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Vitamin E bioaccessibility: Influence of carrier oil type on digestion and release of emulsified α-tocopherol acetate

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ABSTRACT

Vitamin E is an essential micronutrient for humans and animals due to its antioxidant and non-antioxidant biological activities. The α-tocopherol acetate form is often used in foods and other products due to its high biological activity and chemical stability. In this study, we examined the influence of carrier oil type on the bioaccessibility and molecular form of emulsified vitamin E using a simulated gastrointestinal model. Oil-in-water emulsions containing α-tocopherol acetate were prepared using quillaja saponin as a natural surfactant, and either long chain triacylglycerols (LCT) or medium chain triacylglycerols (MCT) as carrier oils. The rate and extent of lipid digestion was higher for MCT- than LCT-emulsions, which was attributed to differences in the water dispersibility of the free fatty acids formed during lipolysis. Conversely, the total bioaccessibility of vitamin E after digestion was higher for LCT- than MCT-emulsions, which was attributed to the greater solubilisation capacity of mixed micelles formed from long chain fatty acids. The conversion of α-tocopherol acetate to α-tocopherol after *in vitro* digestion was also considerably higher for LCT- than MCT-emulsions, which may impact the subsequent absorption of vitamin E. Overall, this research has important implications for the design and fabrication of effective emulsion-based delivery systems for increasing the bioavailability of vitamin E.

1. Introduction

Vitamin E is known to be an essential micronutrient for maintaining the health and wellbeing of humans and other animals due to its antioxidant and non-antioxidant biological activities (Brigelius-Flohe & Galli, 2010; Burton & Traber, 1990; Zingg & Azzi, 2004). Nevertheless, the precise biological mechanisms by which it exerts its beneficial effects are still unclear, and are currently the focus of extensive research efforts (Brigelius-Flohe & Galli, 2010; Golli & Azzi, 2010). The term "vitamin E" actually refers to a group of related fat-soluble molecules that are found in many natural sources and are widely used as functional ingredients in food, pharmaceutical, and cosmetic preparations (Chiu & Yang, 1992; Rigotti, 2007). The most important biologically active form of vitamin E is α-tocopherol, and therefore this form tends to be used in commercial products. There has been considerable interest in fortifying foods, beverages, and supplements with vitamin E due to its beneficial nutritional qualities (McClements, Decker, Park, & Weiss, 2009; Yang & Huffman, 2011). However, there are a number of challenges associated with incorporating vitamin E into commercial products due to its chemical instability, poor water-solubility, and variable bioavailability.

The α-tocopherol form of vitamin E is highly unstable to oxidation and may therefore be lost during the processing, storage, and utilisation of commercial products due to its chemical degradation (Gawrysiak-Witulska, Siger, & Nogala-Kalucka, 2009; Yoon & Choe, 2009). For this reason, a more chemically stable esterified form (α-tocopherol acetate) is typically used in commercial products (rather than the free form) (Lauridsen, Hedemann, & Jensen, 2001). Another challenge associated with the utilisation of vitamin E is that it is a highly lipophilic molecule with a very low water-solubility, which means that it cannot be directly dispersed into aqueous media (Sagalowicz, Leser, Watzke, & Michel, 2006; Velikov & Pelan, 2008). Instead it must either be dispersed into a lipid phase or incorporated into a suitable delivery system (Gonnet, Lethuaut, & Boury, 2010; McClements, Decker, Park, & Weiss, 2009). A well-designed delivery system can encapsulate lipophilic nutraceuticals in a form that that does not adversely affect product quality (such as appearance, taste, texture, or stability), protect them from chemical degradation during storage, and increase their bioavailability after ingestion (Lesmes & McClements, 2009; McClements, Decker, Park, & Weiss, 2009). A wide variety of delivery systems have been developed for encapsulating lipophilic nutraceutical ingredients, including microemulsions, nanoemulsions, emulsions, solid lipid nanoparticles, and polymer particles (Flanagan & Singh, 2006; Matalanis, Jones, & McClements, 2011; McClements, 2011; McClements & Li, 2010b; Velikov & Pelan,

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2008). Each of these delivery systems has specific advantages and disadvantages related to the nature of the nutraceutical to be encapsulated, the food matrix that it is to be dispersed within, and the processing, storage, and transport conditions it is exposed to.

After ingestion, it is important that a nutraceutical component has a high bioavailability so that it can effectively deliver its beneficial biological effects. Previous studies have shown that the bioavailability of vitamin E may be highly variable (ranging from <1% to 100%) depending on its chemical form and the nature of the food matrix it is incorporated within (O'Callaghan & O'Brien, 2010; Reboul et al., 2006). Typically, vitamin E must first be released from the food matrix, then incorporated into mixed micelles, and subsequently transported across the mucous layer before it is absorbed by epithelium cells in the small intestine (Rigotti, 2007; Ullrey, 1972). The mixed micelles are comprised of bile salts and phospholipids from the bile and pancreatic juices, as well as lipid digestion products (free fatty acids and monoacylglycerols) from the action of lipases on ingested triacylglycerol oils (Porter, Trevaskis, & Charman, 2007). The bioaccessibility of lipophilic nutraceuticals and drugs has previously been shown to increase as the total amount of mixed micelles increases, as well as to depend on nature of the mixed micelles formed after lipid digestion (Huo, Ferruzzi, Schwartz, & Failla, 2007; Porter et al., 2007; Pouton & Porter, 2008), which may account for some of the observed variations in vitamin E bioavailability. It has also been reported that the esterified form of vitamin E (α-tocopheryl acetate) is less bioaccessible than the free form (α-tocopherol) (Chung, Mahan, & Lepine, 1992; Eicher, Morrill, & Velazco, 1997; Hidiroglou, Laflamme, & McDowell, 1988; Hidiroglou, McDowell, & Pastrana, 1988), so it has to be converted into α-tocopherol by digestive enzymes (*e.g.*, pancreatic esterases) within the gastrointestinal tract prior to absorption (Burton & Traber, 1990; Herrero-Barbudo, Granado-Lorencio, Blanco-Navarro, Perez-Sacristan, & Olmedilla-Alonso, 2009). However, studies using an *in vitro* digestion model have shown that α-tocopheryl acetate may be absorbed by Caco-2 cells to a similar extent as α-tocopherol (Brisson et al., 2008). Some of the observed variations in vitamin E bioaccessibility may therefore also be due to differences in the chemical nature of the ingested form, *e.g.*, free *versus* esterified. The wide variability reported in the bioavailability of vitamin E makes it difficult to interpret the results of clinical trials on the efficacy of vitamin E supplementation, and to develop appropriate guidelines on the consumption levels of vitamin E (Rigotti, 2007). The development of suitable delivery systems for vitamin E may be able to overcome some of the challenges associated with its varying bioavailability.

