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Food-Grade Titanium Dioxide Particles Decreased the Bioaccessibility of Vitamin D_3 in the Simulated Human Gastrointestinal Tract

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ABSTRACT: Food-grade titanium dioxide (E171) particles, as a "whiteness" additive, are often co-ingested with lipid-rich foods. Therefore, we explored the impact of E171 on lipid digestion and vitamin D_3 (VD_3) bioaccessibility encapsulated within oil-in-water emulsions in a simulated human gastrointestinal tract (GIT) model. VD_3 bioaccessibility significantly decreased from 80 to 74% when raising E171 from 0 to 0.5 wt %. The extent of lipid digestion was reduced by E171 addition in a dose-dependent manner. VD_3 bioaccessibility was positively correlated with the final amount of free fatty acids (FFAs) produced by lipid digestion (R^2 = 0.95), suggesting that the reduction in VD_3 bioaccessibility was due to the inhibition of lipid digestion by E171. Further experiments showed that E171 interacted with lipase and calcium ions, thereby interfering with lipid digestion. The findings of this study enhance our understanding toward the potential impact of E171 on the nutritional attributes of foods for human digestion health.

KEYWORDS: *food-grade titanium dioxide, vitamin D_3, bioaccessibility, lipid digestion, free fatty acids*

INTRODUCTION

Titanium dioxide (TiO_2) is widely used as a whitening agent in dairy, bakery, and confectionery products because of its strong light-scattering properties and resistance to discoloration.[1] The global production of TiO_2 was around 165 million metric tons from 1916 to 2011, and this production rate is predicted to rise until at least 2025.[2,3] As a food additive, TiO_2, designated "E171" by the European Union, is authorized to be used in *quantum satis* levels (as much as sufficient, but not more) in Europe.[4] In the United States, the addition of TiO_2 is permitted up to 1% by overall food weight.[2] A human exposure analysis of TiO_2 use in food showed a U.S. adult's daily consumption at around 1 mg Ti kg^{-1} body weight per day, and that children typically have higher exposure levels than adults due to the greater amounts of TiO_2 found in the foods for children, such as confectionary.[5] Humans are therefore exposed to significant levels of E171 through their diet.[6]

Oil-in-water emulsions consist of an oil phase dispersed within an aqueous phase.[7] In the food industry, this kind of colloidal dispersion is an integral part of many kinds of food products, including beverages, milks, creams, dressings, sauces, soups, and dips.[8] Moreover, they can be used to encapsulate lipophilic bioactive substances, protect them from degradation, and increase their bioaccessibility, e.g., curcumin,[9] carotenoid,[10] and oil-soluble vitamins.[11] Vitamin D_3 (VD_3) is an oil-soluble micronutrient that has essential roles in maintaining bone, teeth, and cartilage health, as well as for the proper function of the immune system and protection against cancer.[12] It has been estimated that one-third of the U.S. population suffers from the risk of vitamin D inadequacy (30−49 nmol L^{-1}) or deficiency (<30 nmol L^{-1}) based on blood levels.[13,14] The relatively poor chemical stability and low bioaccessibility of this oil-soluble vitamin contribute to this problem. It has been reported that oil-in-water emulsions may be used as a vehicle for VD_3 delivery to promote its stability and bioaccessibility in food products.[15]

The bioaccessibility of encapsulated oil-soluble vitamins is highly dependent on the digestion of the lipid droplets encapsulated within emulsions because vitamins need to be released from oil droplets, as well as to be solubilized within the mixed micelles that convey them to the epithelium cells.[16] Consequently, any factor that interferes with lipid digestion would impact vitamin bioaccessibility. When there is insufficient lipase present, the extent of lipid digestion is shown to be relatively low.[17] Certain types of emulsifier may inhibit lipid digestion by interfering with the ability of lipase to access the lipid droplet surfaces, by denaturing lipase or by prompting lipid droplet aggregation.[18] Bile salts also have an essential role in lipid digestion due to their capacity to bind to the lipid droplet surfaces and displace emulsifiers, as well as their function in forming mixed micelles.[19] In addition, calcium is important for lipid digestion because of its ability to remove lipid digestion products (long-chain free fatty acids (FFAs)) from the lipid droplet surfaces.[17]

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E171 particles can be co-ingested with fatty food products, which may contain lipid droplets or form new ones within the human gastrointestinal tract. For instance, E171 particles can be added to powdered milks or sprinkled on bakery products to improve their appearance. Then, these E171 particles could interfere with the digestion of ingested lipids, thereby reducing the bioaccessibility of any oil-soluble vitamins encapsulated within lipid droplets. For instance, the surfaces of TiO_2 particles could bind calcium ions under simulated GIT conditions;[20] anionic bile salts and lipase might adsorb onto positively charged E171 particles through static electrostatic interaction. Furthermore, surface-active agents containing −OH and −COOH functional group, such as Tween 80 (an emulsifier in this study) or bile salts, might interact with TiO_2 via chemical bonding and hydrophilicity.[21] Therefore, we hypothesized that the binding ability of surfactants, lipase, bile salts, and calcium to the surface of E171 particles could interfere with lipid digestion, thereby decreasing VD_3 bioaccessibility.

In this study, we used an in vitro gastrointestinal tract (GIT) model to investigate the potential fate of E171 particles within a simulated human gut, as well as to assess their impact on lipid digestion and the bioaccessibility of VD_3 in model food emulsions. The in vitro simulated GIT included mouth, gastric, and small intestinal phases. According to the E171 amount in food[5] and the doses of E171 used in a previous study,[22] the impacts of E171 (0−0.5 wt %) on lipid digestion as well as VD_3 bioaccessibility were examined first. Then, additional experiments were implemented to detect the physicochemical origin of the observed effects. In particular, the interactions of the E171 particles with the surfactant used to coat the vitamin-loaded lipid droplets were studied, as well as with the bile salts, lipase, and calcium ions in the gastrointestinal fluids. The knowledge from this study is useful for evaluating the potential impact of TiO_2 particles on the nutritional value of foods and human health.

