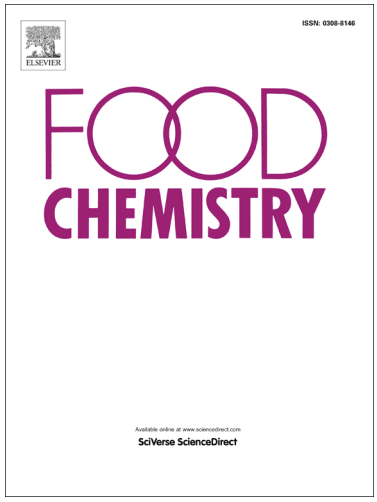


Nanoemulsion Delivery Systems for Oil-Soluble Vitamins: Influence of Carrier Oil Type on Lipid Digestion and Vitamin D_3 Bioaccessibility

Bengu Ozturk, Sanem Argin, Mustafa Ozilgen, David Julian McClements

PII: S0308-8146(15)00611-1
DOI: http://dx.doi.org/10.1016/j.foodchem.2015.04.065
Reference: FOCH 17466

To appear in: *Food Chemistry*

Received Date: 8 January 2015
Revised Date: 23 March 2015
Accepted Date: 16 April 2015

Please cite this article as: Ozturk, B., Argin, S., Ozilgen, M., McClements, D.J., Nanoemulsion Delivery Systems for Oil-Soluble Vitamins: Influence of Carrier Oil Type on Lipid Digestion and Vitamin D_3 Bioaccessibility, *Food Chemistry* (2015), doi: http://dx.doi.org/10.1016/j.foodchem.2015.04.065

Nanoemulsion Delivery Systems for Oil-Soluble Vitamins: Influence of Carrier Oil Type on Lipid Digestion and Vitamin D_3 Bioaccessibility

Bengu Ozturk[a,b,e], Sanem Argin[c], Mustafa Ozilgen[c], David Julian McClements[a,d*]

[a] *Department of Food Science, University of Massachusetts, Chenoweth Laboratory, Amherst, MA, USA*

[b] *Department of Chemical Engineering, Yeditepe University, Kayisdagi, Istanbul, Turkey*

[c] *Department of Food Engineering, Yeditepe University, Kayisdagi, Istanbul, Turkey*

[d] *Production of Bioproducts for Industrial Applications Research Group, Department of Biochemistry, Faculty of Science, King Abdulaziz University, P. O. Box 80203 Jeddah 21589 Saudi Arabia*

[e] *Food Institute, TÜBİTAK Marmara Research Center, P.O. Box 21, 41470 Gebze-Kocaeli, Turkey*

Journal: Food Chemistry

Submitted: January 2015

*Corresponding Author

Address: Department of Food Science, University of Massachusetts, Chenoweth Laboratory, Amherst, MA, 01003 USA

Tel: 413 545 1019; Fax: 413 545 1262; E-mail: mcclements@foodsci.umass.edu

ABSTRACT

The influence of carrier oil type on the bioaccessibility of vitamin D_3 encapsulated within oil-in-water nanoemulsions prepared using a natural surfactant (quillaja saponin) was studied using a simulated gastrointestinal tract (GIT) model: mouth; stomach; small intestine. The rate of free fatty acid release during lipid digestion decreased in the following order: medium chain triglycerides (MCT) > corn oil ≈ fish oil > orange oil > mineral oil. Conversely, the measured bioaccessibility of vitamin D_3 decreased in the following order: corn oil ≈ fish oil > orange oil > mineral oil > MCT. These results show that carrier oil type has a considerable impact on lipid digestion and vitamin bioaccessibility, which was attributed to differences in the release of bioactives from lipid droplets, and their solubilization in mixed micelles. Nanoemulsions prepared using long chain triglycerides (corn or fish oil) were most effective at increasing vitamin bioaccessibility.

Keywords: nanoemulsions; vitamin D_3; cholecalciferol, calcifediol, calcitriol, digestion; quillaja saponin; carrier oil; bioaccessibility; bioavailability.

1. Introduction

Vitamin D is an oil-soluble micronutrient that is essential in the human diet for maintaining good health. It has two major chemical forms: vitamin D_2 (ergocalciferol) and D_3 (cholecalciferol) (Luo, Teng, & Wang, 2012). Vitamin D_3

has more potency than vitamin D_2 in humans and it is usually synthesized in the skin after exposure to light. Vitamin D_3 may exist in a number of different chemical forms depending on environmental conditions, *e.g.* calciol, calcidiol, and calcitriol. Calcitriol (25-dihydroxyvitamin D_3) is the biologically active form of vitamin D_3 and controls calcium and phosphorus homeostasis, intestinal transport, bone metabolism, and renal calcium reabsorption, blood pressure, and insulin secretion (Gonnet, Lethuaut, & Boury, 2010).

Vitamin D_3 deficiency often occurs in people who are not exposed to sufficient sunlight, and in individuals with metabolic (*e.g.* obesity or hyperparathyroidism) or gastrointestinal (*e.g.* celiac or inflammatory bowel disease) disorders (Adams & Hewison, 2010; Holick, Binkley, Bischoff-Ferrari, Gordon, Hanley, Heaney, et al., 2011). Malfunction of the regulation of calcium and phosphorus absorption and bone metabolism can lead to osteoporosis and osteomalacia (pore formation and softening of bones) in adults, while rickets may occur in children in developing countries (Holick, 2007). Vitamin D sources are fairly limited in foods, and include products such as beef liver, dairy products, egg yolk, and fish (Borel, Caillaud, & Cano, 2013; Luo, Teng, & Wang, 2012). Therefore, there is a high demand to enrich food and beverages with vitamin D_3.

Vitamin D_3 is highly sensitive to environmental stresses (such as light, heat, and oxygen) and can easily be oxidized, leading to loss of its functionality and physiological benefits (Luo, Teng, & Wang, 2012). Oil-soluble vitamins are typically absorbed at particular locations in the small intestine along with the digestion products of ingested dietary fats and oils (Goncalves, Roi, Nowicki, Dhaussy, Huertas, Amiot, et al., 2015). They may be absorbed by both passive and active transport mechanisms (Porter, Trevaskis, & Charman, 2007; Reboul,

Goncalves, Comera, Bott, Nowicki, Landrier, et al., 2011). The oil-soluble vitamins are incorporated into chylomicrons (consisting of bile salts, phospholipids, and lipid digestion products), which are released into the systemic circulation *via* the lymphatic system, and then activated in the liver (Porter & Charman, 1997). Due to its poor water-solubility and low oral bioavailability, vitamin D is often encapsulated within lipid-based delivery systems that can improve its bioaccessibility.

A considerable amount of research has been carried out to identify lipid-based delivery systems to encapsulate, protect, and release lipophilic bioactive agents. Nanoemulsions are particularly good candidates for delivery of lipid-soluble bioactives (such as vitamin D) because they can be produced with natural food ingredients using simple production methods, and can be designed to increase both water-dispersibility and oral bioavailability (Guttoff, Saberi, & McClements, 2015; Huang, Yu, & Ru, 2010; W. Li, Peng, Ning, Yao, Luo, Zhao, et al., 2014; Luo, Teng, & Wang, 2012; Nik, Corredig, & Wright, 2011; Teng, Luo, & Wang, 2013).

