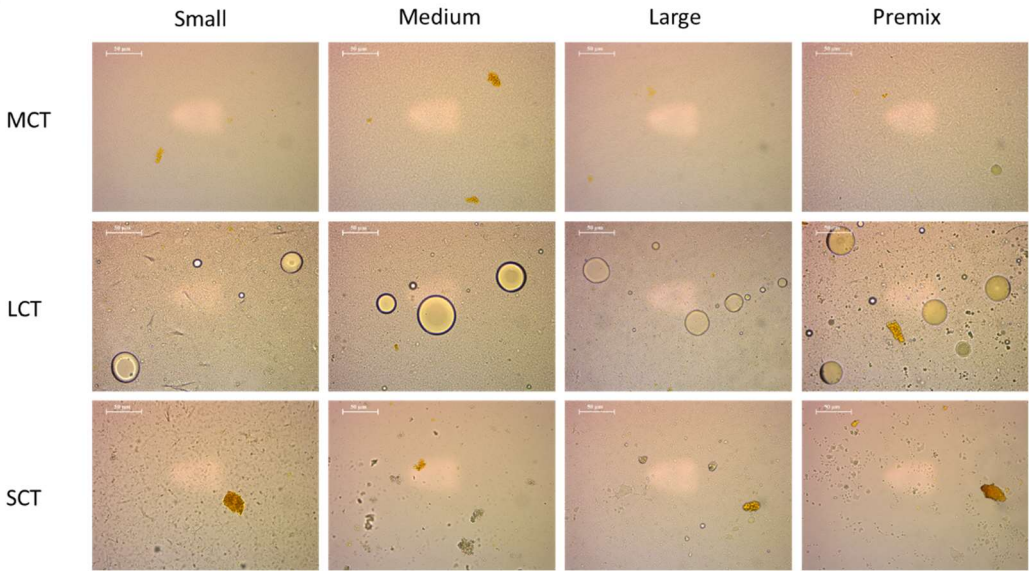


Fig. 3. Confocal fluorescence images of different droplet size (Small, Medium, Large, and Premix) SCT (A), MCT (B) and LCT (C) emulsions after the simulation of stomach and small intestinal phases, and optical microscopy images (D) of different droplet size MCT, LCT and SCT emulsions after the simulation of small intestinal phases. The scale bars represent a length of 50 μm in both confocal fluorescence and optical microscopy images.