MATERIALS AND METHODS

Materials. Food-grade TiO_2 particles (E171, purity 99%) were purchased from Precheza (Přerov, Czech Republic). Vitamin D_3 (1.0 million I.U. g^{-1}) was provided by D·BASF (Ludwigshafen, Germany). Tween 80 (Acros Organics) was purchased from Fisher Scientific (Hampton, NH). Corn oil was purchased from a commercial food supplier. Porcine mucin, porcine pepsin from gastric mucosa (≥250 units mg^{-1}), porcine pancreas lipase (100−500 units mg^{-1}), and porcine bile extract were purchased from Sigma-Aldrich (St. Louis, MO). Other chemicals used were of analytical grade, and ultrapure deionized water was used to prepare all solutions.

Emulsion Preparation. Emulsion preparation was based on a previous study with slight modification.[23] The details are described in Experiment S1. Stock oil-in-water nanoemulsions consisted of 10 wt % oil phase (1 wt % vitamin D_3 + 9 wt % corn oil) and 90 wt % aqueous phase (0.9 wt % Tween 80 + 89.1 wt % 5 mM phosphate buffer solution, pH 7). Emulsions containing 4 wt % oil phase (0.4 wt % vitamin D_3) were obtained by diluting stock emulsion in phosphate buffer solution.

Simulated GIT Model. Food-grade TiO_2 particle suspensions were prepared by adding powdered E171 ingredients into ultrapure deionized water, and then sonicating for 15 min (Fisherbrand 120 Sonic Dismembrator) to reduce particle agglomeration. Model food emulsions containing a range of different E171 concentrations were prepared by mixing (1:1 w/w) aqueous E171 suspensions (0, 0.01, 0.5, 1, 5, 10 wt %) with stock emulsions (0.4 wt % VD_3, 3.6 wt % oil) and then stirred for another 20 min. The final samples used in the simulated GIT experiments therefore contained 0.2 wt % VD_3, 1.8 wt % oil, and 0, 0.005, 0.25, 0.5, 2.5, or 5 wt % E171 particles, respectively.

The test samples were then passed through the simulated GIT to simulate the passage upon mouth (pH 6.8, 2 min), gastric (pH 2.5, 2 h), and intestinal phases (pH 7.0, 2 h). The details and the procedure of this simulated GIT model were based on the previous study and are described in Experiment S2 and Table S1 with some slight modifications.[22] The simulated saliva fluid contained 3 g L^{-1} of mucin; the simulated gastric fluids contained 3.2 g L^{-1} of pepsin; and the small intestinal fluid contained 1.5 mL of salt solution, 3.5 mL of bile salts (53.57 g L^{-1}), and 2.5 mL of lipase (24 g L^{-1}). The percentage of free fatty acids (FFAs) produced was calculated as stated in previous studies.[22]

Particle Size and ζ-Potential. Average hydrodynamic diameters and ζ-potential values were measured by dynamic light scattering and laser Doppler velocimetry by a 90Plus Particle Size Analyzer (Brookhaven). The samples were diluted with buffer solutions or distilled water to provide an optimum light-scattering signal. Diluted mouth and intestinal samples were prepared in phosphate buffer (pH 7.0), and diluted gastric samples were prepared in distilled water (pH adjusted to 2.5).

Vitamin D_3 Bioaccessibility. The bioaccessibility of lipophilic substances is regularly described as the fraction that is dissolved within the mixed micelle phase after small intestine digestion. Raw digesta samples (15 g) were centrifuged at 12 000 rpm, 25 °C for 30 min (Thermo Scientific, CL10 centrifuge). The clear supernatant was obtained as the micelle fraction. According to the previous studies, the bioaccessibility was calculated as the vitamin D_3 concentration in the micelle fraction relative to that in the overall digesta.[16]

Vitamin D_3 Extraction and High-Performance Liquid Chromatography (HPLC) Analysis. The VD_3 concentration in micelle and digesta was determined following the method by Abbasi et al.,[24] and the details are shown in Experiment S3. One milliliters of filtered solution was transferred into a HPLC vial. High-performance liquid chromatography (HPLC, Shimadzu Prominence, Japan) has a reverse-phase C18 column (ZORBAX Eclipse Plus, 4.6 × 150 mm^2, 5.0 μm, Agilent) with a UV detector set at 265 nm. The injection volume was set to 40 μL. The mobile phase was acetonitrile−water (95/5; v/v) at a flow rate 1 mL min^{-1}. The VD_3 calibration curve was established over the range of 2−10 μg g^{-1} with acceptable linearity (R^2 = 0.9989).

Ultraviolet−Visible Spectroscopy Measurements. *Tween 80.* Tween 80 was measured based on the method by Brown et al.[25] and the details are described in Experiment S4. Tween 80 was detected at 318.5 nm using ultraviolet−visible spectroscopy (UV−vis, Agilent 8453). Samples containing 0.36 wt % Tween 80 (a food-grade nonionic surfactant) and various E171 concentrations (0, 0.005, 0.5 wt %) were carried out through the simulated mouth, stomach, and small intestine phases. The standard curve was prepared by eluting Tween 80 in phosphate buffer to various concentrations (0−0.27 mM) (R^2 = 0.997).

