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Nanoemulsion Delivery Systems for Oil-Soluble Vitamins:

Influence of Carrier Oil Type on Lipid Digestion and Vitamin D₃ Bioaccessibility

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ABSTRACT

The influence of carrier oil type on the bioaccessibility of vitamin D₃ 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 D₃ 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 D₃; 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 D₂ (ergocalciferol) and D₃ (cholecalciferol) (Luo, Teng, & Wang, 2012). Vitamin D₃
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.* calcidiol, calcitriol, 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 D₃ 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; 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 D₃, 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 D₃ and mineral oil were purchased from Sigma-Aldrich (St. Louis, MO). Orange oil (10x) 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 D₃ 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 (d₃₂), 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 D₃ 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 D₃.

Mouth stage: Simulated artificial saliva solution (SASS) was prepared according to a previous study (Y. Li & McClements, 2010; Sarkar, Goh, & Singh,
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$_2$O 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
calculated according to Li and McClements (2010). *In vitro* digestion in the small intestine stage lasted for 2 h and then physicochemical and structural characterization of the samples at each stage were performed.

2.2.4. Bioaccessibility determination

The bioaccessibility of lipophilic components is normally defined as the fraction that is solubilized within the mixed micelle phase after lipid digestion (Carbonell-Capella, Buniowska, Barba, Esteve, & Frigola, 2014; Marze, Meynier, & Anton, 2013). After the full digestion, two portions of 10 ml of samples were collected and centrifuged (4000 rpm, Thermo Scientific, CL10 centrifuge) at 25 °C for 40 min. The emulsions separated into an opaque sediment phase at the bottom, a clear micelle phase in the middle, and sometimes a thin creamed phase at the top or on the wall of the centrifuge tube. An aliquot (1 ml) of micelle phase or raw digesta sample was vortexed after adding an organic solvent mixture (1:3 isooctane: ethyl alcohol) at 1:5 to extract the vitamin D₃ and then centrifuged at 1750 rpm for another 10 min. The supernatant phases were used as samples for determination of vitamin D₃ using an UV-Vis Spectrophotometer at 265 nm wavelength (Ultrospec 3000 pro Pharmacia Biotech, Biochrom Ltd., Cambridge, UK).

Absorbances of different concentrations of vitamin D₃ standard were measured at 265 nm wavelength to obtain a calibration curve for vitamin D₃ concentration versus absorbance ($r^2 = 0.9996$).

2.2.5. Microstructural analysis

A Nikon Confocal Fluorescent Microscope (C1 Digital Eclipse, Tokyo, 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
D₃.

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

Again, with the exception of orange oil, there was only a slight increase in the
mean particle diameters of the nanoemulsions after incubation in artificial saliva
and gastric solutions (Figure 1), and the particle size distributions remained
monomodal (data not shown), which suggested that these nanoemulsions were
stable against coalescence under simulated mouth (pH 6.8, 10 min) and stomach
(pH 2.5, 2 h) conditions. This protection can be attributed to the strong steric
stabilizing effect of the natural surfactant (Q-Naturale) used. In a previous study,
we reported that Q-Naturale coated lipid droplets were stable against droplet
coalescence over a wide range of pH conditions i.e., pH 3 to 8 (Ozturk, Argin,
Ozilgen, & McClements, 2014). Moreover, there were no highly surface active
components present within the simulated saliva or gastric environments that might
have caused emulsion instability by adsorbing to the surfaces of the Q-Naturale
coated droplets. Finally, Q-Naturale would be expected to be resistant to pepsin
digestion within the gastric fluids, which may increase the stability of the
emulsions to coalescence in the stomach phase. Having said this, the confocal
microscopy images indicated that appreciable droplet flocculation occurred under
mouth and stomach conditions (Figure 2). Droplet flocculation may have
occurred due to electrostatic screening effects (due to mineral ions), loss of
droplet charge (under acidic conditions), or bridging or depletion attraction (due
to mucin). Presumably, these flocs were relatively weak and broke down when
the emulsions were diluted for the light scattering experiments. In contrast, there
was a relatively large increase in mean particle diameter of the orange oil emulsions as they passed from the initial to mouth to stomach stages, which may be due to Ostwald ripening or coalescence leading to droplet growth. Indeed, large spherical droplets were observed in this sample in the stomach phase (whereas irregular shaped particles were observed in the other nanoemulsions), which suggests that the individual oil droplets had grown in size rather than become flocculated.