Vitamin D_3 is crystalline at ambient temperature and therefore needs to be dissolved within a suitable carrier oil before it can be incorporated into nanoemulsion-based delivery systems. Previous studies have shown that nanoemulsion composition (*i.e.,* emulsifier and carrier oil type) effects lipid digestion and bioavailability (Golding & Wooster, 2010; McClements & Li, 2010; McClements & Xiao, 2012; Mun, Decker, & McClements, 2007; Qian, Decker, Xiao, & McClements, 2012; Rao, Decker, Xiao, & McClements, 2013; Salvia-Trujillo, Qian, Martin-Belloso, & McClements, 2013; Yang & McClements, 2013). In particular, these studies have shown that carrier oil type has a major

impact on the bioaccessibility of lipophilic bioactives. Consequently, it is important to optimize the nature of the carrier oil used to formulate nanoemulsions in order to ensure good bioavailability of any encapsulated lipophilic bioactive components. In this manuscript, we therefore examined the influence of carrier oil type on lipid digestion and vitamin bioaccessibility. Carrier oils were selected that had different susceptibilities to lipase digestion and different molecular characteristics: medium chain triglycerides (MCT), corn oil, fish oil, orange oil, and mineral oil. MCT, corn oil and fish oil are all triglyceride oils that are digestible by lipase. Corn oil and fish oil are both examples of long chain triglycerides (LCT), but corn oil contains a high proportion of monounsaturated fatty acids, whereas fish oil contains a high proportion of polyunsaturated fatty acids. Orange oil and mineral oil are both examples of indigestible oils. This study should have important implications for designing effective nanoemulsion-based delivery systems to increase the bioaccessibility of vitamin D_3, and therefore improve the efficacy of vitamin-enriched functional foods and beverages.

2. Materials and methods

2.1. Materials

Quillaja saponin (Q-Naturale®100) was kindly provided by Ingredion Inc. (Westchester, IL). It is actually a mixture of various saponin components dispersed in water with the major fraction being reported to have a molecular weight of around 1650 g mol^{-1} (Mitra, et al., 1997a). Vitamin D_3 and mineral oil were purchased from Sigma-Aldrich (St. Louis, MO). Orange oil (10×) was purchased from International Flavors and Fragrances (Union Beach, NJ). It Corn

oil (Mazola, ACH Food Companies, Inc., Memphis, TN) was purchased from a local supermarket. The triglycerides in corn oil have been reported to be about 11.2% $C_{16:0}$, 2.2% $C_{18:0}$, 28.9% $C_{18:1}$, 55.5% $C_{18:2}$, 1.1% $C_{18:3}$, and 0.4% $C_{20:1}$ {Yalcin, 2012 #222}. Medium chain triglyceride (MCT) oil (Miglyol 812) was purchased from Coletica (Northport, NY). The triglycerides in MCT were reported to contain around 60% octanoic acid ($C_{8:0}$) and around 40% capric acid ($C_{10:0}$). Fish oil was provided by DSM Nutritional Products Ltd (Basel, Switzerland). Lipase, bile salts, mucin, and pepsin were purchased from the Sigma Chemical Company (St. Louis, MO). All other chemicals used were of analytical grade. Double distilled water was used to prepare all solutions and emulsions.

2.2. Methods

2.2.1. Nanoemulsion preparation

Oil-in-water nanoemulsions were prepared by homogenizing 10% (w/w) oil phase with 90% (w/w) aqueous phase using a well-established two-step procedure {McClements, 2015 #175}. The oil phase consisted of 0.1 w/w % vitamin D_3 dissolved within 99.9 % carrier oil (MCT, corn oil, fish oil, mineral oil, or orange oil). The aqueous phase consisted of 2% w/w surfactant (Q-Naturale) dispersed within 98% w/w buffer solution (10 mM sodium phosphate, pH 7.0). The manufacturer reported that the Q-Naturale ingredient contained 14 wt% active saponins (with the remainder being mainly water), and so the concentration of this surfactant is reported on an active ingredient basis (rather than total mass basis). Coarse emulsions were prepared by blending the oil and aqueous phases together using a high-speed blender (Bamix, Switzerland) for 2 min at room temperature.

Fine emulsions were prepared by passing the coarse emulsion through a high pressure homogenizer (Microfluidics M110L, Newton, MA, USA) for 3 cycles at 12,000 psi.

2.2.2. Particle characterization

The particle size of the vitamin D_3 nanoemulsions was measured using a static light scattering instrument (Mastersizer 2000, Malvern Instruments, Malvern, UK). The particle size of each sample was represented as the surface-weighted mean diameter (d_{32}), which was calculated from the full particle size distribution.

The droplet charge (ζ-potential) of the nanoemulsions was measured using particle microelectrophoresis (Zetasizer Nano ZS-90, Malvern Instruments, Worcestershire, UK). Prior to measurements, dilution was carried out using buffer solutions with the same pH as the samples being tested to avoid multiple scattering effects, *i.e.,* they had the same pH as the appropriate gastrointestinal region (initial, mouth, stomach, or small intestine).

2.2.3. In vitro digestion

The original nanoemulsions, containing 10% (w/w) oil phase (0.01 % vitamin D_3 and 9.99 % carrier oil) and 90% (w/w) aqueous phase (2% Q-Naturale and 98% buffer solution), were diluted five-times in buffer solution so that the samples used in the *in vitro* digestion studies initially contained 2 % oil and 0.002 % vitamin D_3.

Mouth stage: Simulated artificial saliva solution (SASS) was prepared according to a previous study (Y. Li & McClements, 2010; Sarkar, Goh, & Singh,

2009). A 20 ml aliquot of the diluted original nanoemulsions (2% w/w oil phase) was placed in a 125 ml flask and then 20 ml of SASS containing 0.6 g mucin was added into the flask. This mixture was adjusted to pH 6.8 and then shaken continuously at a rate of 100 rpm in a temperature controlled incubator (37 ˚C) for 10 min (Innova Incubator Shaker, Model 4080, New Brunswick Scientific, New Jersey, USA).

Stomach stage: Simulated gastric fluid (SGF) was prepared by dissolving 2 g of NaCl and 7 ml of HCl in water (1 L total volume) and the pH of this solution was adjusted to pH 1.2 using 1M HCl. 20 ml of the sample from the mouth stage was then mixed with 20 ml of SGF containing 0.064 g pepsin, and the mixture was adjusted to pH 2.5. The resulting mixture was then shaken for 2 hours at 37 ˚C at 100 rpm.

Intestine stage: 30 ml of digesta sample from the stomach stage was added into a clean beaker and placed into a water bath (37˚C) connected to an automatic titration unit used as a pH-STAT (Metrohm, USA Inc., Riverview, FL, USA). The sample was adjusted to pH 7.00 using NaOH solutions, and then simulated intestinal fluid containing 1.5 ml salt solution (10 mM $CaCl_2.2H_2O$ and 150 mM NaCl) and 3.5 ml bile salts (5 mg ml^{-1}) were added respectively and the pH was adjusted to 7.00 using HCl and NaOH solutions. Afterwards, freshly prepared 2.5 ml lipase solution (1.6 mg ml^{-1}) was added to the sample and the automatic titration unit was started. The volume of 0.1 N NaOH solution required to maintain the pH of the sample at 7.00 was recorded using the software program. A control study was performed using the buffer solution as the sample, and the amount of alkali solution titrated into the reaction chamber for the control was subtracted from that for the test samples. Free fatty acid (FFA) release was

194 calculated according to Li and McClements (2010). *In vitro* digestion in the small
195 intestine stage lasted for 2 h and then physicochemical and structural
196 characterization of the samples at each stage were performed.