Bile Salts. Bile salts were determined according to Wang et al.,[26] and the details are shown in Experiment S4. The bile salts were detected at a wavelength of 309 nm using a UV−vis. Samples containing 12 mM bile salts were mixed with aqueous E171 suspensions (0, 0.005, or 0.5 wt %) and then passed through the small intestine phase. A standard curve was prepared by diluting a series of bile salt in phosphate buffer solutions (0−7 mM) (R^2 = 0.9994).

Lipase. The amount of lipase was detected by measuring the absorbance at 260 nm using a UV−vis. The details are summarized in Experiment S4. The samples containing 3125 mg L^{-1} lipase were mixed with aqueous E171 suspensions (0−1526.5 mg L^{-1}) and then passed through the small intestine phase. A standard curve was prepared by diluting a series of lipase in phosphate buffer solutions (0−600 mg L^{-1}) (R^2 = 0.9998).

Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) Measurements. The solution composed of saliva, gastric, and small intestinal fluid with 0, 0.005, or 0.5 wt % E171 was passed through the small intestine phase (only mineral ions). After

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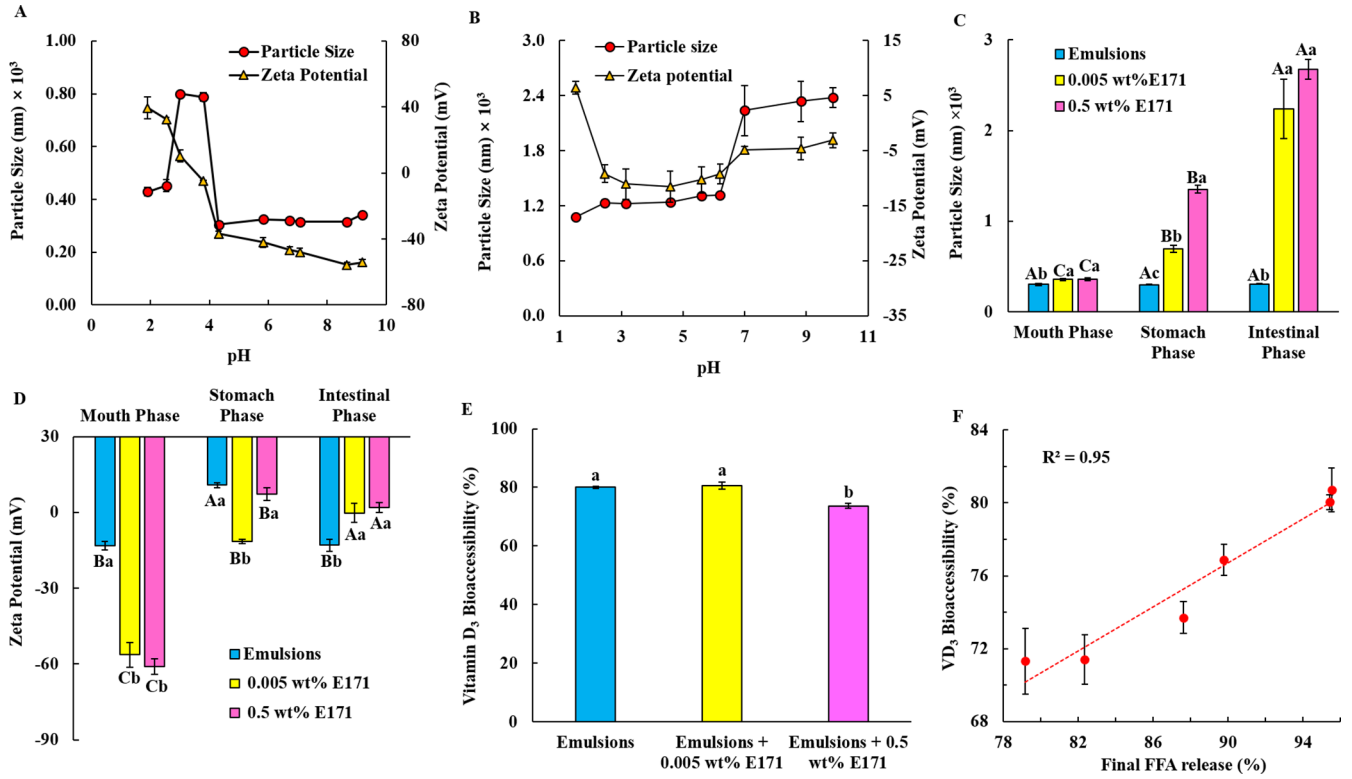

Figure 1. Food-grade TiO_2 (E171) hydrodynamic particle size and ζ-potential values in distilled water (A) and simulated gastrointestinal fluid (B) with only mineral ions as a function of pH. The isoelectric point of E171 is at pH 3.5; (C) particle size (nm) of emulsions, 0.005 and 0.5 wt % E171 in the mouth phase, stomach phase, and small intestine phase (only mineral ions); (D) ζ-potential values (mV) of emulsions, 0.005 and 0.5 wt % E171 in the mouth phase, stomach phase, and small intestine phase (only mineral ions); (E) vitamin D_3 bioaccessibility in emulsions with 0, 0.005, and 0.5 wt % E171; and (F) correlation (R^2 = 0.95) between final free fatty acid (%) released from lipid droplets and vitamin D_3 bioaccessibility (%). Bars with different capital letters (A)−(C) are significantly different ($p < 0.05$) when comparing between different GIT phases for the same treatment. Bars with different lowercase letters (a)−(c) are significantly different ($p < 0.05$) when compared to emulsions, 0.005 and 0.5 wt % E171 in the same region.

incubation in small intestine phase (0, 0.5, or 2 h), the mixtures were centrifuged at 12 000 rpm for 30 min and the supernatants were collected to measure the calcium (Ca) and phosphorus (P) concentration. Briefly, 0.5 g of supernatant was digested with 0.50 mL of HNO_3 at 115 °C for 40 min using a digestion unit (DigiPREP MS Digestor, SCP Science, QC, Canada). Prior to analysis, 0.5 g of sample was diluted to 14 mL using distilled water. Inductively coupled plasma optical emission spectrometry (ICP-OES, PerkinElmer Optima 3300 DV, Norwalk, CT) was used to determine the Ca and P concentration in the supernatant.

