Food & Function

Impact of a plant extract on the gastrointestinal fate of nutraceutical-loaded nanoemulsions: Phytic acid inhibits lipid digestion but enhances curcumin bioaccessibility

Journal:	*Food & Function*
Manuscript ID	FO-ART-03-2019-000545.R1
Article Type:	Paper
Date Submitted by the Author:	29-Apr-2019

Complete List of Authors:	Pei, Yaqiong; Huazhong Agriculture University Ai, Tingyang; Huazhong Agricultural University, College of Food Science and Technology Deng, Ziyu ; Huazhong Agricultural University Wu, Di; Huazhong Agriculture University Liang, Hongshan; Huazhong Agricultural University, College of Food Science and Technology McClements, David; University of Massachusetts, Food Science Li, Bin; Huazhong Agricultural University,

Impact of a plant extract on the gastrointestinal fate of nutraceutical-loaded nanoemulsions: Phytic acid inhibits lipid digestion but enhances curcumin bioaccessibility

Yaqiong Pei [a, b], Tingyang Ai [a, b], Deng Ziyu [a, b], Di Wu [a, b], Hongshan Liang [a, b], David Julian McClements [c*], Bin Li [a, b*]

[a] *College of Food Science and Technology, Huazhong Agricultural University, Wuhan 430070, China*

[b] *Key Laboratory of Environment Correlative Dietology (Huazhong Agricultural University), Ministry of Education, 430070，China*

[c] *Department of Food Science, University of Massachusetts Amherst, Amherst, MA 01003, USA*

*Corresponding author:

E-mail address: libinfood@mail.hzau.edu.cn (Bin Li)

E-mail address: mcclements@foodsci.umass.edu (D.J. McClements)

Abstract

The impact of phytic acid on lipid digestion and curcumin bioaccessibility in oil-in-water nanoemulsions was investigated using a simulated gastrointestinal tract (GIT). The size, charge, and structural organization of the colloidal particles in the system were measured as the curcumin-loaded emulsions (7 mg curcumin/g lipid) were passed through simulated mouth (pH 6.8, 2 min), stomach (pH 2.5, 2 hours), and small intestine (pH 7.0, 2 hours) stages. After the small intestine stage, the level of free fatty acids (FFAs) generated and the bioaccessibility of curcumin were measured. The total amount of FFAs released significantly decreased with increasing phytic acid level, from 105.7 ± 5.9% (control) to 78.4 ± 6.4% (0.5% phytic acid). Conversely, curcumin bioaccessibility significantly increased from 39.4 ± 3.5% (control) to 74.7 ± 2.6% (0.5% phytic acid). The inverse relationship between lipolysis and curcumin bioaccessibility was ascribed to the impact of phytic acid on droplet flocculation and the level of free calcium ions present, which affected the production of mixed micelles capable of solubilizing the nutraceutical. The knowledge obtained here might prove beneficial for the employment of phytic acid as a multifunctional ingredient that inhibits lipid digestion while boosting nutraceutical bioavailability.

Keywords: phytic acid; emulsion; gastrointestinal tract; nutraceuticals; lipid digestion; bioaccessibility.

1. Introduction

The dietary regime followed by many people in developed countries over the past few decades have been linked to the rise in several chronic diseases, such as obesity, Type 2 diabetes, and certain types of cancer [1, 2]. The food industry is, therefore, examining ways of developing a new generation of functional foods specifically designed to tackle these diet-related diseases. Many edible plants, animals, and microorganisms contain substances that are biologically active and may therefore have health benefits when consumed at high enough levels. These nutraceuticals have been demonstrated to display a variety of biological activities, including anti-inflammatory, antioxidant, antibacterial, anticancer, and antiviral [3]. In principle, ingestion of foods enriched with these nutraceuticals may therefore improve human health and performance by preventing chronic diseases at an early stage before therapeutic intervention is required. The widespread availability of efficacious functional foods and beverages would lead to improvements in people's quality of life, as well as to reductions in health care and other costs, which are placing an increasing burden on the economies of many countries.

There are, however, numerous challenges that must be overcome before nutraceutical-enriched functional foods with proven health benefits can be created. In particular, it is important to incorporate the nutraceutical into a food matrix at a sufficiently high level, prevent it from degrading into an inactive form during production and storage, and ensuring it is bioavailable after ingestion [4-6]. The

bioavailability of a nutraceutical depends upon a number of factors: *bioaccessibility* - the fraction released from the food matrix in a soluble form; *stability* – the fraction remaining in a bioactive form after any chemical or biochemical transformations; and, *absorption* – the fraction actually absorbed by the epithelial cells and reaching the site of action [7]. The bioaccessibility, stability, and absorption are highly dependent on nutraceutical type and food matrix effects [7].

Over the past two decades, several food-grade delivery systems have been created that are designed to enhance the bioavailability of encapsulated nutraceuticals, including liposomes, micelles, biopolymer complexes [8-10], emulsions and nanoemulsions [11, 12], and hydrogel beads [13]. These delivery systems consist of particles that may vary considerably in their dimensions, ranging from a few nanometers to a few millimeters, depending on how they are constructed. The composition and structure of the particles can be tailored to modulate the bioaccessibility, stability, and absorption of nutraceuticals, thereby improving their bioavailability [14]. Of the delivery systems available for hydrophobic nutraceuticals, nanoemulsions are particularly suitable for use in functional foods and beverages because of their high encapsulation efficiency, ease of fabrication, good stability characteristics, compatibility with many food matrices, and rapid digestion within the gastrointestinal tract [11]. Previously, researchers have used nanoemulsions to improve the bioavailability of many types of lipophilic nutraceuticals [15-17]. These studies have shown that the efficacy of these delivery systems can be optimized by

carefully controlling emulsion properties, such as droplet size [18, 19], emulsifier type [20], oil phase composition [21], and aqueous phase composition [22-25].