In this study, we utilised an emulsion-based delivery system fabricated from a natural surfactant (quillaja saponin) that we have recently shown to be suitable for encapsulating vitamin E acetate (Yang & McClements, 2013). There is a major drive in the food industry towards developing consumer friendly labels *e.g.*, "all natural" claims. There are few natural small molecule surfactants currently available for utilisation within the food industry. Hence, the availability of a natural surfactant to form and stabilise emulsions would be an advantage for many functional food and beverage applications. Our primary focus was to examine the influence of carrier oil composition (medium chain *versus* long chain fatty acids) on the bioaccessibility of vitamin E acetate using an *in vitro* gastrointestinal tract (GIT) model, since previous studies have shown that the nature of the ingested fatty acids influences the bioaccessibility of lipophilic nutraceuticals and drugs (Ahmed, Li, McClements, & Xiao, 2012; Dahan & Hoffman, 2007; Han et al., 2009; Sek, Porter, Kaukonen, & Charman, 2002). We measured the change in the microstructure and properties of vitamin E-enriched fat droplets throughout the simulated GIT (mouth, stomach, and small intestine). We also monitored the bioaccessibility and conversion of α-tocopherol acetate to α-tocopherol after the small intestine stage. The results of this study provide valuable information about the potential biological fate of ingested emulsion-based delivery systems for vitamin E, as well as their potential for controlling the bioavailability of this important nutrient in functional foods and beverages.

2. Materials and methods

2.1. Materials

Quillaja saponin (Q-Naturale® 200) was provided by National Starch LLC (Bridgewater, NJ). This ingredient is reported to contain 14.1% of saponin dispersed within an aqueous solution. Corn oil (Mazola, ACH Food Companies, Inc., Memphis, TN) was used as an example of a long chain triglyceride (LCT) and was purchased from a local supermarket. The manufacturer reported that the corn oil contained 14% saturated fat, 29% monounsaturated fat, and 57% polyunsaturated fat. Medium chain triglyceride (MCT) oil (Miglyol 812) was purchased from Coletica (Northport, NY). Vitamin E acetate was kindly supplied by BASF (Florham Park, NJ). Lipase from porcine pancreas pancreatin (activity >2.0 USP units/mg, Type II, L3126, Batch # 020M1589) and bile extract (porcine) were also obtained from the Sigma Chemical Company (St. Louis, MO). Type II lipase contains amylase and protease, as well as lipases. All other chemicals used were of analytical grade. Double distilled water was used for the preparation of all solutions and emulsions. Double distilled water was obtained using a Milli-Q® water purification system (Millipore, Billerica, MA).

2.2. Emulsion preparation

Oil-in-water emulsions were prepared by homogenising 10 wt.% lipid phase and 90 wt.% aqueous phase. The lipid phase consisted of vitamin E acetate dispersed in either corn oil (LCT) or medium chain triglyceride oil (MCT). The aqueous phase consisted of surfactant (1 wt.% Q-Naturale®) and buffer solution (10 mM sodium phosphate buffer, pH 7.0). A coarse emulsion premix was prepared by blending the lipid and aqueous phases together using a high-speed mixer (Bamix, Biospec Products, Bartlesville, OK) for 2 min at room temperature. Emulsions containing fine droplets were formed by passing the coarse emulsions through an air-driven microfluidizer (Microfluidics, Newton, MA, USA) four times at 9000 psi. The Q-Naturale® ingredient contained 14.1% of surfactant (active ingredient) dispersed within an aqueous solution, and therefore we reported its concentration based on the amount of active surfactant present (rather than the amount of overall ingredient).

2.3. Particle characterisation

The particle size distributions of the samples were measured using a static light scattering instrument (Mastersizer 2000, Malvern Instruments, Malvern, UK). The droplet charge (ζ-potential) of emulsions was determined using a particle electrophoresis instrument (Zetasizer Nano ZS series, Malvern Instruments, Worcestershire, UK). A refractive index of 1.33 was used for the aqueous phase, and refractive indices of 1.445 and 1.472 were used for oil phases containing MCT/vitamin E and corn oil/vitamin E, respectively. For the droplet charge measurements it was assumed that the physicochemical properties of the continuous phase were the same as pure water. Samples were diluted with buffer solutions of the appropriate pH prior to analysis to avoid multiple scattering effects: *initial* – PBS buffer (pH 7.0); *mouth* – simulated

saliva without mucin (pH 6.8); *stomach* – pH 2.5 buffer; *small intestine* – PBS buffer (pH 7.0).

2.4. In vitro digestion

Each emulsion sample was passed through a three-step simulated GIT model, which includes a mouth, gastric, and small intestine stage.

Mouth stage: Simulated saliva fluid (SSF) was prepared according to a previous study (Sarkar, Goh, & Singh, 2009). 10 ml of emulsion (1 w/w% oil) was placed in a 250 ml flask containing 10 ml of SSF. This mixture was adjusted to pH 6.8 and then shaken continuously at the 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).

Gastric stage: Simulated gastric fluid (SGF) was prepared by placing 2 g NaCl, 7 ml HCl, and 3.2 g pepsin into a flask, adding water to make up to 1 L, and then adjusting the pH to 1.2 using 1.0 M HCl (United States Pharmacopeial Convention, 2000). The sample from the mouth stage was then mixed with SGF at a 1:1 mass ratio so that the final mixture contained 0.5% (w/w) oil. The mixture was then adjusted to pH 2.5 by using 1 M NaOH and incubated with continuous shaking at 100 rpm at 37 °C for up to 2 h to mimic stomach conditions.