Fourier Transform Infrared Spectroscopy (FTIR) and X-Ray Diffraction (XRD). Fourier transform infrared spectroscopy (FT-IR spectrometer, PerkinElmer Spectrum) was used to obtain the spectra of E171 and Tween 80 in each simulated GIT phase, as well as the presence of calcium−phosphate precipitates in the small intestine phase. Transmission spectra were scanned 200 times from 4000 to 500 cm^{-1} at a resolution of 4 cm^{-1}. The composition and crystallinity of raw powder E171, and of calcium phosphates sedimentation in the presence and absence of E171 in the small intestine phase were characterized by X-ray diffraction (XRD), and data were recorded with constant scanning 2θ from 10 to 80° at 0.0263° per step. The details are described in Experiment S5.

Statistical Analysis. Each measurement was carried out on three samples (triplicates). The results were calculated as averages and standard deviations on Microsoft Excel 2019 (Microsoft Corp.). Analysis of variances (ANOVA) was carried out on OriginPro 2018 statistical software (OriginLab Corp.). A Tukey test at a p-value of <0.05 was regarded as significantly different across all treatments.

RESULTS AND DISCUSSION

Characterization of E171 Particles and Emulsion Droplets. *Particle Size and ζ-Potential of E171 Particles.* In the distilled water, the ζ-potential of E171 steadily decreased from highly positive (+39 ± 7 mV) at pH 2 to highly negative (−54 ± 2 mV) at pH 9 (Figure 1A). The isoelectric point (IEP) of the E171 particles was around pH 3.5. At pH close to the IEP, the E171 particles were unstable as indicated by the large increase in hydrodynamic diameter (794 ± 6 nm). In simulated digestion fluids (only mineral ions), the hydrodynamic diameters of E171 particles were relatively large (>1000 nm) at pH < 7, indicating that extensive particle aggregation occurred (Figure 1B). The ζ-potential of E171 particles changed from +10 to −10 mV with increasing the pH in the digestion fluids. The lower surface potential and higher aggregation of the E171 particles in the digestion fluids can be ascribed to the relatively high levels of NaCl and $CaCl_2$ (Table S1). These mineral ions would have decreased the magnitude and range of the electrostatic interactions through electrostatic screening, thereby promoting particle aggregation.[27] At pH ≥ 7, calcium and phosphate formed precipitates on the surfaces of the E171 particles, as confirmed by FTIR and XRD analysis (as presented later), as a result, the large rise in hydrodynamic diameters (>2000 nm) was observed when the pH was increased above 7.

Particle Size and ζ-Potential of E171 Particles and Lipid Droplet Mixtures in Simulated GIT Fluids. The emulsions

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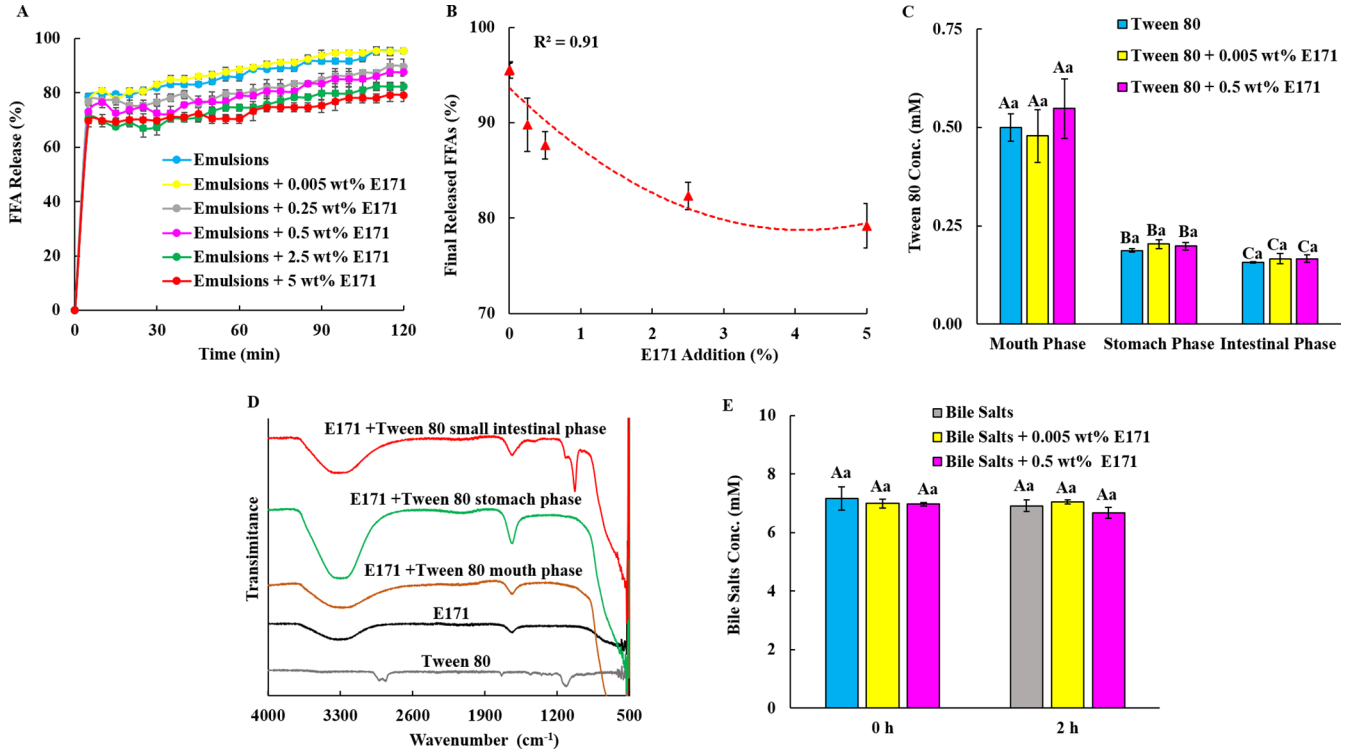