197 *2.2.4. Bioaccessibility determination*

198 The bioaccessibility of lipophilic components is normally defined as the
199 fraction that is solubilized within the mixed micelle phase after lipid digestion
200 (Carbonell-Capella, Buniowska, Barba, Esteve, & Frigola, 2014; Marze, Meynier,
201 & Anton, 2013). After the full digestion, two portions of 10 ml of samples were
202 collected and centrifuged (4000 rpm, Thermo Scientific, CL10 centrifuge) at 25
203 ˚C for 40 min. The emulsions separated into an opaque sediment phase at the
204 bottom, a clear micelle phase in the middle, and sometimes a thin creamed phase
205 at the top or on the wall of the centrifuge tube. An aliquot (1 ml) of micelle phase
206 or raw digesta sample was vortexed after adding an organic solvent mixture (1:3
207 isooctane: ethyl alcohol) at 1:5 to extract the vitamin D_3 and then centrifuged at
208 1750 rpm for another 10 min. The supernatant phases were used as samples for
209 determination of vitamin D_3 using an UV-Vis Spectrophotometer at 265 nm
210 wavelength (Ultrospec 3000 pro Pharmacia Biotech, Biochrom Ltd., Cambridge,
211 UK).

212 Absorbances of different concentrations of vitamin D_3 standard were
213 measured at 265 nm wavelength to obtain a calibration curve for vitamin D_3
214 concentration *versus* absorbance (r^2= 0.9996).

215 *2.2.5. Microstructural analysis*

216 A Nikon Confocal Fluorescent Microscope (C1 Digital Eclipse, Tokyo,
217 Japan) with a 60 × oil immersion objective lens was used to capture images of the

initial emulsions and of samples taken after each stage of digestion. Nile red (a fat soluble fluorescent dye) was excited with a 488 nm argon laser line.

2.3. Statistical analysis

Each experiment was performed at least twice from the beginning, and results are reported as the calculated average and standard deviation of these measurements using Microsoft Excel.

3. Results and discussion

Previous studies have highlighted the potential impact of carrier oil type on lipid digestion and bioactive bioaccessibility (Qian, Decker, Xiao, & McClements, 2012; Rao, Decker, Xiao, & McClements, 2013; Yang & McClements, 2013). In particular, it was found that the bioaccessibility of β-carotene was much higher when a long chain triglyceride (corn oil) was used as a carrier oil, rather than a medium chain triglyceride (MCT) or indigestible oil (flavor oil). In this study, we aimed to determine whether a similar effect was observed for another important type of lipophilic bioactive molecule, *i.e.,* vitamin D_3.

3.1. Influence of carrier oil type on physical stability of nanoemulsions

Nanoemulsions were prepared using both digestible (MCT, corn oil, and fish oil) and non-digestible (orange oil and mineral oil) carrier oils. The mean particle diameter (d_{32}) and microstructure of the initial nanoemulsions and of samples collected after each digestion stage were measured (**Figures 1 and 2**). With the exception of orange oil, all of the other nanoemulsions had relatively small initial mean particle diameters (0.14 – 0.19 μm) and monomodal particle size

241 distributions (see supplementary material). The mean particle diameter of the
242 orange oil nanoemulsions was appreciably higher (0.29 μm) than the other
243 nanoemulsions, which can probably be attributed to Ostwald ripening effects due
244 to the relatively high water-solubility of flavour oils (Rao & McClements, 2012).

245 Again, with the exception of orange oil, there was only a slight increase in the
246 mean particle diameters of the nanoemulsions after incubation in artificial saliva
247 and gastric solutions (**Figure 1**), and the particle size distributions remained
248 monomodal (data not shown), which suggested that these nanoemulsions were
249 stable against coalescence under simulated mouth (pH 6.8, 10 min) and stomach
250 (pH 2.5, 2 h) conditions. This protection can be attributed to the strong steric
251 stabilizing effect of the natural surfactant (Q-Naturale) used. In a previous study,
252 we reported that Q-Naturale coated lipid droplets were stable against droplet
253 coalescence over a wide range of pH conditions *i.e.,* pH 3 to 8 (Ozturk, Argin,
254 Ozilgen, & McClements, 2014). Moreover, there were no highly surface active
255 components present within the simulated saliva or gastric environments that might
256 have caused emulsion instability by adsorbing to the surfaces of the Q-Naturale
257 coated droplets. Finally, Q-Naturale would be expected to be resistant to pepsin
258 digestion within the gastric fluids, which may increase the stability of the
259 emulsions to coalescence in the stomach phase. Having said this, the confocal
260 microscopy images indicated that appreciable droplet flocculation occurred under
261 mouth and stomach conditions (**Figure 2**). Droplet flocculation may have
262 occurred due to electrostatic screening effects (due to mineral ions), loss of
263 droplet charge (under acidic conditions), or bridging or depletion attraction (due
264 to mucin). Presumably, these flocs were relatively weak and broke down when
265 the emulsions were diluted for the light scattering experiments. In contrast, there

266 was a relatively large increase in mean particle diameter of the orange oil
267 emulsions as they passed from the initial to mouth to stomach stages, which may
268 be due to Ostwald ripening or coalescence leading to droplet growth. Indeed,
269 large spherical droplets were observed in this sample in the stomach phase
270 (whereas irregular shaped particles were observed in the other nanoemulsions),
271 which suggests that the individual oil droplets had grown in size rather than
272 become flocculated.

273 There were appreciable changes in the particle size and microstructure of the
274 nanoemulsions samples after they were incubated in the small intestine phases,
275 which depended on carrier oil type (**Figures 1 and 2**). These changes may have
276 occurred for a number of reasons, such as lipid digestion, droplet aggregation,
277 mixed micelle formation, or generation of insoluble sediments (such as calcium
278 soaps). Lipid digestion might be expected to reduce the size of the individual lipid
279 droplets since some of the oil phase would be removed. However, lipid digestion
280 may also promote droplet aggregation (flocculation or coalescence) due to
281 changes in interfacial and core characteristics. In addition, aggregation may be
282 promoted due to droplet interactions with other components within the small
283 intestinal fluids, such as mineral ions, bile salts, or proteins. The mixed micelles
284 formed from lipid digestion products actually consist of a complex mixture of
285 different types of colloidal particles, such as micelles, vesicles, bilayers, and
286 liquid crystals. Finally, any long chain fatty acids generated during lipid digestion
287 may form insoluble calcium soaps. All of these different types of colloidal
288 particles contribute to the light scattering pattern measured in a particle size
289 analyzer, which makes it difficult to accurately interpret particle size
290 measurements made on intestinal digesta (McClements, Decker, Park, & Weiss,

2009; Singh, Ye, & Horne, 2009). With the exception of orange oil, the micelle phases all contained relatively small particles (< 200 nm), which can be attributed to the fact that large particles either creamed or sedimented during centrifugation and were therefore not detected. The micelle phase collected from the orange oil nanoemulsions contained relatively large particles, which may have been due to some droplet coalescence or Ostwald ripening occurring after centrifugation.

The electrical charge on the particles in the various samples was measured after each stage of the model GIT to provide some information about changes in interfacial characteristics (**Figure 3**). Initially, freshly prepared nanoemulsion droplets were highly negatively charged (between -65 and -70 mV) independent of carrier oil type. This result can be attributed to the fact that the electrical characteristics of the droplets were dominated by the presence of the adsorbed surfactant layer. Previous studies have shown that lipid droplets coated with Q-Naturale have a high negative charged at neutral pH (Ozturk, Argin, Ozilgen, & McClements, 2014; Yang, Leser, Sher, & McClements, 2013). An appreciable decrease in the magnitude of the negative charge on the droplets was observed after the mouth stage in all of the samples, which may have been due to electrostatic screening caused by salts in the simulated saliva, or due to adsorption of mucin molecules to the droplet surfaces (van Aken, Vingerhoeds, & de Hoog, 2007; Vingerhoeds, Blijdenstein, Zoet, & van Aken, 2005).