Small intestinal stage: Digesta samples (30 ml) from the simulated gastric stage were added to a clean beaker, then incubated in a water bath (37 °C) for 10 min, and then adjusted to pH 7 using NaOH solution (range from 0.05 to 1 M). The mixture was then incubated for 2 h at 37 °C with simulated small intestinal fluid (SIF) containing 2.5 ml pancreatic lipase (4.8 mg ml^{-1}), 4 ml bile extract solution (5 mg ml^{-1}) and 1 ml calcium chloride solution (750 mM). A pH-stat (Metrohm, USA Inc.) was used to monitor and control the pH (at pH 7.0) of the digestion solution after the SIF was added (Li & McClements, 2010). The amount of alkali solution (0.25 M NaOH) that had to be added to the reaction chamber to maintain the pH at 7.0 was recorded, and used to determine the percentage of free fatty acids (FFA) released from the system (McClements & Li, 2010a). A control (containing Q-Naturale but no oil) was run under the same conditions as the samples, and the amount of alkali titrated into the reaction chamber for the control was subtracted from that for the samples before calculating the FFA released. Samples were also taken for physicochemical and structural characterisation after 2 h *in vitro* digestion in the small intestinal stage.

2.5. Bioaccessibility determination

The bioaccessibility of lipophilic bioactive components is normally defined as the fraction that is solubilised within the mixed micelle phase after lipid digestion (Marze, 2013; Reboul et al., 2006). After the full *in vitro* digestion was completed, 10 ml of sample was 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 oily or creamed phase at the top. An aliquot (0.5 ml) of the micelle phase or the raw digesta was vortexed with an organic solvent mixture (1:3 isopropanol and isooctane) at 1:5 to extract the vitamin E and then centrifuged at 1750 rpm for another 10 min. 1 ml of the top layer was removed and dried using a vacuum centrifuge drier and stored in the −80 °C refrigerator prior to further analysis. Before detection by HPLC, samples were dissolved in 200 μl methanol.

HPLC detection: Vitamin E concentrations in the samples were determined using HPLC (Shimadzu, Kyoto, Japan). A C18 reverse-phase column (150 – 4.6 mm, 5 μm, Beckman Coulter) was used for the chromatographic separation of α-tocopherol acetate and α-tocopherol. The flow rate of the mobile phase was 1.0 ml/min and the sample injection volume was 20 μl. An isocratic elution was carried out using HPLC-grade solvent (95% methanol and 5% double distilled water containing 0.5% phosphoric acid). The α-tocopherol acetate and α-tocopherol contents were determined using a PDA detector at 295 nm. Tocopherol quantification was determined using external standards. The overall bioaccessibility of vitamin E was estimated using the following expression:

$$\text{Bioaccessibility} = \frac{C_{\text{micelle}}}{C_{\text{Total}}} \times 100\% \quad (1)$$

Here C_{micelle} and C_{Total} represent the total concentration of vitamin E (α-tocopherol acetate + α-tocopherol) in the micelle phase and in the overall system after digestion, respectively. The percentage of specific forms of vitamin E solubilised within the micelle phase was also calculated using the same expression, but for α-tocopherol acetate and for α-tocopherol separately. The conversion of α-tocopherol acetate to α-tocopherol in the overall system after digestion was calculated from the following expression:

$$\text{Conversion} = \frac{C_{\text{VE}}}{C_{\text{Total}}} \times 100\% \quad (2)$$

Here C_{VE} and C_{Total} represent the concentration of α-tocopherol and the total concentration of vitamin E (α-tocopherol acetate + α-tocopherol) in the overall system after digestion, respectively.

A preliminary experiment was carried out to estimate the recovery of the total tocopherols using the solvent extraction and HPLC analysis method described above. The recovery of the total tocopherols (VE + VE acetate) was always >90%, which indicates that the methods used were appropriate.

2.6. Statistical analysis

All measurements were performed on at least two freshly prepared samples (*i.e.*, new samples were prepared for each series of experiments) and are reported as means and standard deviations. One-Way Analysis of Variance (ANOVA) of independent variables followed by a Tukey HSD test was carried out on the measurements to establish significant differences ($p < 0.05$).

3. Results and discussion

3.1. Impact of carrier oil type on biological fate of emulsions

3.1.1. Influence on particle size

Initially, we studied the impact of vitamin E encapsulation and carrier oil type on the potential biological fate of emulsion-based delivery systems using a simulated gastrointestinal tract (GIT) model. A series of emulsions containing 2.5 wt.% lipid phase were produced using either long chain triglycerides (LCT) or medium chain triglycerides (MCT). Emulsions containing lipid phases with four different compositions were prepared: LCT (100% LCT); vitamin E in LCT (25% α-tocopherol acetate + 75% LCT); MCT (100% MCT); and, vitamin E in MCT (25% α-tocopherol acetate + 75% MCT).

The influence of vitamin E encapsulation and carrier oil type on the mean particle diameter after each stage of the simulated GIT model was measured (Fig. 1a). The full particle size distributions of selected samples were also compared: (i) emulsions containing different carrier oil types after the small intestine stage (Fig. 1b); emulsions containing LCT at different stages of the GIT model (Fig. 1c). All the initial emulsions had monomodal particle size distributions and relatively small mean particle diameters (d_{32} = 228 to 270 nm). The droplets in the LCT emulsions were slightly larger than those in the MCT emulsions ($p < 0.1$), and the droplets in the emulsions containing vitamin E were significantly larger than

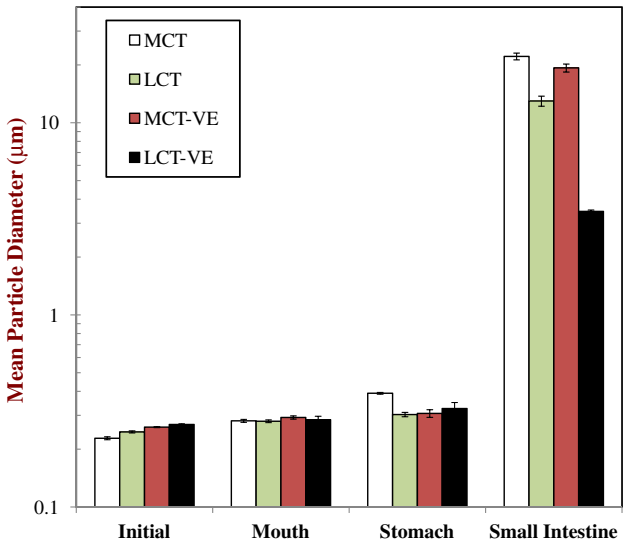

Fig. 1a. Influence of vitamin E encapsulation and carrier oil type on the mean particle diameter (d_{32}) of oil-in-water emulsions passing through different stages of a simulated gastrointestinal tract. *Key:* LCT = 100% LCT; LCT-VE = 25% α-tocopherol acetate + 75% LCT; MCT = 100% MCT; MCT-VE = 25% α-tocopherol acetate + 75% MCT.