Figure 2. (A) Free fatty acid release from lipid droplets in the small intestine phase over 2 h under 0, 0.005, 0.25, 0.5, 2.5, and 5 wt % E171; (B) correlation (R^2 = 0.91) between E171 addition (wt %) released from lipid droplets and final released FAAs (%); (C) Tween 80 concentration (mM) in the mouth phase (2 min), stomach phase (2 h), and small intestine phase (2 h) under 0, 0.005, and 0.5 wt % E171 (only mineral ions), respectively; (D) FTIR spectra of Tween 80, E171, E171-Tween 80 samples in the mouth, stomach, small intestine phases, respectively; (E) bile salt concentration (mM) in the small intestine phase (0 and 2 h) under 0, 0.005, and 0.5 wt % E171. Bars with different capital letters (A)−(C) are significantly different ($p < 0.05$) when compared between different GIT phases for the same treatment. Bars with the same capital letters are not significantly different ($p < 0.05$) when compared between 0 and 2 h (same treatment); bars with the same lowercase letters are not significantly different when compared 0, 0.005, and 0.5 wt % E171 in the same phase or the same time period.

were stable in each phase because Tween 80 (nonionic surfactant)-coated lipid droplets were able to prevent droplet aggregation by generating strong steric repulsive forces (Figure 1C,D). Even though Tween 80 is supposed to be nonionic, the ζ-potentials of the emulsions in the mouth and intestinal phases were -13 ± 1 and -13 ± 2 mV, respectively. It has been reported that lipid droplets emulsified by nonionic surfactants are negatively charged at neutral pH likely due to impurities presented in the surfactants and/or oil, or the preferential adsorption of hydroxyl groups onto the droplet surfaces.[18] The E171 particles rapidly formed large agglomerates after exposure to stomach and small intestine phases. Under these conditions, the ζ-potentials of E171 particles became close to zero, which was likely due to the high ionic strength (Figure S1).

Impact of E171 Particles on Vitamin D_3 Bioaccessibility. The impact of E171 particles on the bioaccessibility of VD_3 in the emulsions was measured upon passage through the simulated GIT (Figure 1E). VD_3 bioaccessibility was around 80% in the control, consistent with a previous study.[23] In comparison with oil type, lipid-bioactive bioaccessibility was higher in long-chain triglycerides (LCT) than medium-chain triglycerides (MCT).[28] In this study, corn oil, as LCT, was used to solubilize VD_3 for emulsion preparation as a result of high bioaccessibility. Additionally, Tween 80 as emulsifier used in this study prompted bioaccessibility since small lipid droplets were stable entering the small intestine phase,[29] which is consistent with the stable particle size of emulsions in the three phases (Figure 1C). The bioaccessibility of VD_3 was 81 and 74% in the presence of 0.005 and 0.5 wt % of E171, respectively, and the latter treatment was significantly lower than the control. These results indicate that comparatively high amounts of E171 particles can cause a significant reduction in VD_3 bioaccessibility. Previous studies have demonstrated that the bioaccessibility of nutraceuticals is strongly linked to the extent of lipid-phase digestion they are dissolved in.[30−32] In our study, the bioaccessibility of VD_3 was positively correlated (R^2 = 0.95) with the final extent of FFAs released from the lipid droplets (Figure 1F). It is observed that at a higher level of final FFAs releases, the VD_3 bioaccessibility was above 80%, while VD_3 bioaccessibility was less than 72% at the lower level of final FFAs released. The VD_3 bioaccessibility is likely to depend on lipid digestion: since lipid phase must be digested before the vitamin is released from the droplets, then VD_3 could be solubilized in mixed micelles to become accessible.[33] We therefore hypothesized that the reduction in VD_3 bioaccessibility was a result of inhibition of lipid digestion by E171. In addition to droplet characteristics (oil phase composition, emulsifier type, and particle size), the digestion of the lipid droplets is also regulated by gastrointestinal conditions, i.e., lipase, bile salts, and calcium level.[16] Thus, we further explored the impact of the E171 particles on lipid digestion, as well as interaction between E171 and some critical constituents known to impact lipid digestion (such as emulsifier, bile salts, calcium ions, and lipase).