In this study, we explored the possibility of improving the bioavailability of a lipophilic nutraceutical (curcumin) using nanoemulsion-based delivery systems by adding a natural excipient compound. Phytic acid (PA), also referred to as inositol hexaphosphate, naturally exists at relatively high levels (0.5-5% w/w) in certain cereals, legumes, and oil seeds [26]. This highly charged anionic molecule can interact with various other substances in foods. In particular, it can strongly bind multivalent metal cations, such as calcium or magnesium, because it has a strong negative charge over the pH range typically found in foods. These binding reactions affect the solubility, chemical reactivity, and aggregation state of other food ingredients, as well as the activity of enzymes found in foods and the human body [27, 28]. These interactions may, therefore, have a negative effect on the digestion and absorption of both macronutrients and micronutrients [29]. Conversely, the presence of phytic acid has also been reported to have some positive effects, such as antioxidant and anticancer activities, inhibition of kidney stone formation, and protection against chronic diseases, such as diabetes, atherosclerosis, and cardiovascular disease [30-32]. Furthermore, phytic acid has been reported to improve the bioavailability of anthocyanins by reducing gastrointestinal motility and prolonging the time available for absorption in the GIT [33]. Some researchers have also reported that phytic acid can enhance the bioavailability of flavones by increasing the permeability of the

epithelium cell membranes [34, 35].

The ability of phytic acid to enhance the bioavailability of nutraceuticals may also be attributed to its effects on the various processes occurring within the GIT. After ingestion, lipophilic nutraceuticals pass through the mouth, stomach, and small intestine where their molecular structure and physiochemical properties may be changed. After release into the gastrointestinal fluids, nutraceuticals may be solubilized within mixed micelles, transported across the intestinal mucus layer, absorbed by epithelial cells, packed into lipoproteins (such as chylomicrons), and then released into the lymphatic system [7]. Finally, the nutraceutical-loaded lipoproteins are released into the bloodstream where they can be distributed throughout the body.

In the current work, we researched the impact of phytic acid addition on a number of the physicochemical changes taking place within the gastrointestinal tract using a simulated GIT. A model lipophilic nutraceutical (curcumin) was loaded within a nanoemulsion-based delivery system and then passed through a GIT model containing mouth, stomach, and small intestine stages. The influence of phytic acid addition on the size, charge, and structural organization of the particles within the system was investigated, as well as its impact on lipid digestion and curcumin bioaccessibility. Our study may guide the rational application of phytic acid as an excipient ingredient in functional foods.

2. Materials and Methods

2.1 Materials

Powdered whey protein isolate (WPI, dry basis content 97.6%) was acquired from Davisco Foods International Inc. (Le Sueur, MN). Curcumin (98%) was purchased from Aladdin Industrial Corporation (Shanghai China). Corn oil (ACH Food Companies, Memphis, USA) was acquired from a grocer. Phytic acid (PA, 98%; Lot #BCBR3133V) from rice, mucin from porcine stomach, pepsin from porcine gastric mucosa (250 units/mg), pancreatin from porcine pancreas (8×USP; Lot # SLBW3957), and porcine bile extract were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other reagents were of analytical grade. All solutions and emulsions were prepared from double distilled water produced using a laboratory-grade water-purification system (Barnstaed International, USA).

2.2 Curcumin-loaded nanoemulsion preparation

Curcumin-loaded oil-in-water nanoemulsions stabilized by WPI were fabricated using the pH-driven method according to our previous research, with some modifications [36].

Initially, a protein emulsifier stock solution was prepared by dispersing 5.0% w/w WPI powder into water with constant stirring (600 rpm) at 25 ^{o}C for 2 h. This solution was then stored at 4 ^{o}C for 12 h to ensure full dissolution of the protein. Coarse emulsions were prepared by blending 90% (w/w) aqueous phase (1% w/w WPI, pH 7.0) and 10% (w/w) oil phase (corn oil) using a high-shear mixer

(M133/1281-0, Biospec Product, Inc., Switzerland) for 2 min. Nanoemulsions were obtained by passing the coarse emulsions through a high-pressure homogenizer three times at 12,000 psi (Microfluidizer M-110Y, Microfluidics, USA).

Curcumin was loaded into the nanoemulsions using the pH-driven method described previously [37]. Powdered curcumin (6 mg/g) was dissolved into sodium hydroxide solution (0.1 N NaOH,) and then the system was vortexed for 1 min in the dark at 25 ^{o}C. The resulting alkaline curcumin solution (pH 12.0) was then immediately added to the neutral nanoemulsion to avoid curcumin degradation under alkaline conditions. Different amounts of curcumin solution were added to obtain a range of final curcumin levels in the nanoemulsions (1, 3, 5 and 7 mg curcumin per g oil). The curcumin was loaded inside the oil droplets in the emulsions by rapidly adjusting the pH back to 7.0 by adding small aliquots of acid solution (2 N HCl). The resulting samples were then stirred (600 rpm) at 25 ^{o}C for 30 min in the dark. The change in polarity of the curcumin, from hydrophilic at alkaline conditions to hydrophobic at neutral conditions, drives the curcumin from the aqueous phase into the oil droplets [37]. Curcumin-loaded nanoemulsions containing 2% (w/w) corn oil were obtained by diluting with buffer solutions containing different levels of phytic acid. This led to the production of nanoemulsions with a range of phytic acid concentrations (0.05%, 0.1%, 0.2% and 0.5% w/w). A curcumin-loaded nanoemulsion without phytic acid was used as a control.

2.3 *In vitro* GIT experiments

Curcumin-loaded nanoemulsions were subjected to the three-stage static *vitro* GIT model (mouth, stomach and small intestine) as described in our previous study [36]. The various stages involved are briefly summarized here:

2.3.1. Initial stage

20 mL of curcumin-loaded nanoemulsion (containing 2% w/w corn oil) was placed in an incubator (Innova Incubator Shaker, New Brunswick Scientific, USA) and swirled for 10 min at 37 ^{o}C.

2.3.2. Mouth stage

20 mL of simulated saliva fluid (SSF) containing 3 mg/mL mucin (37℃) was added to 20 mL of the initial phase. The resulting sample (containing 1% w/w corn oil) was then adjusted to pH 6.8 and placed in the incubator shaker (100 rpm, 37 °C) for 2 min.

2.3.3. Stomach stage

25 mL of simulated gastric fluid (SGF) containing 2 mg/ml NaCl, 7 mg/mL HCl, and 3.2 mg/mL pepsin (37 °C) was added to 25 mL sample resulting from the mouth phase. The resulting mixture (containing 0.5% w/w corn oil) was adjusted to pH 2.5 by adding 2 N NaOH solution. Then the system was shaken in the incubator shaker mentioned earlier for 2 h at 37 °C (100 rpm).