The magnitude of the negative charges decreased further after exposure to simulated gastric conditions, which can be mainly attributed to changes in the electrical characteristics of the Q-Naturale at low pH values. Previous studies have shown that this surfactant loses its negative charge under highly acid conditions due to protonation of the carboxyl groups on the quillaja saponin

316 molecules (Ozturk, Argin, Ozilgen, & McClements, 2014; Yang, Leser, Sher, &
317 McClements, 2013). There were some differences in the magnitude of the
318 electrical charge on droplets containing different types of carrier oil (**Figure 3**).
319 This may have been due to differences in the susceptibility of the emulsions to
320 competitive adsorption effects.

321 After exposure to the simulated small intestine stage, the magnitude of the
322 negative charge on all the nanoemulsions increased appreciably. This change in
323 droplet electrical characteristics can be attributed to various factors (Qian, Decker,
324 Xiao, & McClements, 2012; Yang & McClements, 2013). First, the increase in
325 pH would cause the surfactant molecules to become more negatively charged.
326 Second, the adsorption of anionic phospholipids and bile salts to the droplet
327 surfaces would lead to a negative charge. Third, the generation of anionic free
328 fatty acids during lipid digestion of triglyceride oils would contribute to the
329 negative surface charge. The particles in the micelle phase were also strongly
330 negatively charged, which can be attributed to the fact that they consisted of
331 particles whose surfaces contained anionic phospholipids, bile salts, and possibly
332 free fatty acids.

333 **3.2. Influence of carrier oil type on in vitro digestion**

334 The influence of carrier oil type on the rate and extent of lipid digestion of
335 vitamin-loaded nanoemulsions under simulated small intestinal conditions was
336 investigated using the pH-STAT method. The percentage of free fatty acids (FFA)
337 released was calculated according to the following equation (Y. Li &
338 McClements, 2010):

339 $$FFA(\%) = 100 \times (V_{NaOH} \times m_{NaOH} \times M_{Lipid}) / (w_{Lipid} \times 2) \quad (1)$$

Where V_{NaOH} is the volume of sodium hydroxide required to neutralize the FFAs produced (L), m_{NaOH} is the molarity of sodium hydroxide solution used (in M), M_{Lipid} is the molecular weight of the triacylglycerol oil (in g/mol), and w_{Lipid} is the total mass of triacylglycerol oil initially present in the digestion cell (in g). Blanks were performed using solutions with the same composition as the samples, except that they contained no oil, and these values were subtracted from the values measured on the samples. The lipid digestion profiles are reported as FFA (%) values *versus* digestion time (min).

The digestion profiles could be divided into two groups: (i) digestible oils (corn oil, fish oil, MCT); (ii) indigestible oils (mineral oil and orange oil). For all the digestible oils, there was initially a rapid increase in FFAs produced during the first 10 minutes after lipase addition, which suggested that the lipase rapidly adsorbed to the droplet surfaces and converted the triglycerides to free fatty acids and monoacylglycerols. At longer times (10 to 120 minutes), the amount of FFAs increased only slowly or remained relatively constant, suggesting that the lipid digestion process was complete. The final extent of lipid digestion was appreciably higher for the MCT nanoemulsions than the ones containing LCT (corn oil or fish oil). This effect can be attributed to the fact that long chain FFAs released from corn oil or fish oil may have accumulated at the lipid droplet surfaces and therefore inhibited further lipase action (Devraj, Williams, Warren, Mullertz, Porter, & Pouton, 2013). On the other hand, medium chain FFAs rapidly moved into the aqueous phase after formation, making it easier for the lipase to continue working (Sek, Porter, Kaukonen, & Charman, 2002). The lipid digestion profiles of fish oil and corn oil nanoemulsions were fairly similar, which is probably because they both contained long chain triglycerides. Orange oil and

365 mineral oil would not be expected to be digested by lipase, which would account
366 for the fact that there was little increase in the calculated FFAs released over time
367 (**Figure 4**).

368 **3.3. Influence of carrier oil type on vitamin D3 bioaccessibility**

369 In this series of experiments, the influence of carrier oil type on vitamin D_3
370 bioaccessibility after full digestion by the simulated GIT system was studied. The
371 bioaccessibility of vitamin D_3 was determined by measuring its concentrations in
372 the micelle phase and the total digesta using solvent extraction and UV-Vis
373 spectrophotometry. The bioaccessibility was then calculated using the equation
374 below:

375 $$\text{Bioaccessibility} = 100 \times (C_{Micelle} / C_{Raw\ Digesta}) \quad (2)$$

376 Where $C_{Micelle}$ and $C_{Raw\ Digesta}$ are the concentrations of vitamin D_3 in the micelle
377 and total digesta samples after the small intestine stage (end of pH-STAT
378 experiment). The bioaccessibility was also measured after filtration of the micelle
379 phase (with 0.45μm pore size). In the human body, mixed micelles and other
380 colloidal particles must pass through the mucus layer that lines the GIT prior to
381 absorption, which has been reported to have a pore size around 0.4 μm (Cone,
382 2009). Consequently, the filtration step used in this study may have removed
383 some of the larger particles that would not be expected to pass through the mucus
384 layer.

385 The bioaccessibility was highly dependent on the type of carrier oil present in
386 the nanoemulsions (**Figure 5**). For the digestible oils, the bioaccessibility of
387 vitamin D_3 was appreciably higher from the nanoemulsions containing LCTs
388 (corn oil and fish oil) than from those containing MCTs (orange oil and mineral

oil), with the magnitude of the effect depending on filtration. Before filtration there were much larger differences in bioaccessibility than after filtration. It is possible that filtration removed relatively large vitamin-loaded vesicles or undigested lipid droplets from the LCT nanoemulsions. Indeed, the micelle phase collected from these nanoemulsions was relatively turbid prior to filtration, suggesting that it did contain some large particles (but not large and/or dense enough to be removed by centrifugation) (**Table 1**). Conversely, there was little change in vitamin bioaccessibility when the micelle phase collected from the MCT nanoemulsions was filtered. The reason for this effect is that the micelle phase in these systems was transparent, which suggested that it did not contain any large particles that would be removed by filtration.

The higher bioaccessibility of vitamin D_3 in the LCT nanoemulsions can be attributed to the solubilization capacity of the micelles formed after digestion. Corn oil and fish oil contain long chain fatty acids (*e.g.* C_{16} to C_{18}) whereas MCT contains medium chain fatty acids (*e.g.* C_8 to C_{10}). Long chain fatty acids form mixed micelles (micelles and vesicles) that have larger non-polar regimes capable of accommodating large lipophilic bioactive molecules (Qian, Decker, Xiao, & McClements, 2012). Conversely, large lipophilic bioactives cannot easily be accommodated into the smaller non-polar regimes found in mixed micelles formed by medium chain fatty acids. Consequently, the mixed micelles formed from long chain FFAs tend to have higher solubilization capacities than those formed by medium chain FFAs.

Indigestible oils (such as mineral and orange oil) do not form free fatty acids in the presence of lipase. As mentioned earlier, free fatty acids can combine with bile salts and phospholipids to form mixed micelles that can solubilize

hydrophobic molecules. Consequently, one would expect the solubilization capacity of the intestinal fluids formed from nanoemulsions containing indigestible oils to be relatively low. Surprisingly, we found that the bioaccessibility of the vitamin D_3 was relatively high in the micelle phase collected from the orange oil nanoemulsion (**Figure 5**). Indeed, the bioaccessibility appeared to be higher for both orange oil and mineral oil than for MCT, despite the fact that MCT was fully digestible. This effect may be an artefact of the method used to measure vitamin bioaccessibility. There may have been relatively small non-digested lipid droplets containing vitamin D_3 in the micelle phases collected from the orange oil and mineral oil nanoemulsions. These droplets may have been so small that they were not removed by centrifugation or filtration. This result raises an interesting question: would these lipid droplets pass through the mucus layer and be adsorbed by the epithelium cells in the human body? Further studies are clearly needed to establish the influence of lipid digestibility on the biological fate of oil-soluble vitamins encapsulated in nanoemulsions.