There were appreciable changes in the particle size and microstructure of the nanoemulsions samples after they were incubated in the small intestine phases, which depended on carrier oil type (Figures 1 and 2). These changes may have occurred for a number of reasons, such as lipid digestion, droplet aggregation, mixed micelle formation, or generation of insoluble sediments (such as calcium soaps). Lipid digestion might be expected to reduce the size of the individual lipid droplets since some of the oil phase would be removed. However, lipid digestion may also promote droplet aggregation (flocculation or coalescence) due to changes in interfacial and core characteristics. In addition, aggregation may be promoted due to droplet interactions with other components within the small intestinal fluids, such as mineral ions, bile salts, or proteins. The mixed micelles formed from lipid digestion products actually consist of a complex mixture of different types of colloidal particles, such as micelles, vesicles, bilayers, and liquid crystals. Finally, any long chain fatty acids generated during lipid digestion may form insoluble calcium soaps. All of these different types of colloidal particles contribute to the light scattering pattern measured in a particle size analyzer, which makes it difficult to accurately interpret particle size measurements made on intestinal digesta (McClements, Decker, Park, & Weiss, 2012).
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
molecules (Ozturk, Argin, Ozilgen, & McClements, 2014; Yang, Leser, Sher, & McClements, 2013). There were some differences in the magnitude of the electrical charge on droplets containing different types of carrier oil (Figure 3). This may have been due to differences in the susceptibility of the emulsions to competitive adsorption effects.

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

3.2. Influence of carrier oil type on in vitro digestion

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

$$\text{FFA(\%)} = 100 \times \left( V_{\text{NaOH}} \times m_{\text{NaOH}} \times M_{\text{Lipid}} \right) / \left( w_{\text{Lipid}} \times 2 \right)$$  

(1)
Where $V_{\text{NaOH}}$ is the volume of sodium hydroxide required to neutralize the FFAs produced (L), $m_{\text{NaOH}}$ is the molarity of sodium hydroxide solution used (in M), $M_{\text{Lipid}}$ is the molecular weight of the triacylglycerol oil (in g/mol), and $w_{\text{Lipid}}$ 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
mineral oil would not be expected to be digested by lipase, which would account for the fact that there was little increase in the calculated FFAs released over time (Figure 4).

3.3. Influence of carrier oil type on vitamin D3 bioaccessibility

In this series of experiments, the influence of carrier oil type on vitamin D₃ bioaccessibility after full digestion by the simulated GIT system was studied. The bioaccessibility of vitamin D₃ was determined by measuring its concentrations in the micelle phase and the total digesta using solvent extraction and UV-Vis spectrophotometry. The bioaccessibility was then calculated using the equation below:

\[
\text{Bioaccessibility} = 100 \times \frac{C_{\text{Micelle}}}{C_{\text{Raw Digesta}}} \tag{2}
\]

Where \(C_{\text{Micelle}}\) and \(C_{\text{Raw Digesta}}\) are the concentrations of vitamin D₃ in the micelle and total digesta samples after the small intestine stage (end of pH-STAT experiment). The bioaccessibility was also measured after filtration of the micelle phase (with 0.45 µm pore size). In the human body, mixed micelles and other colloidal particles must pass through the mucus layer that lines the GIT prior to absorption, which has been reported to have a pore size around 0.4 µm (Cone, 2009). Consequently, the filtration step used in this study may have removed some of the larger particles that would not be expected to pass through the mucus layer.

The bioaccessibility was highly dependent on the type of carrier oil present in the nanoemulsions (Figure 5). For the digestible oils, the bioaccessibility of vitamin D₃ was appreciably higher from the nanoemulsions containing LCTs (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 D₃ 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. C₁₆ to C₁₈) whereas MCT contains medium chain fatty acids (e.g. C₈ to C₁₀). 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 D₃ 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 D₃ 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 D₃) 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 D₃ 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

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the phase behaviour of their lipolytic products. *Journal of Pharmacy and Pharmacology, 54*(1), 29-41.


Figure 1. Influence of gastrointestinal tract (GIT) stage and oil type on the mean particle diameter ($d_{32}$) of oil-in-water emulsions after exposure to different stages of a simulated gastrointestinal model.
<table>
<thead>
<tr>
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<th>Initial</th>
<th>Mouth</th>
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<th>Intestine</th>
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Figure 2. Influence of oil type and gastrointestinal tract stage on microstructure (confocal fluorescence) of oil-in-water nanoemulsions containing Vitamin D₃.

Figure 3. Influence of gastrointestinal tract (GIT) stage and oil type on the 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.
Figure 5. Influence of carrier oil type on the bioaccessibility of vitamin D₃ initially encapsulated within oil-in-water nanoemulsions. Measurements were made before and after the micelle phase samples were filtered.
**Table 1**: Digital photographs of raw digesta (R) and the micelle phase (M), which was collected after centrifugation (4000 rpm, 40 min, 25°C).

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Nanoemulsion Delivery Systems for Oil-Soluble Vitamins: Influence of Carrier Oil Type on Lipid Digestion and Vitamin D₃ Bioaccessibility

Ozturk et al.  
*Food Chemistry*

**Highlights**

- Vitamin-enriched nanoemulsions were prepared using a natural surfactant
- Lipid digestion and vitamin D₃ 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