2.3.4. Small intestine phase

30 mL of sample resulting from the stomach phase was adjusted to pH 7.0 using 2 N NaOH. Firstly, 1.5 mL of simulated intestinal fluid (0.5 N $CaCl_2$ and 7.5 N NaCl) was added to the sample, followed by 3.5 mL of bile salt solution (375 mg bile extract) with constant stirring. The resulting system was then adjusted to pH 7.0 again. Finally, 2.5 mL of pancreatin solution (48 mg/mL) was added to the system, and the automatic titrator (Metrohm, USA) was initiated to monitor and keep the sample pH at 7.0 by titrating aliquots of 0.1 M NaOH solution into the reaction chamber for 2 h at 37 °C.

2.4 Droplet size and charge measurements

The particle size distribution (PSD) and mean particle diameter ($D_{4,3}$) of the samples was measured using laser diffraction (MasterSizer 3000, Malvern Instruments, UK). The surface potential (ζ-potential) of the particles was determined using Zetasizer Nano ZS（Malvern Instruments, UK). The refractive indexes of corn oil and water used in calculations were 1.474 and 1.33, respectively. Before particle size and ζ-potential tests, nanoemulsions were diluted with aqueous solutions at the same pH as them to reduce multiple scattering effects. Samples collected from the stomach phase were diluted with pH 2.5-distilled water, whereas samples collected from the initial, mouth, and intestinal phases were diluted with pH 7.0-distilled water.

2.5 Microstructure analysis

The microstructures of samples were examined using laser scanning confocal microscopy (LSCM) with a 40× objective and 10× eyepiece lens (Nikon D-Eclipse C1 80i, Nikon). Prior to measurement, 1 mL of samples collected from various stage digestion was added with 60 μL of Nile Red solution (1 mg/mL ethanol, corn oil stain: excitation and emission wavelengths = 543 and 605 nm, respectively). A drop of nanoemulsion was injected onto a glass slide and then covered with a coverslip. Microstructure images were captured and processed using image analysis software (NIS-Elements, Nikon).

2.6 Curcumin bioaccessibility and transformation

The term *bioaccessibility* was identified as the fraction of the "ingested" nutraceuticals that reached the small intestine and was solubilized by the mixed micelles [38]. The term *transformation* was identified as the fraction of nutraceuticals that reached the small intestine in an active form compared to that in the initial sample[38]. The amount of curcumin in the samples was measured using an UV spectrophotometric method as previously described [36] with some slight modifications. After the small intestine phase, the samples were collected and divided into two portions. One portion was taken to be the "digesta" sample and the other portion was used to obtain the "mixed micelle" sample. This was achieved by centrifuging (18,000 rpm, 25 ^{o}C) the sample for 50 min (Thermo Scientific, Waltham) to remove any insoluble matter and then collecting the clear upper layer to isolate the mixed

micelles where the curcumin was solubilized. Each phase (1 mL) was added to 4 mL ethyl alcohol (containing 5 mM HCl to protect the curcumin from degradation during measurement), vortexed for 1 min, and then centrifuged at 5000 rpm for 15 min. The absorbance of the supernatant was then examined using an UV−visible spectrophotometer (Cary 100 UV−vis, Agilent Technologies, USA) at a wavelength of 419 nm. The concentration of the curcumin was calculated according to a curcumin standard curve.

2.7 Statistical analysis

All experiments were repeated in triplicates. The results are expressed as means ± standard deviations (SD). The differences between samples were calculated using LSD (SPSS 22.0). The letters a-d or A-D were used to suggest significant differences between samples ($p<0.05$). Samples designated with the same letter represent no significant difference.

3. Results and discussion

We hypothesized that phytic acid would interact with some of the key components in the gastrointestinal environment, which would affect the gastrointestinal fate of the emulsion-based delivery systems, as well as the bioaccessibility and transformation of the encapsulated curcumin. For this reason, the influence of phytic acid on the size, charge, and structural organization of the colloidal particles in the emulsions was examined after they passed through each stage of the *vitro* GIT model. In addition, the influence of phytic acid on lipid digestion

and curcumin bioaccessibility was also evaluated.

3.1 Impact of phytic acid on physical and structural properties of nanoemulsions in simulated GIT

3.1.1. Initial phase

Initially, the mean particle diameters ($D_{4,3}$) of curcumin-loaded nanoemulsions containing different levels of phytic acid were all around 0.16 μm and the PSDs were all monomodal (**Figures 1 and 2A**). This result suggests that addition of phytic acid at neutral pH had no appreciable effect on the stability of the WPI-stabilized nanoemulsions. The microscopy images showed that all the initial nanoemulsions contained small oil droplets that were equally spread throughout the entire system (**Figure 3**), which is consistent with the particle size results. The initial emulsions all had strong negative charges (-45 to -55 mV) because the solution pH (pH 7.0) was well above the isoelectric point of the adsorbed proteins (pH 5.0) (**Figure 4**). The magnitude of the ζ-potential increased as the phytic acid concentration was increased. This change may be because some of the anionic phytic acid molecules became associated with the surfaces of the protein-coated oil particles or because dissolved phytic acid molecules contributed to the electrophoretic signal used to calculate the ζ-potential [39]. Alternatively, it may be due to the phytic acid molecules in the aqueous phase pulling off cationic mineral ions (such as Ca^{2+}, Fe^{2+} or Fe^{3+} iron ions) from the oil droplet surfaces [40, 41].

Overall, these results suggest that, owing to a strong electrostatic repulsion

between the small protein-coated oil droplets, phytic acid did not promote droplet aggregation in the nanoemulsions under neutral pH conditions.

3.1.2. Mouth phase

After exposure to simulated oral conditions, the PSDs of all the nanoemulsions remained monomodal and the mean particle diameter did not change appreciably (Figures 1 and 2B). These results indicate that most of the oil droplets in the nanoemulsions remained stable after incubation in simulated saliva. The microstructure of all the nanoemulsions were similar to the initial samples (Figure 3). The magnitude of the negative charges on all the nanoemulsions was reduced by about 5 mV after incubation in the simulated saliva (Figure 4). This result may have been because of adsorption of mucin molecules onto the oil droplet surfaces [42], which would have modulated their surface potentials. Alternatively, it may have been due to electrostatic screening of the droplet surface potential by counter-ions in the simulated saliva.

Overall, these results suggest that phytic acid addition did not have a strong impact on the aggregation stability of the nanoemulsions in the oral stage, which may be important for ensuring they have an appropriate mouthfeel.