4. Conclusions

In this study, the influence of the type of carrier oil used to formulate nanoemulsion-based delivery systems, on the bioaccessibility of an important oil-soluble bioactive (vitamin D_3) was examined. As observed with other highly lipophilic bioactive agents, it was found that the nature of the carrier oil had a major influence on the bioaccessibility of vitamin D_3 measured using a simulated gastrointestinal model.

The rate and extent of lipid digestion was higher for MCT nanoemulsions than LCT nanoemulsions (corn oil and fish oil), which was attributed to accumulation of long chain FFAs at the lipid droplet surfaces inhibiting lipase activity. As expected, indigestible lipids (orange oil and mineral oil) did not produce FFAs when exposed to lipase. Vitamin bioaccessibility was higher in LCT nanoemulsions than in MCT nanoemulsions, presumably due to the higher solubilization capacity for vitamin D_3 of mixed micelles formed by long chain FFAs. Surprisingly, a relatively high bioaccessibility for the nanoemulsions prepared from indigestible oils was found, which may have been an experimental artefact associated with the presence of vitamin-containing small lipid droplets in the micelle phase. Alternatively, it may be possible for these small lipid droplets to be adsorbed by the human body, and therefore increase vitamin bioavailability. In summary, LCT nanoemulsions were found to be the most suitable for increasing the bioaccessibility of vitamin D_3. These results are important for formulating nanoemulsion-based delivery systems for oil-soluble vitamins and other lipophilic nutraceuticals.

5. Acknowledgements

This material is based upon work supported by the Cooperative State Research, Extension, Education Service, United State Department of Agriculture, Massachusetts Agricultural Experiment Station (Project No. 831) and by the United States Department of Agriculture, NRI Grants (2011-03539, 2013-03795, 2011-67021, and 2014-67021). The authors also acknowledge TUBITAK 2214-A Program for providing financial support for Bengu Ozturk.

6. References

Adams, J. S., & Hewison, M. (2010). Update in Vitamin D. *Journal of Clinical Endocrinology & Metabolism, 95*(2), 471-478.

Borel, P., Caillaud, D., & Cano, N. J. (2013). Vitamin D bioavailability: State of the art. *Crit Rev Food Sci Nutr*.

Carbonell-Capella, J. M., Buniowska, M., Barba, F. J., Esteve, M. J., & Frigola, A. (2014). Analytical Methods for Determining Bioavailability and Bioaccessibility of Bioactive Compounds from Fruits and Vegetables: A Review. *Comprehensive Reviews in Food Science and Food Safety, 13*(2), 155-171.

Cone, R. A. (2009). Barrier properties of mucus. *Adv Drug Deliv Rev, 61*(2), 75-85.

Devraj, R., Williams, H. D., Warren, D. B., Mullertz, A., Porter, C. J. H., & Pouton, C. W. (2013). In vitro digestion testing of lipid-based delivery systems: Calcium ions combine with fatty acids liberated from triglyceride rich lipid solutions to form soaps and reduce the solubilization capacity of colloidal digestion products. *International Journal of Pharmaceutics, 441*(1-2), 323-333.

Golding, M., & Wooster, T. J. (2010). The influence of emulsion structure and stability on lipid digestion. *Current Opinion in Colloid & Interface Science, 15*(1-2), 90-101.

Goncalves, A., Roi, S., Nowicki, M., Dhaussy, A., Huertas, A., Amiot, M.-J., & Reboul, E. (2015). Fat-soluble vitamin intestinal absorption: Absorption sites in the intestine and interactions for absorption. *Food Chem, 172*, 155-160.

Gonnet, M., Lethuaut, L., & Boury, F. (2010). New trends in encapsulation of liposoluble vitamins. *J Control Release, 146*(3), 276-290.

Guttoff, M., Saberi, A. H., & McClements, D. J. (2015). Formation of vitamin D nanoemulsion-based delivery systems by spontaneous emulsification: factors affecting particle size and stability. *Food Chem, 171*, 117-122.

Holick, M. F., Binkley, N. C., Bischoff-Ferrari, H. A., Gordon, C. M., Hanley, D. A., Heaney, R. P., Murad, M. H., Weaver, C. M., & Endocrine, S. (2011). Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab, 96*(7), 1911-1930.

Huang, Q., Yu, H., & Ru, Q. (2010). Bioavailability and delivery of nutraceuticals using nanotechnology. *J Food Sci, 75*(1), R50-57.

Li, W., Peng, H., Ning, F., Yao, L., Luo, M., Zhao, Q., Zhu, X., & Xiong, H. (2014). Amphiphilic chitosan derivative-based core-shell micelles: synthesis, characterisation and properties for sustained release of Vitamin D3. *Food Chem, 152*, 307-315.

Li, Y., & McClements, D. J. (2010). New mathematical model for interpreting pH-stat digestion profiles: impact of lipid droplet characteristics on in vitro digestibility. *J Agric Food Chem, 58*(13), 8085-8092.

Luo, Y., Teng, Z., & Wang, Q. (2012). Development of zein nanoparticles coated with carboxymethyl chitosan for encapsulation and controlled release of vitamin D3. *J Agric Food Chem, 60*(3), 836-843.

Marze, S., Meynier, A., & Anton, M. (2013). In vitro digestion of fish oils rich in n-3 polyunsaturated fatty acids studied in emulsion and at the oil-water interface. *Food Funct, 4*(2), 231-239.

McClements, D. J., Decker, E. A., Park, Y., & Weiss, J. (2009). Structural design principles for delivery of bioactive components in nutraceuticals and functional foods. *Crit Rev Food Sci Nutr, 49*(6), 577-606.

McClements, D. J., & Li, Y. (2010). Structured emulsion-based delivery systems: controlling the digestion and release of lipophilic food components. *Adv Colloid Interface Sci, 159*(2), 213-228.

McClements, D. J., & Xiao, H. (2012). Potential biological fate of ingested nanoemulsions: influence of particle characteristics. *Food Funct, 3*(3), 202-220.

Mun, S., Decker, E. A., & McClements, D. J. (2007). Influence of emulsifier type on in vitro digestibility of lipid droplets by pancreatic lipase. *Food Research International, 40*(6), 770-781.

Nik, A. M., Corredig, M., & Wright, A. J. (2011). Release of lipophilic molecules during in vitro digestion of soy protein-stabilized emulsions. *Mol Nutr Food Res, 55 Suppl 2*, S278-289.

Ozturk, B., Argin, S., Ozilgen, M., & McClements, D. J. (2014). Formation and stabilization of nanoemulsion-based vitamin E delivery systems using natural surfactants: Quillaja saponin and lecithin. *Journal of Food Engineering, 142*, 57-63.

Porter, C. J. H., & Charman, W. N. (1997). Uptake of drugs into the intestinal lymphatics after oral administration. *Adv Drug Deliv Rev, 25*(1), 71-89.

Porter, C. J. H., Trevaskis, N. L., & Charman, W. N. (2007). Lipids and lipid-based formulations: optimizing the oral delivery of lipophilic drugs. *Nature Reviews Drug Discovery, 6*(3), 231-248.