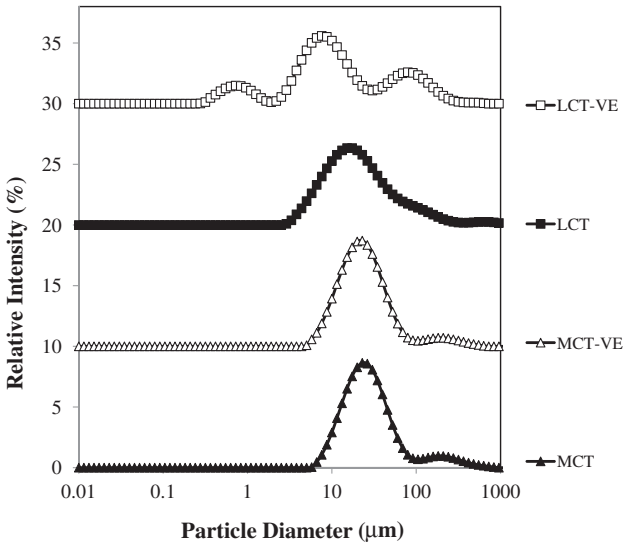

Fig. 1b. Influence of vitamin E encapsulation and carrier oil type on the particle size distributions of emulsions after passage through the small intestinal stage of a simulated gastrointestinal model. See caption of Fig. 1a for *Key*.

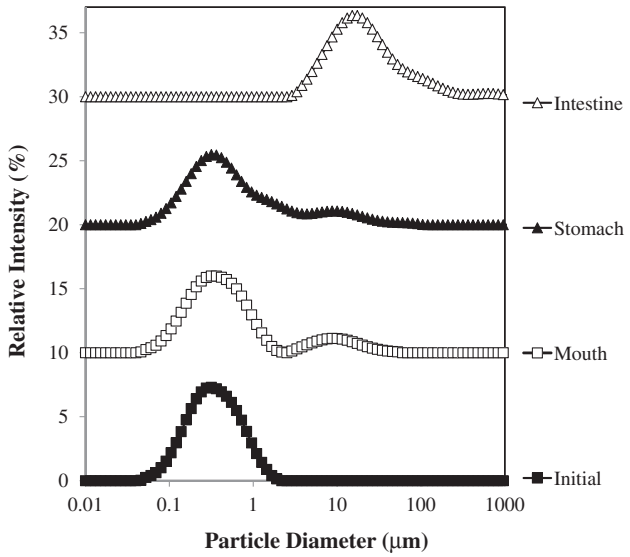

Fig. 1c. Particle size distributions of emulsions containing LCT at different stages of a simulated gastrointestinal tract.

those containing no vitamin E ($p < 0.05$), which may have been due to differences in oil phase viscosities. The size of the droplets produced by high pressure homogenisation is known to increase as the viscosity of the oil phase increases (Cheng & McClements, 2011; Walstra, 1993). After passage through the simulated mouth and gastric stages, the mean particle diameter remained relatively small ($d_{32} < 400$ nm), suggesting that the oil droplets were fairly stable to aggregation under these conditions (Figs. 1a–c). Recent studies have shown that oil droplets coated by the same quillaja saponin extract used in this work (Q-Naturale®) were relatively stable to changes in pH and salt concentration (Yang, Leser, Sher, & McClements, 2013). Any saponin molecules adsorbed to fat droplet surfaces would not be expected to be hydrolysed by proteases (pepsin) in the gastric juices, which can cause instability of protein-coated fat droplets. In addition, we would not have expected any saponin molecules adsorbed to the fat droplet surfaces to be displaced due to competitive adsorption processes since the oral and gastric fluids used in this study did not contain any highly surface-active substances.

After passage through the simulated small intestinal stage there was a large increase in mean particle diameter (Fig. 1a) and evidence of large particles in the particle size distributions (Fig. 1b) for all emulsions studied. These results indicate that there was a marked reduction in the physical stability of the emulsions after exposure to small intestinal conditions. Several physicochemical phenomena may explain the observed reduction in emulsion stability in the small intestinal stage. First, the intestinal juices contained bile salts and phospholipids that are highly surface active molecules that may have displaced some or all of the quillaja saponins from the oil droplet surfaces. Second, the intestinal juices contained pancreatic lipase that is capable of adsorbing to oil droplet surfaces and converting triacylglycerols (such as those in LCT or MCT oils) into free fatty acids (FFAs) and monoacylglycerols (MAGs). The digestion products of lipolysis may accumulate at the oil–water interface or move into the surrounding aqueous phase depending on their molecular weight and the presence of bile salts, phospholipids, and calcium (Porter et al., 2007; Pouton & Porter, 2008). The oil droplet size may therefore change appreciably as a result of alterations in their internal and interfacial compositions. One would expect that removal of FFA and MAG digestion products from fat droplets would lead to a reduction in their size (McClements, Decker, & Park, 2009; Singh, Ye, & Horne, 2009). On the other hand, one would expect that droplet coalescence, promoted by lipid digestion, would lead to an increase in their size (McClements, Decker, & Park, 2009; Singh et al., 2009). Consequently, the overall influence of lipid digestion on fat droplet size may be complicated. It should also be noted that particle size measurements made on complex colloidal systems containing mixtures of different particles (such as those produced by lipid digestion) should be treated with some caution. The software used by commercial particle sizing instruments to analyse light scattering measurements usually assumes that the particles are spherical, dilute, and have well-defined refractive indices. In practice, the colloidal suspension resulting from lipid digestion may contain undigested fat droplets, partially digested fat droplets, mixed micelles

and various other colloidal structures. In addition, each of these particles may be non-spherical and have different refractive indices, and may change due to dilution.