3.1.3. Stomach phase

For all of the nanoemulsions, a significant increase in the particle size after incubation in the simulated gastric fluids occurred, with the mean particle diameter

increasing from around 0.16 μm in the oral phase to around 50 μm at the end of the stomach phase (Figure 1). As displayed in Figure 2C, the PSDs of the samples became bimodal, with a large number of particles with diameters from 10 to 100 μm and a smaller number of particles with diameters from 3 to 10 μm. Overall, the mean particle diameters and PSDs of the nanoemulsions were very similar and independent of the level of phytic acid present (Figure 2C). The microstructure results suggested that there was considerable aggregation of the oil droplets after passing through the simulated stomach stage (Figure 3). The large particles observed in the images consisted of numerous oil droplets clustered together, rather than a few large individual droplets, which suggests that droplet aggregation was mainly due to flocculation rather than coalescence.

There are a number of factors that contribute to droplet flocculation in simulated stomach conditions [39]. First, the pepsin present in the gastric fluids may partially hydrolyze the WPI molecules at the lipid droplet surfaces, thereby reducing their aggregation stability. Second, anionic mucin molecules may adsorb to the cationic WPI-coated lipid droplet surfaces due to electrostatic attraction, thereby partially neutralizing their charge. As a result, there would have been a reduction in the long-range electrostatic repulsive forces acting among the lipid droplets. Third, the anionic mucin molecules may have bound to the multiple cationic lipid droplet surfaces, thereby leading to bridging flocculation. Finally, the high level of ionic species in the gastric fluids may have decreased the electrostatic repulsion among the

oil droplets due to screening effects.

After incubation in simulated stomach conditions (pH 2.5), the magnitude of the ζ-potentials of all the samples was reduced to around zero (about -2 mV), regardless of initial phytic acid level (Figure 4). This effect was primarily attributed to the protonation of the carboxyl groups on the protein molecules and the binding of anionic mucin or phytic acid molecules to the surfaces of the cationic WPI-coated oil droplets, which led to charge neutralization. In addition, the observed reduction in ζ-potential may have partially been because of electrostatic screening effects [43].

3.1.4. Intestinal phase

After digestion in the simulated small intestine, the mean particle diameters of all the samples significantly decreased, with the effect being most pronounced at the highest phytic acid level (Figure 1). This decrease in particle size may be the result of various effects [12, 44, 45]. First, the pH changes from 2.5 (stomach) to 7.0 (small intestine), which alters the electrostatic interactions among the WPI-coated lipid droplets. Second, lipase adsorbs onto the lipid droplet surfaces and hydrolyzes the encapsulated lipids, thereby changing lipid droplet size. Third, the free fatty acids and monoglycerides produced by lipid digestion form micelles, calcium soaps, vesicles, and other colloidal particles. Fourth, there may be some protein fragments left over after digestion of the oil droplets. As a result, there was a change in the dimensions of the particles remaining in the system after digestion.

Compared to the samples in the stomach phase, the PSDs of all the samples in

the small intestine were broad and unimodal, with most of the particles falling in the range from 2 to 70 μm, with the exception of the sample containing 0.5% phytic acid where there was a significant fraction of particles with dimensions in the range from 0.3 to 2 μm (Figure 2). The microstructure images of the samples indicated that most of the oil droplets had been hydrolyzed, but there remained a few relatively large oil-rich particles (Figure 3), which may have been large vesicles or insoluble calcium soaps.

Taken together, these results indicate that there were numerous different kinds of colloidal particles with different dimensions present in the digesta, such as micelles, non-digested oil droplets, calcium soaps, and vesicles [20]. The microstructure images also suggested that the number of large aggregates in the digesta gradually decreased as the concentration of phytic acid increased (Figure 3), which is consistent with the particle size results. A possible explanation for this phenomenon is that the phytic acid bound some of the calcium ions, which reduced the level of insoluble calcium soaps formed [46]. This explanation is supported by visual observations of the digesta after centrifugation: the level of insoluble sediment gradually decreased as the phytic acid content increased (Figure 5A), whereas the turbidity of the micelle phase increased (Figure 5B). This suggests that there may have been more smaller particles containing long-chain fatty acids in the micelle phase at higher phytic acid levels.

Compared to the particles in the simulated gastric phase, the particles in the final

digesta had a higher negative charge (Figure 4). This result may have been because of the presence of different forms of anionic biopolymers or colloidal particles (such as micelles, liquid crystals, vesicles, or undigested lipid droplets) in the digesta, which are assembled from anionic bile acids, free fatty acids, phytic acid, phospholipids, peptides, or proteins [20]. No significant difference in the ζ-potentials of the samples containing different phytic acid levels was observed, suggesting that the overall charge characteristics of the digesta were dominated by other anionic species.

3.2 Lipid Digestion Profile

The free fatty acid (FFA) release curves of emulsions with different phytic acid levels were fairly similar: rapid digestion initially followed by slower digestion later (Figure 6A). This result is consistent with previous reports of the enzymatic digestion of nanoemulsions, which contain small lipid droplets that have a large surface area of oil exposed to lipase [18, 46, 47]. There were obvious differences in the lipid digestion profiles of the nanoemulsions depending on the level of phytic acid present (Figure 6A).

The levels of FFAs released from nanoemulsions containing different phytic acid levels were therefore compared at 5 and 120 min. Interestingly, we found that the amounts of FFAs released within the first 5 min significantly increased as the phytic acid concentration increased (Figure 6B). The initial rate of lipid digestion depends on the rate that lipase adsorbs to the surfaces of lipid droplets and hydrolyzes the triglycerides inside them. Typically, lipid digestion occurs more slowly as the oil

droplets become more flocculated because then it is harder for the enzyme to access the droplets in the interior of the flocs [48, 49]. In this study, we found that the oil droplets in the stomach were highly flocculated. Consequently, their digestion rate will depend on how quickly the flocs are broken down when the nanoemulsions enter the small intestine. We postulate that the presence of phytic acid facilitated the rapid dissociation of the flocs in the simulated small intestine condition. Under neutral conditions, the anionic phytic acid may have adsorbed to the surfaces of the oil droplets and increased the electrostatic repulsion between them, thereby allowing lipase to access the encapsulated triglycerides more easily. This phenomenon would account for the increase in FFAs generated within the first 5 min of digestion as the phytic acid level was increased. The microstructure images show that the aggregates present within the intestinal fluids became smaller as the phytic acid level was increased (Fig. 2), which supports this mechanism.