535 Qian, C., Decker, E. A., Xiao, H., & McClements, D. J. (2012). Nanoemulsion
536 delivery systems: influence of carrier oil on beta-carotene bioaccessibility.
537 *Food Chem, 135*(3), 1440-1447.
538 Rao, J., Decker, E. A., Xiao, H., & McClements, D. J. (2013). Nutraceutical
539 nanoemulsions: influence of carrier oil composition (digestible versus
540 indigestible oil) on beta-carotene bioavailability. *J Sci Food Agric, 93*(13),
541 3175-3183.
542 Rao, J., & McClements, D. J. (2012). Impact of lemon oil composition on
543 formation and stability of model food and beverage emulsions. *Food*
544 *Chem, 134*(2), 749-757.
545 Reboul, E., Goncalves, A., Comera, C., Bott, R., Nowicki, M., Landrier, J. F.,
546 Jourdheuil-Rahmani, D., Dufour, C., Collet, X., & Borel, P. (2011).
547 Vitamin D intestinal absorption is not a simple passive diffusion:
548 Evidences for involvement of cholesterol transporters. *Mol Nutr Food Res,*
549 *55*(5), 691-702.
550 Salvia-Trujillo, L., Qian, C., Martin-Belloso, O., & McClements, D. J. (2013).
551 Modulating beta-carotene bioaccessibility by controlling oil composition
552 and concentration in edible nanoemulsions. *Food Chem, 139*(1-4), 878-
553 884.
554 Sarkar, A., Goh, K. K. T., & Singh, H. (2009). Colloidal stability and interactions
555 of milk-protein-stabilized emulsions in an artificial saliva. *Food*
556 *Hydrocolloids, 23*(5), 1270-1278.
557 Sek, L., Porter, C. J. H., Kaukonen, A. M., & Charman, W. N. (2002). Evaluation
558 of the in-vitro digestion profiles of long and medium chain glycerides and

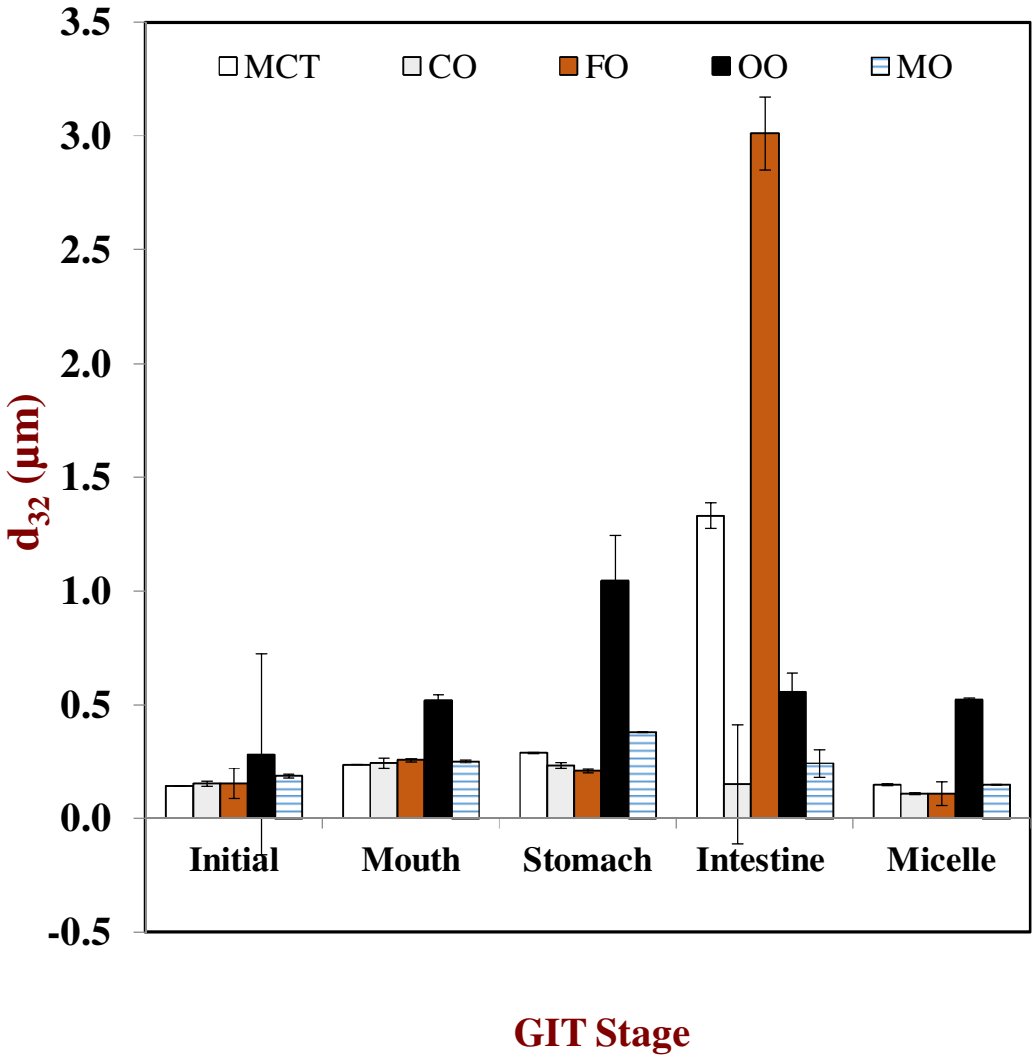
559 the phase behaviour of their lipolytic products. *Journal of Pharmacy and*
560 *Pharmacology, 54*(1), 29-41.
561 Singh, H., Ye, A., & Horne, D. (2009). Structuring food emulsions in the
562 gastrointestinal tract to modify lipid digestion. *Prog Lipid Res, 48*(2), 92-
563 100.
564 Teng, Z., Luo, Y., & Wang, Q. (2013). Carboxymethyl chitosan-soy protein
565 complex nanoparticles for the encapsulation and controlled release of
566 vitamin D(3). *Food Chem, 141*(1), 524-532.
567 van Aken, G. A., Vingerhoeds, M. H., & de Hoog, E. H. A. (2007). Food colloids
568 under oral conditions. *Current Opinion in Colloid & Interface Science,*
569 *12*(4-5), 251-262.
570 Vingerhoeds, M. H., Blijdenstein, T. B. J., Zoet, F. D., & van Aken, G. A. (2005).
571 Emulsion flocculation induced by saliva and mucin. *Food Hydrocolloids,*
572 *19*(5), 915-922.
573 Yang, Y., Leser, M. E., Sher, A. A., & McClements, D. J. (2013). Formation and
574 stability of emulsions using a natural small molecule surfactant: Quillaja
575 saponin (Q-Naturale®). *Food Hydrocolloids, 30*(2), 589-596.
576 Yang, Y., & McClements, D. J. (2013). Vitamin E bioaccessibility: influence of
577 carrier oil type on digestion and release of emulsified alpha-tocopherol
578 acetate. *Food Chem, 141*(1), 473-481.