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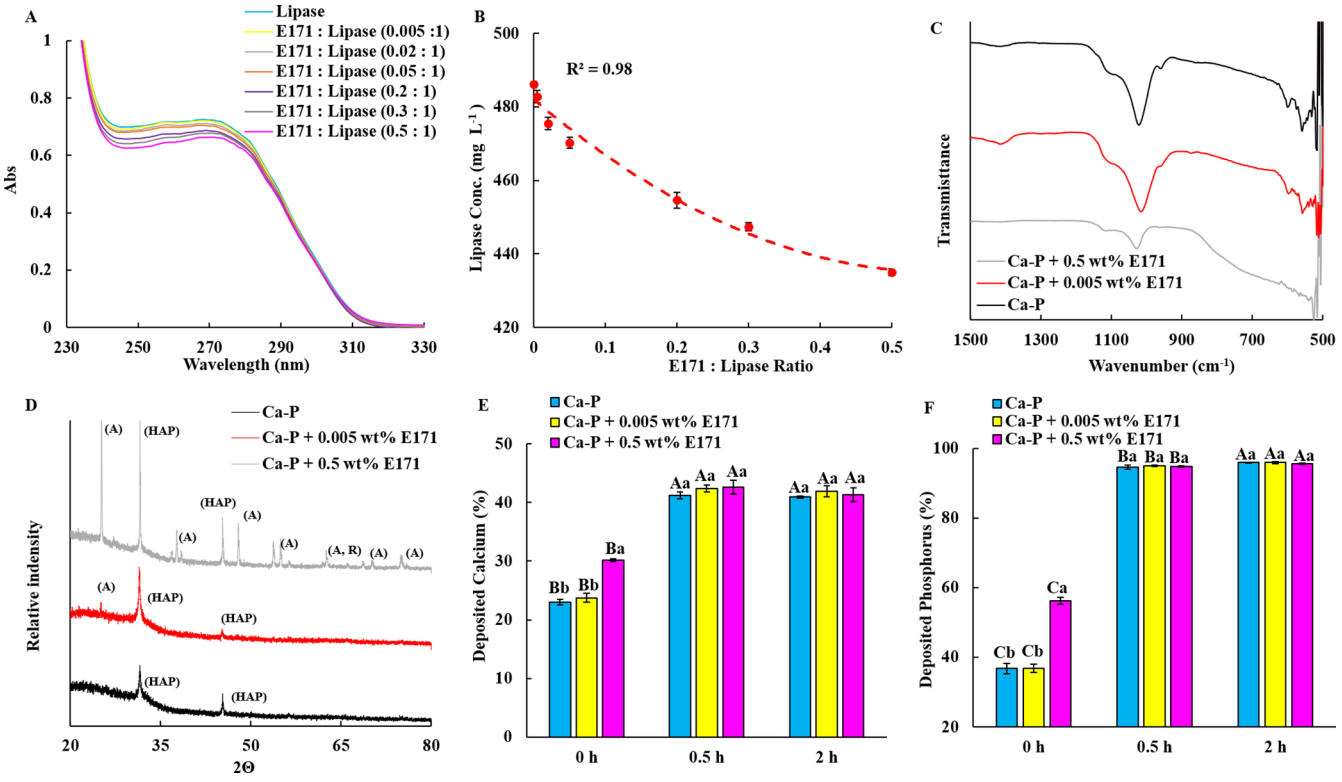

Figure 3. (A) UV−vis spectra of lipase (initial concentration 3125 mg L^{-1}) under various initial concentrations of E171 (0, 15.625, 62.5, 156.25, 625, 937.5, 1562.5 mg L^{-1}) in the small intestine phase; (B) correlation (R^2 = 0.98) between lipase concentration (mg L^{-1}) in the supernatant and initial concentration ratio of E171 to lipase (0, 0.005, 0.02, 0.05, 0.2, 0.3, 0.5); (C) FTIR spectra and (D) XRD patterns of Ca−P precipitates at 0, 0.005, and 0.5 wt % E171; (E) deposited calcium (%) and (F) phosphorous (%) in the small intestine phase under 0, 0.005, and 0.5 wt % E171 after 0, 0.5, and 2 h. Bars with different capital letters (A)−(C) are significantly different ($p < 0.05$) when compared between different GIT phases for the same treatment. Bars with lowercase letters (a), (b) are significantly different ($p < 0.05$) when compared 0, 0.005, and 0.5 wt % E171 at the same time period.

Impact of E171 Particles on Lipid Digestion. The effect of E171 particles on lipid digestion was determined by measuring the generation of FFAs produced from the lipid droplets during the small intestine phase. The lipid digestion profiles of VD_3 emulsions exhibited a similar pattern in the absence or presence of E171 (Figure 2A). More than 70% of the FFAs were released during the first 10 min, with the remainder being released more slowly at longer times, until a comparatively steady level was reached after 2 h. The maximum amount of FFAs released ranged from around 79 to 95% depending on the E171 concentration (Figure 2A). In the absence of E171 particles, the maximum extent of the FFAs released was around 95%. The addition of 0.005 wt % of E171 had little influence on lipid digestion. However, the addition of 0.5 wt % of E171 led to an appreciable decrease to 88% by the end of digestion. Figure 2B shows that the final extent of FFAs released reduced with increasing E171 concentration (R^2 = 0.91). These results indicate that high amounts of E171 particles inhibited lipid digestion during the intestinal phase. Li et al. reported that emulsions with TiO_2 particles showed a significant decrease in final extents of lipid digestion compared to the one in the absence of TiO_2 particles.[22] In addition, pectin, as a dietary fiber, significantly decreased the rate and extent of lipid digestion, which was attributed to its binding with gastrointestinal components, i.e., bile salts, calcium, and lipase.[34] Given this reason, we implemented further experiments to identify the origin of these effects.