The time required for lipid digestion to transition from the rapid phase to the slow phase ($Time_{balance}$) was estimated from the FFA-time profiles, and then the FFA level ($FFA_{balance}$) at this time was determined (Figure 6C). The time required to reach the balance point decreased from around 20.7 ± 0.6 min for the control to 11.7 ± 1.1 min for the nanoemulsion containing 0.5% phytic acid, which again shows that the initial rate of lipid digestion was faster for samples containing phytic acid. Conversely, the amount of FFAs released at the balance point significantly ($P < 0.05$) decreased with increasing phytic acid level, from around 86.1 ± 2.7% (control) to 69.9

± 2.7% (0.5% phytic acid) (Figure 6C). This finding suggests that although the initial rate of lipid digestion was faster for samples containing phytic acid, the total fraction of triglycerides hydrolyzed by the end of digestion was reduced. This effect was also seen when the total amounts of FFAs generated after 2 hours of digestion were plotted against phytic acid level (Figure 6D). The final level of FFAs produced decreased from around 105.7 ± 5.9% (control) to 78.4 ± 6.4% (0.5% phytic acid). The main reason that the measured amount of FFAs released was higher than 100% is that the whey proteins in the system were hydrolyzed and contributed to the pH stat measurements [50, 51]. Previous studies have reported that phytic acid can decrease the activity of lipase [52, 53], but the physiological and biochemical mechanisms responsible for this effect are still unclear. In our study, it is therefore possible that phytic acid slowly deactivated the lipase, thereby lowering the amount of FFAs generated at longer digestion times.

Alternatively, this effect may be related to changes in the level of free calcium ions (Ca^{2+}) present in the gastrointestinal fluids. Calcium ions are key players in the lipid digestion process: (1) they are cofactors for pancreatic lipase; (2) they interact with long-chain anionic FFAs that normally accumulate at the interface and remove them through a precipitation mechanism, thereby allowing the lipid digestion process to continue [46, 51]. If these long-chain FFAs remain at the interface, they prevent the lipase from reaching the triglycerides below, thereby inhibiting digestion. Consequently, any components in the gastrointestinal fluids that affect the level of

free calcium ions present within the small intestine will impact lipid digestion, for instance chelating agents, polysaccharides, proteins, and peptides [24]. Anionic phytic acid molecules are known to strongly bind calcium ions [54]. We therefore hypothesize that increasing the concentration of phytic acid within the nanoemulsions decreased the concentration of free calcium ions present. As a result, they prevented the calcium ions from removing the long-chain FFAs from the lipid droplet surfaces, thereby inhibiting the later stages of lipid digestion.

In summary, the addition of phytic acid had diverse effects on lipid digestion – increasing the initial rate, but decreasing the total amount of FFAs released. These results suggest that phytic acid might be useful as an effective lipid-lowering agent that could be incorporated into functional foods to reduce the total number of calories extracted or to prevent spikes in blood lipid levels.

3.3 Curcumin bioaccessibility and transformation

Finally, we evaluated the influence of phytic acid on the bioaccessibility and transformation of the encapsulated curcumin at the end of the full GIT model. The bioaccessibility of the curcumin depended on the amount added initially, as well as the level of phytic acid present (Figure 7).

At the same phytic acid level, the bioaccessibility of the nutraceutical significantly decreased as the initial level of curcumin was increased from 1 to 7 mg/g lipid. In other words, the higher the initial amount of curcumin present, the lower the fraction solubilized within the mixed micelles. This phenomenon may have

occurred because a higher fraction of curcumin was present at the lipid droplet surfaces at lower concentrations and was therefore more readily liberated and solubilized during lipid digestion. Alternatively, the mixed micelles may have become saturated with curcumin at higher nutraceutical levels.

At the same curcumin level, the bioaccessibility tended to increase as the phytic acid concentration increased (Figure 7). For instance, the bioaccessibility increased from 39.5 ± 3.5% (control) to 74.7 ± 2.6% (0.5% phytic acid) for the nanoemulsion containing 7 mg CUR/g lipid. Usually, the bioaccessibility of lipophilic nutraceuticals exhibits a positive correlation with the final degree of lipid digestion. This has been attributed to two effects: (i) more of the triglycerides are converted into FFAs, which then leave the droplets, and so there is less room for the nutraceuticals to be accommodated; (ii) there are more FFAs available to produce mixed micelles that can solubilize the released nutraceuticals [18, 47, 55]. Interestingly, we observed the opposite effect in this study - the bioaccessibility of curcumin increased significantly as the degree of lipid digestion decreased (Figure 8). As an example, the highest degree of lipolysis was observed in the control sample (105.7 ± 5.9%) but it had the lowest curcumin bioaccessibility (39.5 ± 3.5%). Conversely, the lowest extent of lipolysis was observed in the sample containing 0.5% phytic acid (78.4 ± 6.4%), but it had the highest bioaccessibility (74.7 ± 2.6%).

Measurements of the total content of curcumin present after incubation within the small intestine phase showed that appreciable nutraceutical degradation had

occurred (with only around 50% curcumin remaining), but that there was no significant difference between samples with different phytic acid levels. This suggests that phytic acid neither promote nor inhibited the chemical transformation of the curcumin under simulated GIT conditions.

Based on our experimental results and knowledge of the colloid aspects of lipid digestion, we propose the following explanation for our observations. The addition of phytic acid increased the dissociation rate of the flocs entering the small intestine stage, which increased the initial rate of lipid digestion. Curcumin is a surface-active molecule that is likely to accumulate near the surfaces of the oil droplets. Consequently, it can be rapidly liberated during the early stages of lipid digestion. In contrast, the presence of phytic acid reduces the level of free calcium ions present within the system, and may inhibit lipase activity, which reduce the total amount of lipid digestion that occurs. However, most of the curcumin has already been released in the early stages of lipid digestion so that this effect has less impact on bioaccessibility.

In addition, the binding of phytic acid to calcium ions reduces the amount of insoluble calcium soaps formed. As a consequence, more FFAs and bile acids are available that can be incorporated into mixed micelles, which increases their ability to solubilize and transport curcumin. This hypothesis is supported by visual observations of the digesta after the small intestine stage, which show that the level of insoluble sediment formed decreased as the phytic acid level increased (Figure 5A).

Some recent studies have reported a similar phenomenon for other lipophilic nutraceuticals: the bioaccessibility of β-carotene [46], vitamin E [56], and carotenoids [57] encapsulated in emulsions decreased with increasing calcium concentration due to precipitation of the FFAs and bile salts needed to form mixed micelles. Moreover, previous studies have reported that phytic acid itself can form nano-sized micelles [58, 59], which might increase the solubilization of curcumin within the small intestine stage [60].