579

580

581

582



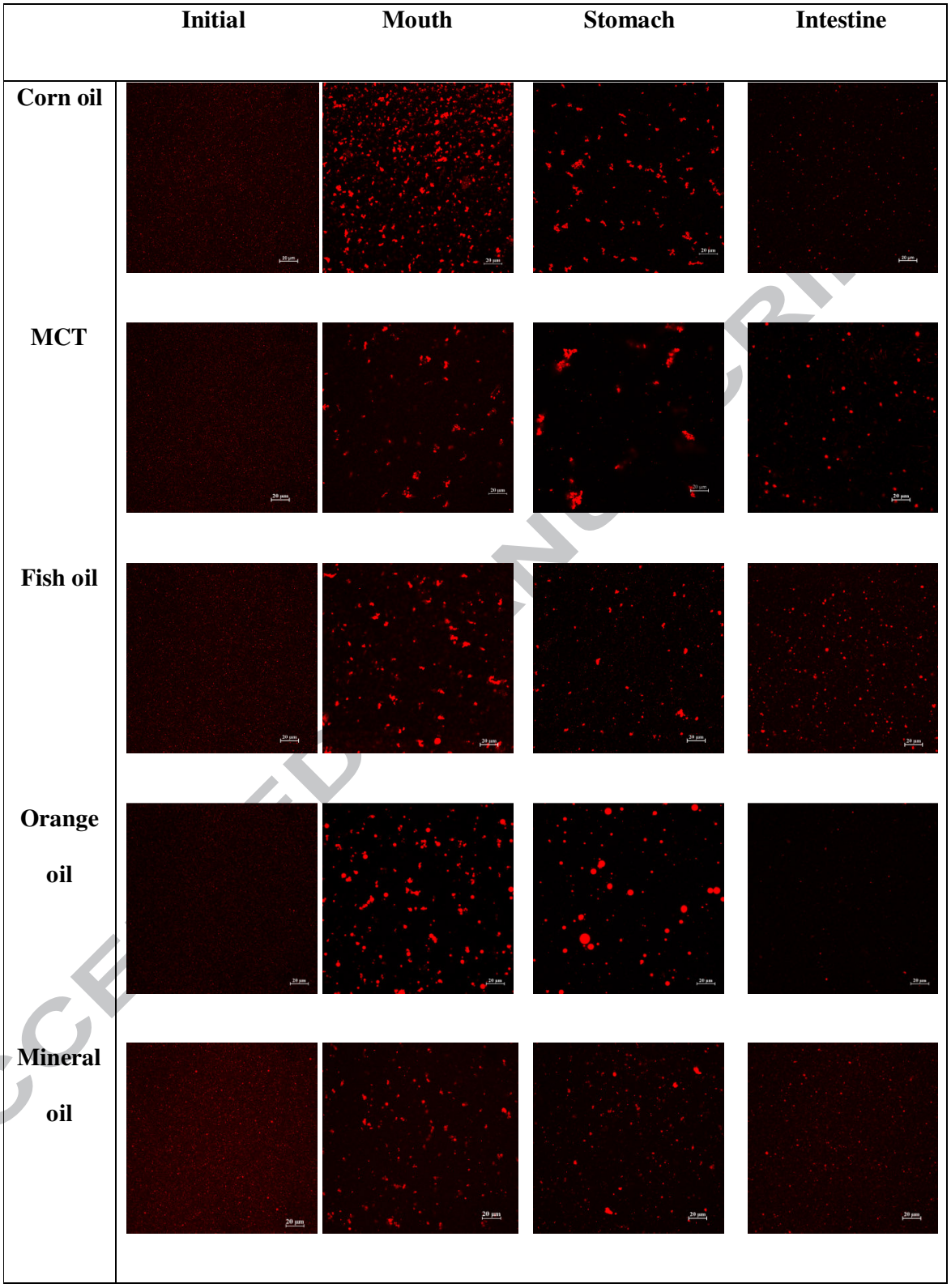
583

584

585 **Figure 1.** Influence of gastrointestinal tract (GIT) stage and oil type on the mean
586 particle diameter (d_{32}) of oil-in-water emulsions after exposure to different stages
587 of a simulated gastrointestinal model.

588

589

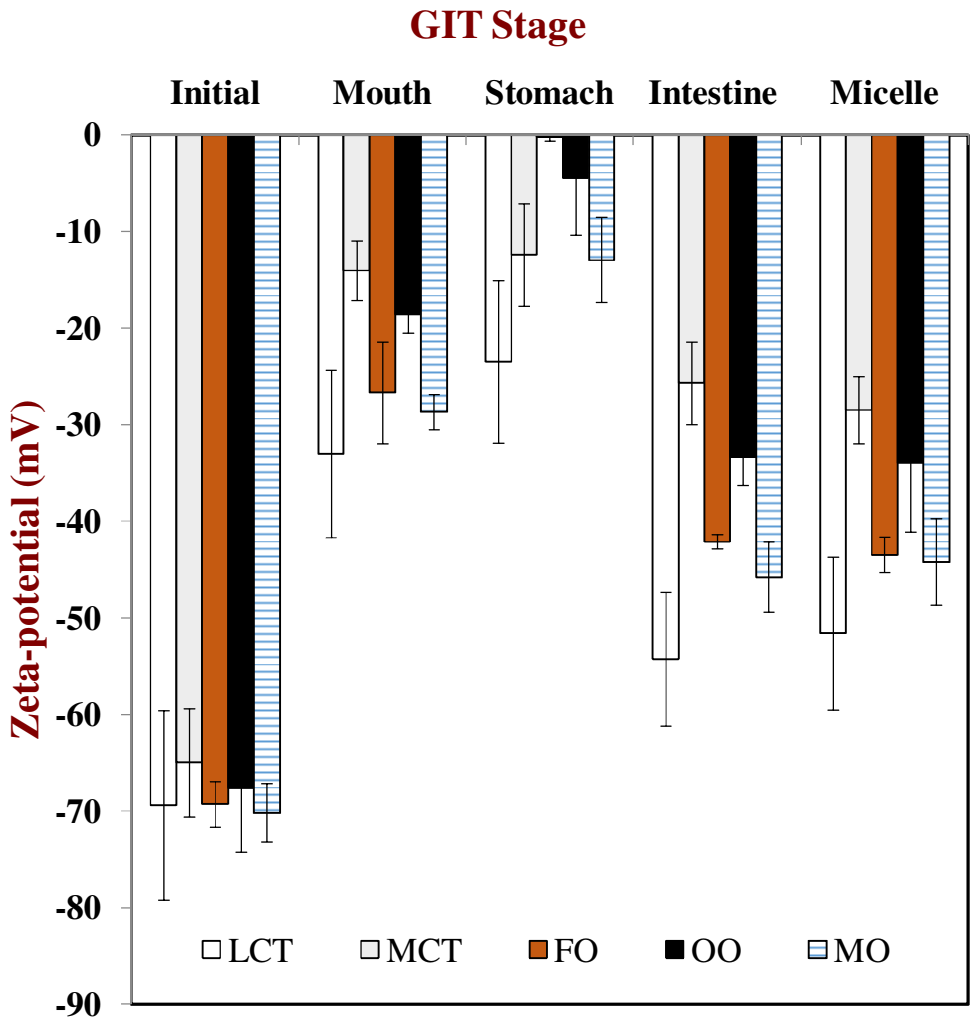


590

591 **Figure 2.** Influence of oil type and gastrointestinal tract stage on microstructure
592 (confocal fluorescence) of oil-in-water nanoemulsions containing Vitamin D_3.

593

594



595

596

597 **Figure 3.** Influence of gastrointestinal tract (GIT) stage and oil type on the
598 particle charge (ζ-potential) of oil-in-water nanoemulsions.