3.1.2. Influence on particle charge characteristics

Changes in the electrical characteristics of the particles in the emulsions were also measured after each stage in the simulated gastrointestinal tract to provide some information about changes in interfacial properties (Fig. 2). Initially, all of the oil droplets coated by quillaja saponin were highly negatively charged (−40 to −46 mV), which has previously been attributed to the ionisation of carboxyl groups (–COO^-) on the saponins at neutral pH (Yang et al., 2013). All of the droplets remained highly negative after passage through the simulated oral stage, although there was some reduction in the magnitude of their negative charge (Fig. 2). The fact that the charge remained highly negative can be attributed to the neutral conditions within the mouth, whereas the slight changes in charge may be due to adsorption of some ionised components from the simulated saliva to the droplet surfaces, *e.g.*, minerals or mucin (Sarkar et al., 2009).

The magnitude of the electrical charge on the droplets decreased appreciably after they were incubated in simulated gastric fluid, and the charge depended somewhat on carrier oil type (Fig. 2). The emulsions containing MCT had a slightly negative charge (≈−6 mV), whereas those containing LCT had a slightly positive charge (≈+0.5 mV). The origin of this difference in the charge on droplets containing different types of oils is currently unknown. One possible explanation is that the different oils phases contained different types and concentrations of ionic impurities (such as free fatty acids or phospholipids). Previous studies have shown that the electrical charge on oil droplets coated by quillaja saponin goes from highly negative at pH 7 to close to zero at pH 2, which was attributed to protonation of the carboxyl groups (–COOH) at pH values below their pK_a values (Yang et al., 2013). The low net charge on the oil droplets under gastric conditions can therefore be largely attributed to this effect. The fact that there was little change in the mean particle diameter of the droplets under simulated gastric conditions (Fig. 1a), suggests that the droplets were still coated by a layer of surface active material that prevented aggregation.

The electrical charge on the particles in all of the emulsions became strongly negative after they were incubated within simulated small intestinal fluid, and the charge depended somewhat on encapsulation of vitamin E and carrier oil type (Fig. 2). The negative charge on the droplets in the small intestine can be attributed to a number of different factors. First, the quillaja saponin has a relatively high negative charge at neutral pH (Yang et al., 2013), so if any of these molecules remained at the particle surfaces they would impart a negative charge. Second, the small intestinal fluids contained phospholipids and bile salts which are anionic surface active substances that may adsorb to particle surfaces and generate a negative charge. Third, lipase converts neutral triacylglycerol molecules into anionic free fatty acids, so if any of these molecules remain at the droplet surfaces they will also produce a negative charge. As mentioned earlier, it is not clear exactly what types of particles are detected by an electrophoresis instrument in a complex medium that contains many different types of charged particles that can scatter light.

3.2. Impact of encapsulation and carrier oil type on lipid digestion

The influence of vitamin E encapsulation and carrier oil type on the rate and extent of lipid digestion was measured using a pH-stat method. This approach is widely used by pharmaceutical and food researchers for the *in vitro* characterisation of lipid digestion under simulated small intestinal conditions (Dahan & Hoffman, 2008; McClements & Li, 2010a; Porter & Charman, 2001). The pH-stat method measures the amount of alkali (0.25 M NaOH) that had to be titrated into the digestion mixture to maintain neutral pH after addition of simulated small intestinal fluids containing lipase, bile salts, and $CaCl_2$.

In general, there was a rapid initial production of free fatty acids during the first few minutes of the digestion reaction, followed by a more gradual increase at longer times (Fig. 3), indicating that lipase was able to rapidly interact with the emulsified TAGs and convert them into FFAs and MAGs. Nevertheless, there were some distinct differences between emulsions prepared using different oil phases: (i) The extent of lipid digestion was higher for MCT- than LCT-emulsions; (ii) the extent of lipid digestion was higher for emulsions containing vitamin E than those containing no vitamin E. A

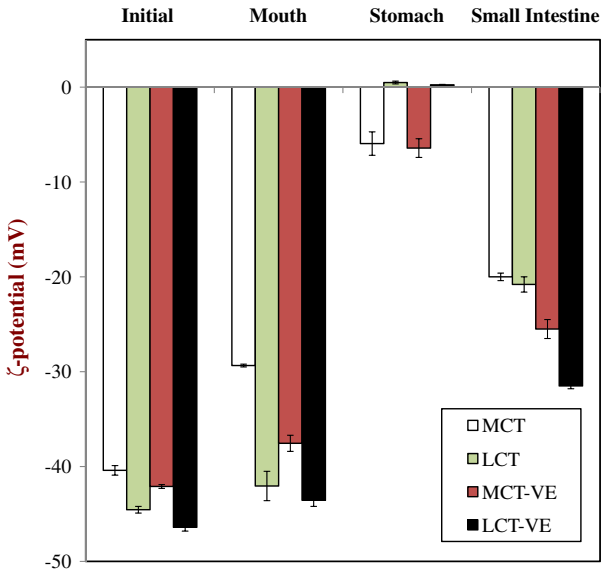

Fig. 2. Influence of vitamin E encapsulation and carrier oil type on the electrical characteristics ζ-potential of the particles in oil-in-water emulsions passed through different stages of a simulated gastrointestinal tract. See caption of Fig. 1a for *Key*.

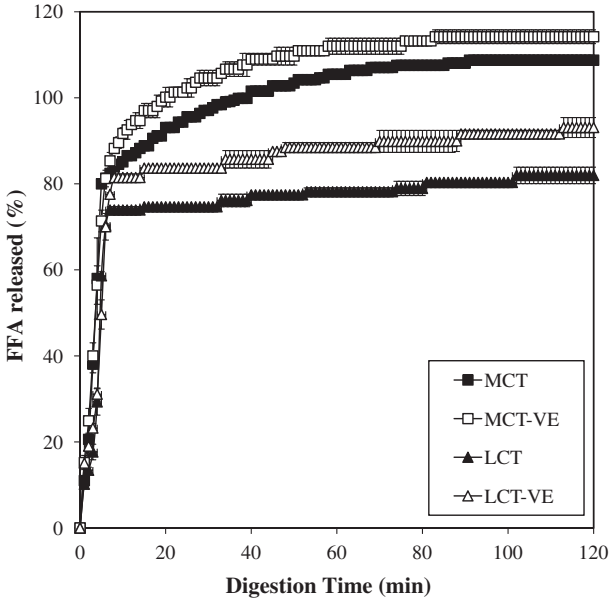

Fig. 3. Effect of vitamin E encapsulation and carrier lipid type on rate and extent of lipid digestion measured using an *in vitro* digestion model.