Our results therefore suggest that addition of phytic acid to the nanoemulsions affects the bioaccessibility of curcumin through various mechanisms, including altering the extent of droplet flocculation, deactivating lipase, sequestrating free calcium ions, and forming phytic acid micelles. However, further work is still needed to determine whether these vehicles (micelles and complexes) can encapsulate curcumin and transport it through the intestinal mucous layer and into the apical surfaces of the epithelial cells. This kind of study would need to be carried out using Caco-2 models, animal feeding tests, or human studies.

4. Conclusions

In conclusion, phytic acid was shown to have an appreciable effect on the *in vitro* digestibility of protein-stabilized nanoemulsions, as well as on the bioaccessibility of curcumin loaded in these delivery systems. Phytic acid promoted droplet flocculation under acidic gastric conditions, resulting in an increase in particle size. Conversely, it reduced the degree of flocculation under neutral small intestine

conditions, which enlarged the surface area of lipids available for the lipase to digest, thereby leading to an enhancement in the initial rate of lipid digestion. However, phytic acid could also bind calcium ions, thereby reducing their ability to precipitate long chain fatty acids from the interfaces. Moreover, other studies have shown that phytic acid can inhibit the activity of digestive enzymes. As a consequence, lipid digestion was inhibited by phytic acid in the later stages. Interestingly, the presence of the phytic acid led to an increase in the bioaccessibility of curcumin, even though the final level of lipid digestion was reduced. We postulate that the curcumin was located near the surfaces of the oil droplets and so was mostly released during the early stages of oil digestion. Moreover, there would have been more FFAs available to fabricate mixed micelles that could solubilize the curcumin released.

Overall, our study has shown that the addition of phytic acid to emulsions decreases lipid digestion but increases curcumin bioaccessibility. These results suggest that phytic acid could be used as a lipid-lowering agent as well as a bioavailability enhancer. This information may be important for the application of phytic acid as an excipient ingredient that can be used to create emulsion-based delivery systems for use in functional foods and beverages.

Acknowledgements

The authors acknowledge the National Key R&D Program of China (Program No. 2017YFD0400205), Technical Innovation Program of Hubei province (Program No. 2017ABA150) & the Wuhan Yellow Crane Special Talents Program. This material

was also partly based upon work supported by the National Institute of Food and Agriculture, USDA, Massachusetts Agricultural Experiment Station (MAS00491).

Conflict of interest statement

There are none potential conflicts between authors and others that bias our work.

References

1. K. H. Wagner and H. Brath, A global view on the development of non communicable diseases, *Prev Med*, 2012, **54 Suppl**, S38-41.
2. P. G. Kopelman, Obesity as a medical problem, *Nature*, 2000, **404**, 635-643.
3. J. P. Gleeson, S. M. Ryan and D. J. Brayden, Oral delivery strategies for nutraceuticals: Delivery vehicles and absorption enhancers, *Trends in Food Science & Technology*, 2016, **53**, 90-101.
4. N. P. Aditya, Y. G. Espinosa and I. T. Norton, Encapsulation systems for the delivery of hydrophilic nutraceuticals: Food application, *Biotechnol Adv*, 2017, **35**, 450-457.
5. N. M. Zaki, Progress and Problems in Nutraceuticals Delivery: An Expert Review, *J Bioequiv Availab*, 2014, **6**, 075-077.
6. Y. Ting, Y. Jiang, C.-T. Ho and Q. Huang, Common delivery systems for enhancing in vivo bioavailability and biological efficacy of nutraceuticals, *Journal of Functional Foods*, 2014, **7**, 112-128.
7. D. J. McClements, F. Li and H. Xiao, in *Annual Review of Food Science and Technology, Vol 6*, eds. M. P. Doyle and T. R. Klaenhammer, 2015, vol. 6, pp. 299-327.
8. F. Liu, C. Ma, Y. Gao and D. J. McClements, Food-Grade Covalent Complexes and Their Application as Nutraceutical Delivery Systems: A Review, *Comprehensive Reviews in Food Science and Food Safety*, 2017, **16**, 76-95.
9. L. Chen, G. E. Remondetto and M. Subirade, Food protein-based materials as nutraceutical delivery systems, *Trends in Food Science & Technology*, 2006, **17**, 272-283.
10. M. H. Abd El-Salam and S. El-Shibiny, Formation and potential uses of milk proteins as nano delivery vehicles for nutraceuticals: A review, *International Journal of Dairy Technology*, 2012, **65**, 13-21.
11. D. J. McClements, Nanoemulsion-based oral delivery systems for lipophilic bioactive componnets: nutraceuticals and pharmaceuticals, *Therapeutic delivery*, 2013, **4**, 841-857.
12. A. Araiza-Calahorra, M. Akhtar and A. Sarkar, Recent advances in emulsion-based delivery approaches for curcumin: From encapsulation to bioaccessibility, *Trends in Food Science & Technology*, 2018, **71**, 155-169.
13. D. J. McClements, Recent progress in hydrogel delivery systems for improving nutraceutical bioavailability, *Food Hydrocolloids*, 2017, **68**, 238-245.
14. D. J. McClements, Enhancing nutraceutical bioavailability through food matrix design, *Current Opinion in Food Science*, 2015, **4**, 1-6.