It is known that surfactants, bile salts, lipase, and calcium ions impact the rate and extent of lipid digestion in oil-in-water emulsions, as well as the bioaccessibility of nutraceuticals.[16,17,19] We hypothesized that increasing the amount of E171 might inhibit lipid digestion, thereby decreasing vitamin bioaccessibility, through various mechanisms: (1) E171 particles might adsorb Tween 80 molecules to their surfaces, altering the aggregation stability of lipid droplets, which can change the surface area of lipids exposed to lipase; (2) E171 particles might interact with bile salts, reducing bile salt adsorption onto the lipid droplet surfaces and vitamin solubilization within the intestinal fluids; (3) E171 might interact with lipase and decrease the amount available to digest the lipids; and (4) E171 particles might interact with calcium ions to reduce their ability on removal of long-chain FFAs from the lipid droplet surfaces. A sequence of experiments was therefore executed to establish the relative importance of these different mechanisms.

Tween 80. Tween 80 is a nonionic surfactant commonly used as an emulsifier in the food industry.[18] In this study, vitamin-loaded lipid droplets were coated with this surfactant. The concentrations of Tween 80 in simulated GIT fluids containing different E171 levels were measured (Figure 2C). For each GIT phase, no significant difference in Tween 80 concentration was seen in samples containing different E171 levels, suggesting that there was no appreciable surfactant adsorption onto the E171 particles. The Tween 80 concentration used in these experiments was above the critical

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micelle concentration (around 13 μg mL^{-1}),[35] suggesting that the surfactant should form micelles in the mouth, gastric, and intestinal phases. The significant differences in different GIT phases were due to the dilution factor: 0.5 for the stomach phase and 0.8 for the small intestine phase.

FTIR analysis provided additional evidence that no Tween 80 was adsorbed onto the E171 particles in any of the simulated GIT phases. The FTIR spectrum of pure Tween 80 is shown in Figure 2D. The peak at 1737 cm^{-1} is consistent with a C═O stretching vibration associated with the ester structure.[36] Interestingly, no characteristic band associated with Tween 80 was found in the mixed E171 particles/Tween 80 system after incubation in the mouth (2 min), gastric (2 h), and intestinal (2 h) phases. The peak around 3500−3000 cm^{-1} and the peak around 1650−1600 cm^{-1} are associated with hydroxyl groups from water molecules adsorbed onto the titanium dioxide surfaces.[37] After passing through the small intestine phase, the spectrum of the E171 particles/Tween 80 system showed distinct peaks around 1200−900 cm^{-1}, which can be attributed to the P−O bands arising from calcium−phosphate precipitates formed in the intestinal phase.[38] Hence, there was no strong interaction between the Tween 80 and E171 in any of the simulated GIT phases.

Bile Salts. Bile salts are natural anionic surfactants that have hydrophilic and hydrophobic regions on their surfaces. During the lipid digestion, bile salts can bind to lipid droplet surfaces and remove the original emulsifiers from the oil−water interface (such as proteins, phospholipids, or surfactants), thereby facilitating the capability of lipase to bind to the droplet surfaces and digest the oil phase.[39] In addition, bile salts promote lipase activity by removing lipid digestion products, such as monoglycerides and FFAs, from the lipid droplet surfaces.[40] This is because accumulated lipolysis products can impede the access of lipase to the droplet surfaces and slow down digestion.[41] The lipid digestion products can also form mixed micelles that increase the solubility of VD_3 in intestinal liquids.[40] No significant difference in the concentrations of bile salts was observed when different levels of E171 were used or across the different time points (Figure 2E). Thus, there was no interaction between E171 particles and bile salts. Interestingly, the measured bile salts concentrations (<8 mM) were appreciably lower than the initial concentrations (12 mM) in the small intestine phase, indicating that some of the bile salts settled down to the bottom of the tubes after centrifugation. This effect can be attributed to the formation of electrostatic complexes ("calcium soaps") between cationic calcium ions and anionic bile salts, which was supported by the change in the ζ-potential and concentration of the bile salt solutions with or without calcium (Figure S2). Without calcium ions, the concentration of bile salts was around 11 mM, close to the initial concentration, suggesting that the anionic E171 particles did not strongly bind the anionic bile salts. In the presence of 10 mM calcium, bile salt concentrations decreased to around 8 mM, suggesting that calcium ions could bind to bile salts and precipitate.

Lipase. Lipase plays a critical role in lipid digestion by converting triglycerides to monoglycerides and FFAs.[17] A recent study reported that microplastics (MPs) reduce lipid digestion via two mechanisms: one is the formation of lipid−MPs aggregates decreasing the bioavailability of lipid droplets, and another is the adsorption of lipase onto microplastics reducing enzyme activity.[42] In our study, Tween 80, as a surfactant to coat on the lipid surface, showed no interaction with E171 across all of the phases. However, the free lipase concentration in the small intestine phase decreased as the concentration of E171 particles increased (R^2 = 0.98). In particular, the presence of 0.5 wt % E171 particles decreased the lipase concentration by 11% (Figure 3A,B). Thus, the ability of E171 to bind lipase could be responsible for the observed decrease in lipid digestion observed in our study.