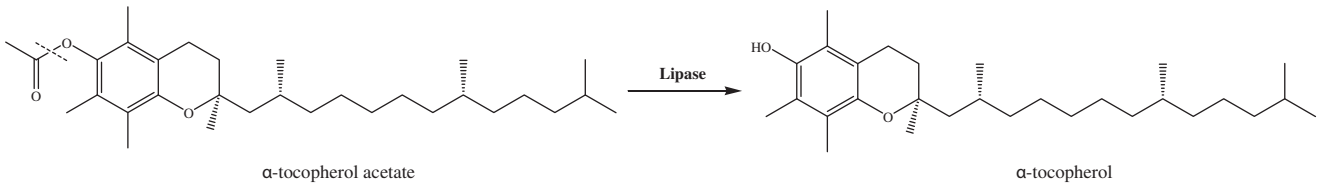

Fig. 4. Conversion of α-tocopherol acetate to α-tocopherol during *in vitro* digestion.

number of earlier studies have reported that lipids containing medium-chain fatty acids are digested more rapidly and extensively than those containing long-chain fatty acids (Li, Hu, & McClements, 2011; Porter et al., 2007; Pouton & Porter, 2008). A number of physiochemical explanations have been proposed to account for this phenomenon. First, medium-chain FFAs have a higher water dispersibility than long-chain FFAs (Porter et al., 2007; Pouton & Porter, 2008). Consequently, the medium-chain FFAs produced during MCT digestion rapidly migrate into the aqueous phase surrounding the droplets, thereby allowing lipase to continue reacting with undigested MCT molecules within the oil droplet core. Conversely, the long-chain FFAs produced during LCT digestion tend to accumulate at the droplet surfaces, thereby inhibiting the ability of lipase to interact with the undigested LCT molecules within the droplet core (Sek et al., 2002). Efficient digestion of LCT requires that there are enough bile salts and free calcium ions present within the aqueous phase to ensure that the long-chain fatty acids are effectively removed from the droplet surfaces through solubilisation or precipitation (Li et al., 2011). The fact that the measured FFAs released was higher for the emulsions containing vitamin E than the equivalent emulsions containing no vitamin E may be attributed to the ability of lipase to cleave the ester bond of the α-tocopherol acetate molecule, thereby releasing α-tocopherol (Fig. 4).

Our results suggest that >100% of FFAs were released from the MCT samples at longer digestion times (Fig. 3). There are a number of possible reasons for this phenomenon associated with the method used to calculate the percentage of FFAs released from the lipid droplets during digestion. There are two main assumptions underlying this calculation: (i) only two FFA molecules are released per TAG molecule; (ii) there are no other components within the system that release protons during the *in vitro* digestion process. In practice, these assumptions may not be completely valid (Ahmed et al., 2012). Some of the MAGs produced during digestion may have been further hydrolysed to FFAs and glycerol, so that more than two FFA molecules were formed from each initial TAG. In addition, there may have been other components within the system that produced protons during the digestion reaction (*e.g.*, proteins, phospholipids, or vitamin E), which would require the addition of extra alkali to neutralise them. However, we did run a control experiment using the surfactant alone, so that this effect should be less important.

3.3. Impact of carrier oil type on vitamin E bioaccessibility

In this section, we examined the impact of carrier oil type on the bioaccessibility of vitamin E after passage through the simulated GIT. After the small intestine stage, digesta were collected and centrifuged, which led to the formation of two distinct layers: a white "sediment" phase at the bottom and a watery "micelle" phase above it. Visually, the micelle phases were either optically transparent (MCT and MCT-VE) or slightly turbid (LCT and LCT-VE). The micelle phase was collected and its particle size distribution was measured before and after filtration. The bioaccessibility of the vitamin E was determined by measuring the concentrations

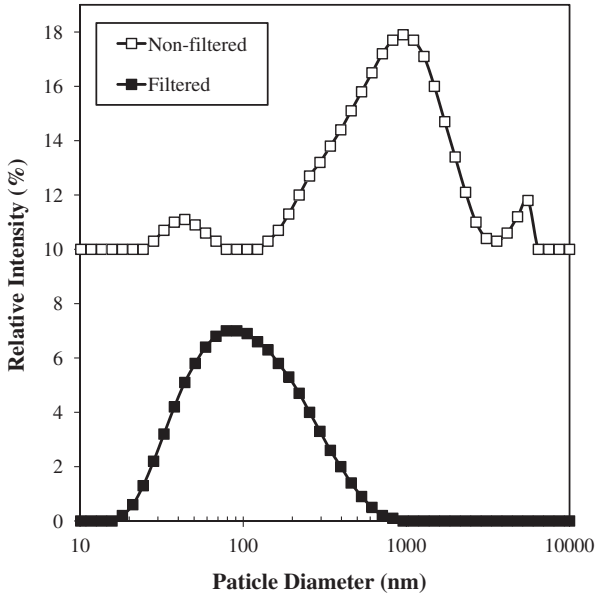

Fig. 5. Particle size distribution of the "micelle" phase collected after digestion and centrifugation of LCT-emulsions. Measurements were made before and after filtration (0.45 μm pore size) of the micelle phase.

of α-tocopherol and α-tocopherol acetate in the micelle phase and the total digesta using solvent extraction and HPLC.

The particle size distribution of the micelle phase isolated from the digesta of the LCT-emulsion before and after filtration using a 0.45 μm pore size filter is shown in Fig. 5. In the unfiltered samples, a broad range of differently sized particles was observed with diameters ranging from around 140 to 5500 nm, which could have been due to the presence of mixed micelles, vesicles, and non-digested oil droplets. As expected, there was an appreciable decrease in the number of large particles present in the micelle phase after filtration. In practice, the mixed micelles and vesicles containing lipid digestion products must pass through the mucous layer that coats the epithelium cells prior to absorption. The mucous layer can be considered to act like a biological filter, with a critical cut-off point for particle penetration being reported to be around 400 nm (Cone, 2009). Filtering the micelle layer prior to analysis may therefore provide a more accurate representation of the potential bioavailability of a lipophilic compound that must be carried by colloidal structures through the mucous layer to the epithelium cells. For this reason, we filtered the micelle phases collected from the digesta prior to further analysis.