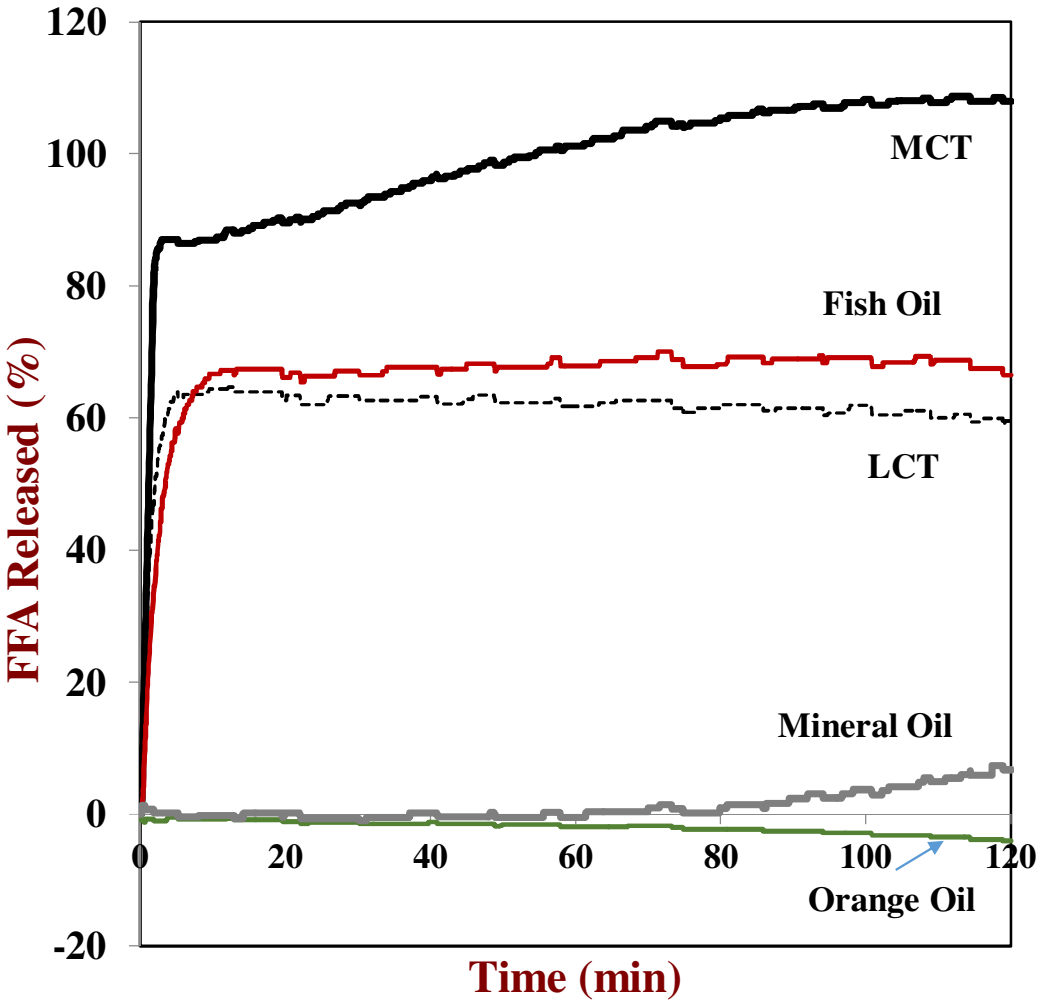


Figure 4. Release of free fatty acids (FFA) from nanoemulsions containing different oil types after exposure to simulated small intestine conditions.

607

608

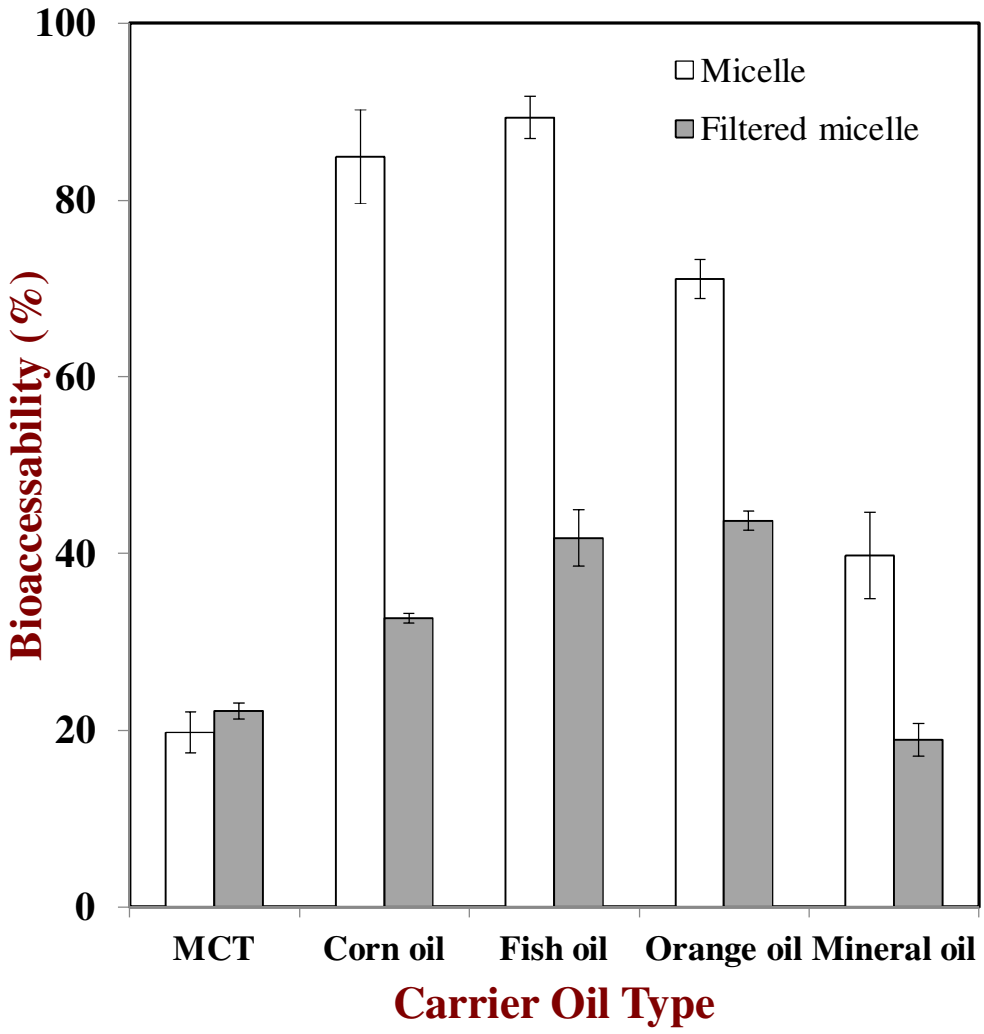
609

610

611

612

613



614

615 **Figure 5.** Influence of carrier oil type on the bioaccessibility of vitamin D_3
616 initially encapsulated within oil-in-water nanoemulsions. Measurements were
617 made before and after the micelle phase samples were filtered.

618

619

620

621

622

623

624 **Table 1:** Digital photographs of raw digesta (R) and the micelle phase (M), which

625 was collected after centrifugation (4000 rpm, 40 min, 25˚ C).

MCT		Corn oil		Fish oil		Orange oil		Mineral oil	
R	**M**	**R**	**M**	**R**	**M**	**R**	**M**	**R**	**M**
									

626

627

Nanoemulsion Delivery Systems for Oil-Soluble Vitamins: Influence of Carrier Oil Type on Lipid Digestion and Vitamin D_3 Bioaccessibility

Ozturk *et al.*

Food Chemistry

Highlights

- Vitamin-enriched nanoemulsions were prepared using a natural surfactant
- Lipid digestion and vitamin D_3 bioaccessibility strongly depend on carrier oil type
- Medium chain triglycerides were rapidly digested, but gave low bioavailability
- Long chain triglycerides were most effective at increasing vitamin bioaccessibility