15. S. Akhavan, E. Assadpour, I. Katouzian and S. M. Jafari, Lipid nano scale cargos for the protection and delivery of food bioactive ingredients and nutraceuticals, *Trends in Food Science & Technology*, 2018, **74**, 132-146.
16. A. Acevedo-Fani, R. Soliva-Fortuny and O. Martin-Belloso, Nanostructured emulsions and nanolaminates for delivery of active ingredients: Improving food safety and functionality, *Trends in Food Science & Technology*, 2017, **60**, 12-22.
17. L. Salvia-Trujillo, R. Soliva-Fortuny, M. A. Rojas-Grau, D. J. McClements and O. Martin-Belloso, in *Annual Review of Food Science and Technology, Vol 8*, eds. M. P. Doyle and T. R. Klaenhammer, 2017, vol. 8, pp. 439-466.
18. L. Salvia-Trujillo, C. Qian, O. Martin-Belloso and D. J. McClements, Influence of particle size on lipid digestion and beta-carotene bioaccessibility in emulsions and nanoemulsions, *Food Chem*, 2013, **141**, 1472-1480.
19. L. Zou, B. Zheng, W. Liu, C. Liu, H. Xiao and D. J. McClements, Enhancing nutraceutical bioavailability using excipient emulsions: Influence of lipid droplet size on solubility and bioaccessibility of powdered curcumin, *Journal of Functional Foods*, 2015, **15**, 72-83.
20. S. Park, S. Mun and Y.-R. Kim, Emulsifier Dependent in vitro Digestion and Bioaccessibility of β-Carotene Loaded in Oil-in-Water Emulsions, *Food Biophysics*, 2018, **13**, 147-154.
21. T. A. J. Verrijssen, K. H. G. Smeets, S. Christiaens, S. Palmers, A. M. Van Loey and M. E. Hendrickx, Relation between in vitro lipid digestion and β-carotene bioaccessibility in β-carotene-enriched emulsions with different concentrations of l-α-phosphatidylcholine, *Food Research International*, 2015, **67**, 60-66.
22. T. A. J. Verrijssen, S. H. E. Verkempinck, S. Christiaens, A. M. Van Loey and M. E. Hendrickx, The effect of pectin on in vitro β-carotene bioaccessibility and lipid digestion in low fat emulsions, *Food Hydrocolloids*, 2015, **49**, 73-81.
23. V. Ajay Mallia and R. G. Weiss, Correlations between thixotropic and structural properties of molecular gels with crystalline networks, *Soft Matter*, 2016, DOI: 10.1039/c6sm00377j.
24. M. Hu, Y. Li, E. A. Decker and D. J. McClements, Role of calcium and calcium-binding agents on the lipase digestibility of emulsified lipids using an in vitro digestion model, *Food Hydrocolloids*, 2010, **24**, 719-725.
25. L. Q. Zou, W. Liu, C. M. Liu, H. Xiao and D. J. McClements, Utilizing Food Matrix Effects To Enhance Nutraceutical Bioavailability: Increase of Curcumin Bioaccessibility Using Excipient Emulsions, *Journal of Agricultural and Food Chemistry*, 2015, **63**, 2052-2062.
26. Y. H. Wang, Z. L. Wan, X. Q. Yang, J. M. Wang, J. Guo and Y. Lin, Colloidal complexation of zein hydrolysate with tannic acid: Constructing peptides-based nanoemulsions for alga oil delivery, *Food Hydrocolloids*, 2016, **54**, 40-48.
27. J. W. Bye, N. P. Cowieson, A. J. Cowieson, P. H. Selle and R. J. Falconer, Dual effects of sodium phytate on the structural stability and solubility of proteins, *J Agric Food Chem*, 2013, **61**, 290-295.
28. A. L. de la Garza, F. I. Milagro, N. Boque, J. Campion and J. A. Martinez, Natural inhibitors of pancreatic lipase as new players in obesity treatment, *Planta Med*, 2011, **77**, 773-785.
29. V. Kumar, A. K. Sinha, H. P. S. Makkar and K. Becker, Dietary roles of phytate and phytase in human nutrition: A review, *Food chemistry*, 2010, **120**, 945-959.

30. U. Schlemmer, W. Frølich, R. M. Prieto and F. Grases, Phytate in foods and significance for humans: Food sources, intake, processing, bioavailability, protective role and analysis, *Molecular Nutrition & Food Research*, 2009, **53**, S330-S375.
31. Y. Zhou, M. Wu and H. Jiang, Effects of inositol 1,2,6-triphosphate on human liver cancer SMMC-7721 cells, *Medicinal Chemistry Research*, 2011, **21**, 4069-4073.
32. C. N. Kunyanga, J. K. Imungi, M. W. Okoth, H. K. Biesalski and V. Vadivel, Antioxidant and type 2 diabetes related functional properties of phytic acid extract from Kenyan local food ingredients: effects of traditional processing methods, *Ecology of food and nutrition*, 2011, **50**, 452-471.
33. H. Matsumoto, K. Ito, K. Yonekura, T. Tsuda, T. Ichiyanagi, M. Hirayama and T. Konishi, Enhanced Absorption of Anthocyanins after Oral Administration, *Journal of Agricultural and Food Chemistry*, 2007, **55**, 2489-2496.
34. Q. Fu, H. Wang, M. Xia, B. Deng, H. Shen, G. Ji, G. Li and Y. Xie, The effect of phytic acid on tight junctions in the human intestinal Caco-2 cell line and its mechanism, *European journal of pharmaceutical sciences : official journal of the European Federation for Pharmaceutical Sciences*, 2015, **80**, 1-8.
35. Y. Xie, H. Luo, J. Duan, C. Hong, P. Ma, G. Li, T. Zhang, T. Wu and G. Ji, Phytic acid enhances the oral absorption of isorhamnetin, quercetin, and kaempferol in total flavones of Hippophae rhamnoides L, *Fitoterapia*, 2014, **93**, 216-225.
36. B. Zheng, S. Peng, X. Zhang and D. J. McClements, Impact of Delivery System Type on Curcumin Bioaccessibility: Comparison of Curcumin-Loaded Nanoemulsions with Commercial Curcumin Supplements, *J Agric Food Chem*, 2018, **66**, 10816-10826.
37. B. J. Zheng, S. F. Peng, X. Y. Zhang and D. J. McClements, Impact of Delivery System Type on Curcumin Bioaccessibility: Comparison of Curcumin-Loaded Nanoemulsions with Commercial Curcumin Supplements, *Journal of Agricultural and Food Chemistry*, 2018, **66**, 10816-10826.
38. R. Zhang, Z. Zhang, L. Zou, H. Xiao, G. Zhang, E. A. Decker and D. J. McClements, Enhancing Nutraceutical Bioavailability from Raw and Cooked Vegetables Using Excipient Emulsions: Influence of Lipid Type on Carotenoid Bioaccessibility from Carrots, *J Agric Food Chem*, 2015, **63**, 10508-10517.
39. Y. Pei, J. Wan, M. You, D. J. McClements, Y. Li and B. Li, Impact of whey protein complexation with phytic acid on its emulsification and stabilization properties, *Food Hydrocolloids*, 2019, **87**, 90-96.
40. L. Y. Mei, E. A. Decker and D. J. McClements, Evidence of iron association with emulsion droplets and its impact on lipid oxidation, *Journal of Agricultural and Food Chemistry*, 1998, **46**, 5072-5077.
41. L. Y. Mei, D. J. McClements and E. A. Decker, Lipid oxidation in emulsions as affected by charge status of antioxidants and emulsion droplets, *Journal of Agricultural and Food Chemistry*, 1999, **47**, 2267-2273.
42. A. Sarkar, K. K. T. Goh and H. Singh, Colloidal stability and interactions of milk-protein-stabilized emulsions in an artificial saliva, *Food Hydrocolloids*, 2009, **23**, 1270-1278.