The mean particle diameters and turbidities of the micelle phases collected from the LCT-emulsions were larger than those collected from the MCT-emulsions (Fig. 6), which indicates that they contained larger particles. These larger particles may have been comprised of non-digested triacylglycerol oil, since the pH-stat measurements indicated that not all of the LCT was digested after the small intestine stage (Fig. 3). Alternatively, they may have been due to the formation of larger colloidal structures by the LCT

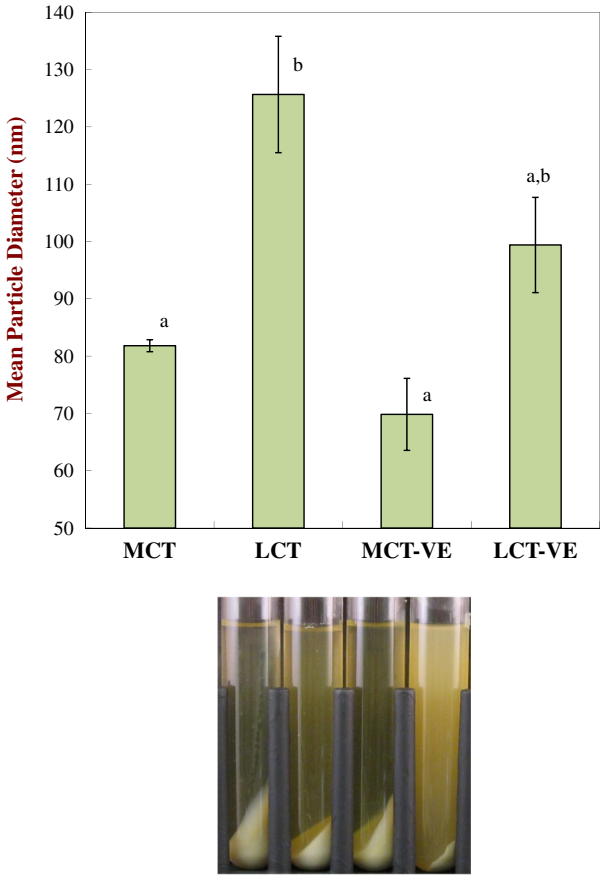

Fig. 6. Influence of carrier oil type on the mean particle diameter (measured after filtration) and appearance of the micelle phase after *in vitro* digestion. Letters show samples with significant differences ($p < 0.05$).

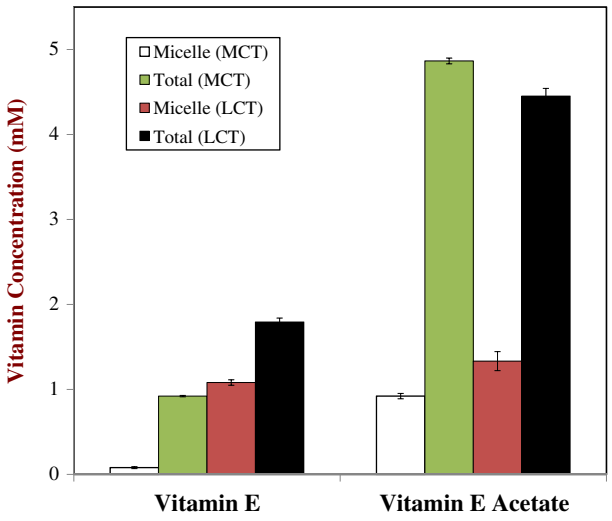

Fig. 7. Influence of carrier oil type on the concentrations of α-tocopherol and α-tocopherol acetate within the micelle phase and the total digesta after *in vitro* digestion of emulsions.

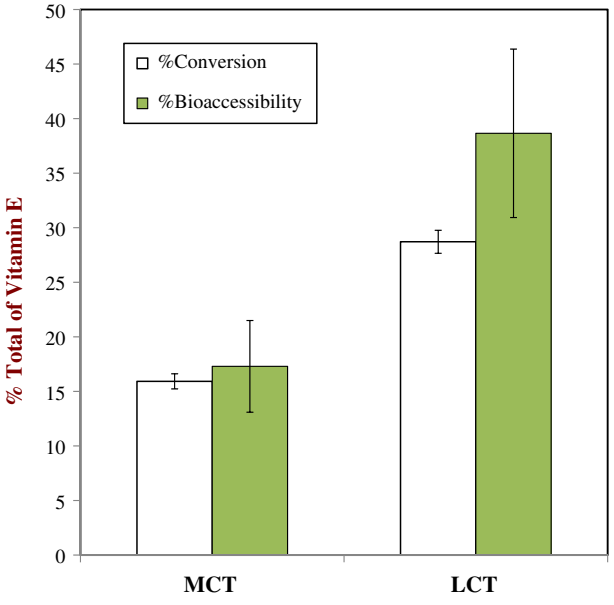

Fig. 8. Influence of carrier oil type on the total bioavailability of Vitamin E (α-tocopherol + α-tocopherol acetate) and the conversion of α-tocopherol acetate to α-tocopherol after *in vitro* digestion of emulsions.

digestion products. The mean droplet diameters were slightly smaller for emulsions containing vitamin E than for emulsions containing no vitamin E, which suggested that the presence of this lipophilic molecule altered the structure of the non-digested oil droplets or the colloidal particles formed from digestion products (Fig. 6).