To better understand the origin of the interaction between lipase and E171 particles, we examined the role of calcium ions. Without calcium ions, lipase concentration decreased significantly with increasing the concentration ratio (E171 over lipase) larger than 0.05, and free lipase decreased by 2% with increasing the ratio at 0.5; however, in the presence of calcium ions, the lipase concentration decreased significantly with increasing the ratio beyond 0.005, and free lipase decreased by 11% at the ratio of 0.5 (Figure S3a). The ζ-potential determination exhibited that the magnitude of the negative charge on E171 particles decreased appreciably with increasing $CaCl_2$ concentration, from around −20 to nearly 0 mV (Figure S2a). In comparison with E171, the ζ-potential of lipase was significantly decreased from −21.5 ± 2.2 to −14.4 ± 0.8 mV by adding 10 mM calcium (Figure S3b). These results suggest that the cationic calcium ions can reduce the surface potential of E171 particles by charge screening and ion binding.[43] In addition, the calcium ions may act as bridges between the surfaces of the anionic lipase and anionic E171, thereby holding them together. After incubating with 10 mM $CaCl_2$, E171 immediately formed large agglomerates. Thus, lipase binding to the flocs could impede the capability of lipase to access the lipid droplet surfaces, further decreasing lipid digestion.[34]

Calcium Ions. Characteristic peaks that appeared around 1200−900 cm^{-1} were assigned to P−O bonds across all three levels of E171 in the small intestine phase (Figure 3C).[38] Significantly, the spectra of these specific peaks were similar to the FTIR spectrum of hydroxyapatite,[44] suggesting calcium−phosphate deposited as hydroxyapatite in the small intestine phase. The strongest peak for all of the treatments was observed in the range from 1030 to 1010 cm^{-1}, which corresponds to the ν_3 PO_4^{3-} band of apatite. A shoulder peak corresponding to the ν_1 mode of PO_4^{3-} was observed at approximately 960 cm^{-1} in the samples containing 0 and 0.005 wt % E171. In addition, an obvious peak was present between 1120 and 1110 cm^{-1} in the samples containing 0.5 wt % E171, but only a broad shoulder was seen in the other treatments. This peak can be found in the poorly crystalline hydroxyapatite and may correspond to the ν_6' and ν_6'' PO_4^{3-} degenerate stretching of HPO_4^{2-}.[44]

Importantly, XRD analysis showed peaks at 2Θ = 32 and 45°, which are characteristics of hydroxyapatite (Figure 3D).[45] Also, precipitates formed in the presence of 0.005 and 0.5 wt % E171 particles had a strong peak at 2Θ = 24.5°, which is consistent with the anatase form of titanium dioxide. Thus, both FTIR and XRD analysis demonstrated the presence of calcium−phosphate precipitates (hydroxyapatite) in the small intestine phase. In addition, XRD analysis of the powdered E171 particles indicated that the major component of the E171 ingredient used in our experiments was anatase rather than rutile (Figure S4). It has been reported that apatite preferably deposited on the surfaces of anatase gels rather than rutile gels, implying that TiO_2 with anatase crystal structure could be more effective for apatite deposition.[46]

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The amount of phosphorus deposited at the initial stage of the small intestine phase increased with E171 concentration, being 37, 37, and 57% for 0, 0.005, and 0.5 wt % E171, respectively (Figure 3F). This suggests that some of the phosphate anions were able to adsorb onto the E171 surface. The amount of calcium present in the precipitates also increased with increasing E171 concentration, being 23, 24, and 30% for 0, 0.005, and 0.5 wt % E171, respectively (Figure 3E). Thus, our results suggest that calcium−phosphate precipitated onto the surface of E171 particles at the beginning of small intestine phase. Previous researchers have reported that titanium dioxide can serve as a reactive substrate to accelerate calcium−phosphate precipitation.[47] Calcium and phosphate deposition reached maximum levels (around 41 and 95%, respectively) after 30 min in the small intestine phase for each treatment. Nevertheless, the percentages of deposited calcium and phosphate by the end of the small intestine phase were not significantly different, indicating no extra adsorption of calcium in the presence of E171 particles. Thus, the mechanism by which E171 decreased the VD_3 bioaccessibility was that lipase could adsorb onto the surface of E171 via electrostatic attraction in the presence of calcium ions, then inhibiting lipid digestion and reducing the bioaccessibility of VD_3. Our findings improved our understanding of the interaction mechanism of E171 particles with gastrointestinal constituents during lipid digestion, as well as the impact of E171 on lipid digestion profile and nutraceutical bioaccessibility. Further experiments should be conducted using animals (i.e., in vivo studies) to provide a better understanding of the impacts of E171 on the nutritional attributes of foods for human health and wellness.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jafc.0c06644.

Table S1: chemical composition of simulated gastrointestinal fluids (stock solution) used to prepare the mouth, stomach, and small intestine phases of the simulated gastrointestinal tract (GIT) model. Figure S1: the ζ-potentials of E171 in DI water at pH 7.0 as a function of $CaCl_2$ concentration (0−10 mM). Figure S2: (a) ζ-potentials of bile salts and E171 under 0, 10 mM $CaCl_2$ in the small intestine phase and (b) bile salt concentration in the small intestine phase (0 and 2 h) under 0.5 wt % E171, 10 mM $CaCl_2$, and 0.5 wt % E171 + 10 mM $CaCl_2$. Figure S3: (a) Lipase concentration (mg L^{-1}) under various ratios of initial concentrations of E171 to lipase (0, 0.005, 0.02, 0.50, 0.2, 0.3, 0.5) in the absence or presence of calcium (0, 10 mM) and (b) ζ-potentials of lipase under 0, 10 mM $CaCl_2$ in the small intestine phase. Figure S4: XRD pattern of the E171 particles (PDF)

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Notes

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■ ABBREVIATIONS

E171, food-grade titanium dioxide; TiO_2, titanium dioxide; VD_3, vitamin D_3; GIT, gastrointestinal tract; FFAs, free fatty acids; HPLC, high-performance liquid chromatography; UV−vis, ultraviolet−visible spectroscopy; ICP-OES, inductively coupled plasma optical emission spectrometry; FTIR, Fourier transform infrared spectroscopy; XRD, X-ray diffraction

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