The concentrations of α-tocopherol acetate and α-tocopherol in the micelle phase and within the total digesta were measured after *in vitro* digestion (Fig. 7). These measurements indicated that some of the α-tocopherol acetate was converted to α-tocopherol during the *in vitro* digestion process, and that the amount of vitamin E solubilised within the micelle phase depended on carrier oil type and the molecular form of the vitamin E. The α-tocopherol acetate concentration was significantly higher ($p < 0.05$) than the α-tocopherol concentration in the micelle phase and in the total system for MCT, and was also significantly higher ($p < 0.05$) in the total system for LCT. The α-tocopherol concentration in the micelle phase and the total system was significantly higher ($p < 0.05$) in LCT than in MCT. On the other hand, the α-tocopherol acetate concentration in the micelle phase was significantly higher ($p < 0.05$) in LCT than in MCT, but the opposite was true for the total system. The influence of carrier oil type on the overall bioaccessibility of vitamin E and the total amount of α-tocopherol acetate converted to α-tocopherol is shown in Fig. 8. The overall bioaccessibility of vitamin E was around 17% when MCT was used as the carrier oil but around 39% when LCT was used. The solubilisation of lipophilic components in the micelle phase usually increases as the total amount of mixed micelles available increases. Our lipid digestion experiments indicated that MCT was actually digested to a greater extent than LCT (Fig. 3) and therefore the total concentration of FFAs and MAGs that could form mixed micelles should have been larger for MCT. On the other hand, the solubilisation capacity of mixed micelles also depends on the nature of the FFAs and MAGs present. Long chain fatty acids can form mixed micelles that have a larger solubilisation capacity for some lipophilic substances than medium chain fatty acids, presumably because they can accommodate larger non-polar molecules. Corn oil contains relatively long chain fatty acids (*e.g.*, C_{16} and C_{18}) while MCT contains medium chain ones (*e.g.*, C_8 and C_{10}), which would therefore account for the observed difference in solubilisation capacity for vitamin E. The α-tocopherol molecule has a non-polar chain that is 14 carbon atoms in length (Fig. 4). This non-polar chain may be too long to easily be accommodated within the monolayers formed by the medium chain fatty acids resulting from MCT digestion, but it

may be able to fit within the monolayers formed by the long chain fatty acids resulting from LCT digestion. The fact that the colloidal structures were larger in the LCT-emulsions than in the MCT-emulsions after lipid digestion (Fig. 6) provides some support for the differences in the structure of the mixed micelles formed. Our results are in agreement with research in the pharmaceutical industry that has shown that many highly lipophilic drugs have a higher bioaccessibility when administered with LCT than with MCT (Kossena, Boyd, Porter, & Charman, 2002; Nielsen, Müllertz, Norling, & Kristensen, 2001).

There were appreciable amounts of sediment formed in many of the samples after centrifugation (Fig. 6). This sediment contains dense insoluble matter formed during the passage of the samples through the simulated gastrointestinal tract, such as aggregated digestive enzymes, bile salts, minerals, and calcium salts of free fatty acids. It is possible that some of the vitamin E acetate or vitamin E was trapped within the sediment phase. In future studies, it would be useful to examine the total amount of vitamin E within the sediment, as well as the nature of its interactions with the other components in the sediment.

3.4. Impact of carrier oil type on α-tocopheryl acetate to α-tocopherol conversion

As mentioned earlier, vitamin E is often used in an esterified form within foods and other commercial products because the free form is highly unstable to oxidation (Gawrysiak-Witulska et al., 2009; Yoon & Choe, 2009). Previous studies suggest that the bioaccessibility of vitamin E may depend on its molecular form, *i.e.*, α-tocopherol *versus* α-tocopherol acetate (Chung et al., 1992; Eicher et al., 1997; Hidiroglou, Laflamme, et al., 1988; Hidiroglou, McDowell, et al., 1988) Therefore, we calculated the amounts of α-tocopherol acetate that had been converted to α-tocopherol after *in vitro* digestion (Fig. 8). Carrier oil type had a significant influence ($p < 0.05$) on this process, with the extent of conversion being about 29% for the LCT-emulsion and 17% for the MCT-emulsion. This result suggests that the conversion of α-tocopherol acetate to α-tocopherol occurred more readily when LCT was used as the carrier oil than when MCT was used. Interestingly, there was a close correlation between the extent of conversion and overall vitamin E bioaccessibility (Fig. 8). A possible explanation for this phenomenon is that vitamin E acetate incorporated into mixed micelles is more susceptible to de-esterification by lipase than vitamin E acetate encapsulated within oil droplets. The fact that a greater fraction of vitamin E was present within the micelle phase for the LCT-emulsion would then account for the observed increase in the hydrolysis of the acetate form. Alternatively, there may have been a competition between the triacylglycerol and the vitamin molecules for hydrolysis by lipase. The MCT molecules were digested more readily than the LCT molecules (Fig. 3), and therefore the rate of vitamin E acetate hydrolysis may have been less when MCT molecules were present in the oil phase due to this competitive effect.

The data presented in Fig. 7 was used to calculate the fraction of vitamin E present in the micelle phase for α-tocopherol and α-tocopherol acetate for both the LCT- and MCT-emulsions (Table 1). This data showed that a higher fraction of the free form (α-tocopherol) was present in the micelle phase than the esterified form (α-tocopherol acetate) for LCT, but the opposite was true for MCT. At present, the molecular or physicochemical basis of this phenomenon is unknown, but it may be related to the compatibility of vitamin E molecules for mixed micelles and other different kinds of colloidal structures. For example, the removal of the acetate group may change the interaction of the vitamin E molecule with the polar head groups of the surface-active molecules that form the palisade layer of the mixed micelles.

Table 1
Percentage of vitamin E present in the micelle phase after lipid digestion and centrifugation of the emulsions.

	MCT	LCT
Vitamin E	8.6 ± 0.1	60.3 ± 3.4
Vitamin E acetate	18.9 ± 0.9	29.9 ± 4.3

4. Conclusions

The purpose of this study was to determine the influence of carrier lipid type on the bioaccessibility and hydrolysis of emulsified α-tocopherol acetate using a simulated gastrointestinal tract. We have shown that α-tocopherol acetate can be effectively encapsulated within oil-in-water emulsions using either medium chain triacylglycerols (MCT) or long chain triacylglycerols (LCT) as a carrier oil. The rate and extent of lipid digestion was higher for MCT-emulsions than for LCT-emulsions, which was attributed to differences in the water dispersibility of the medium and long chain fatty acids formed during lipolysis. On the other hand, the total bioaccessibility of vitamin E after digestion was higher for LCT-emulsions than for MCT-emulsions, which was attributed to the greater solubilisation capacity of mixed micelles formed from long chain fatty acids due to their ability to better accommodate lipophilic vitamin E molecules. Moreover, the conversion of α-tocopherol acetate to α-tocopherol after *in vitro* digestion was also considerably higher for LCT-emulsions than for MCT-emulsions.

In summary, our results suggest that LCT was a more effective carrier lipid than MCT for increasing the overall bioaccessibility of emulsified vitamin E. These results have important implications for designing effective emulsion-based delivery systems for oil-soluble vitamins.

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