43. L. Zou, B. Zheng, R. Zhang, Z. Zhang, W. Liu, C. Liu, H. Xiao and D. J. McClements, Food Matrix Effects on Nutraceutical Bioavailability: Impact of Protein on Curcumin Bioaccessibility and Transformation in Nanoemulsion Delivery Systems and Excipient Nanoemulsions, *Food Biophysics*, 2016, **11**, 142-153.
44. D. J. McClements, Enhanced delivery of lipophilic bioactives using emulsions: a review of major factors affecting vitamin, nutraceutical, and lipid bioaccessibility, *Food & Function*, 2018, **9**, 22-41.
45. L. K. Mao and S. Miao, Structuring Food Emulsions to Improve Nutrient Delivery During Digestion, *Food Engineering Reviews*, 2015, **7**, 439-451.
46. Q. Lin, R. Liang, A. Ye, H. Singh and F. Zhong, Effects of calcium on lipid digestion in nanoemulsions stabilized by modified starch: Implications for bioaccessibility of β -carotene, *Food Hydrocolloids*, 2017, **73**, 184-193.
47. J. Yi, Y. Li, F. Zhong and W. Yokoyama, The physicochemical stability and in vitro bioaccessibility of beta-carotene in oil-in-water sodium caseinate emulsions, *Food Hydrocolloids*, 2014, **35**, 19-27.
48. M. Primozic, A. Duchek, M. Nickerson and S. Ghosh, Formation, stability and in vitro digestibility of nanoemulsions stabilized by high-pressure homogenized lentil proteins isolate, *Food Hydrocolloids*, 2018, **77**, 126-141.
49. C. E. Gumus, G. Davidov-Pardo and D. J. McClements, Lutein-enriched emulsion-based delivery systems: Impact of Maillard conjugation on physicochemical stability and gastrointestinal fate, *Food Hydrocolloids*, 2016, **60**, 38-49.
50. B. R. Shah, C. Zhang, Y. Li and B. Li, Bioaccessibility and antioxidant activity of curcumin after encapsulated by nano and Pickering emulsion based on chitosan-tripolyphosphate nanoparticles, *Food Research International*, 2016, **89**, 399-407.
51. Y. Li, M. Hu and D. J. McClements, Factors affecting lipase digestibility of emulsified lipids using an in vitro digestion model: Proposal for a standardised pH-stat method, *Food Chemistry*, 2011, **126**, 498-505.
52. B. E. Knuckles, Effect of Phytate and Other Myo-lnositol Phosphate Esters on Lipase Activity, *Journal of food science* 1988, **53**, 250-252.
53. V. Denstadli, A. Skrede, Å. Krogdahl, S. Sahlstrøm and T. Storebakken, Feed intake, growth, feed conversion, digestibility, enzyme activities and intestinal structure in Atlantic salmon (Salmo salar L.) fed graded levels of phytic acid, *Aquaculture*, 2006, **256**, 365-376.
54. D. D. Maenz, C. M. Engele-Schaan, R. W. Newkirk and H. L. Classen, The effect of minerals and mineral chelators on the formation of phytase-resistant and phytase-susceptible forms of phytic acid in solution and in a slurry of canola meal, *Animal Feed Science and Technology*, 1999, **81**, 177-192.
55. Q. Lin, R. Liang, F. Zhong, A. Ye and H. Singh, Effect of degree of octenyl succinic anhydride (OSA) substitution on the digestion of emulsions and the bioaccessibility of β-carotene in OSA-modified-starch-stabilized-emulsions, *Food Hydrocolloids*, 2018, **84**, 303-312.
56. Y. Yang, E. A. Decker, H. Xiao and D. J. McClements, Enhancing vitamin E bioaccessibility: factors impacting solubilization and hydrolysis of alpha-tocopherol acetate encapsulated in emulsion-based delivery systems, *Food Funct*, 2015, **6**, 84-97.

57. J. Corte-Real, M. Iddir, C. Soukoulis, E. Richling, L. Hoffmann and T. Bohn, Effect of divalent minerals on the bioaccessibility of pure carotenoids and on physical properties of gastro-intestinal fluids, *Food Chem*, 2016, **197**, 546-553.
58. N. Wang, H. F. Yang, X. Zhu, R. Zhang, Y. Wang, G. F. Huang and Z. R. Zhang, Synthesis of anti-aggregation silver nanoparticles based on inositol hexakisphosphoric micelles for a stable surface enhanced Raman scattering substrate, *Nanotechnology*, 2009, **20**, 315603.
59. Y. Liu, J. Wang, H. Lu, M. Li, P. Chen and L. Fang, Facile synthesis of visible-light-driven Ag3PO4 nanocrystals base on IP6 micelles, *Catalysis Communications*, 2014, **55**, 65-69.
60. M. Cheryan, Phytic acid interactions in food systems, *Critical reviews in food science and nutrition*, 1980, **13**, 297-335.

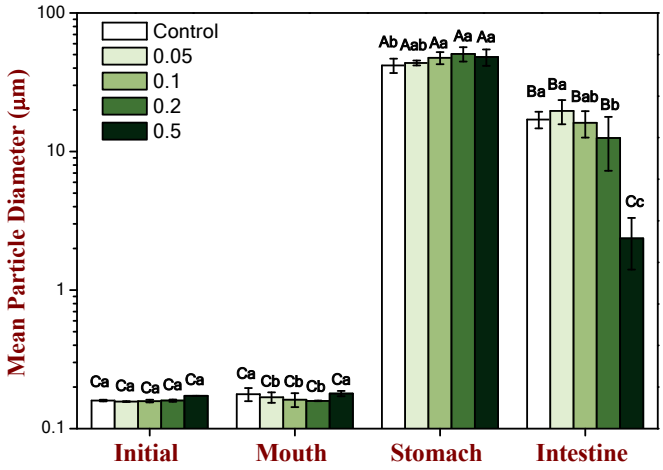

Figure 1. Influence of phytic acid level (control=0, 0.05, 0.1, 0.2, and 0.5%) on the mean droplet diameter ($D_{4,3}$) of curcumin loaded-emulsion at different simulated gastrointestinal phases. Different capital letters (A, B, C) mean significantly different (LSD, $p < 0.05$) between different GIT phases (same phytic acid level). Different lower-case letters (a, b, c) mean significantly different (LSD, $p < 0.05$) between different phytic acid levels (same GIT phase).

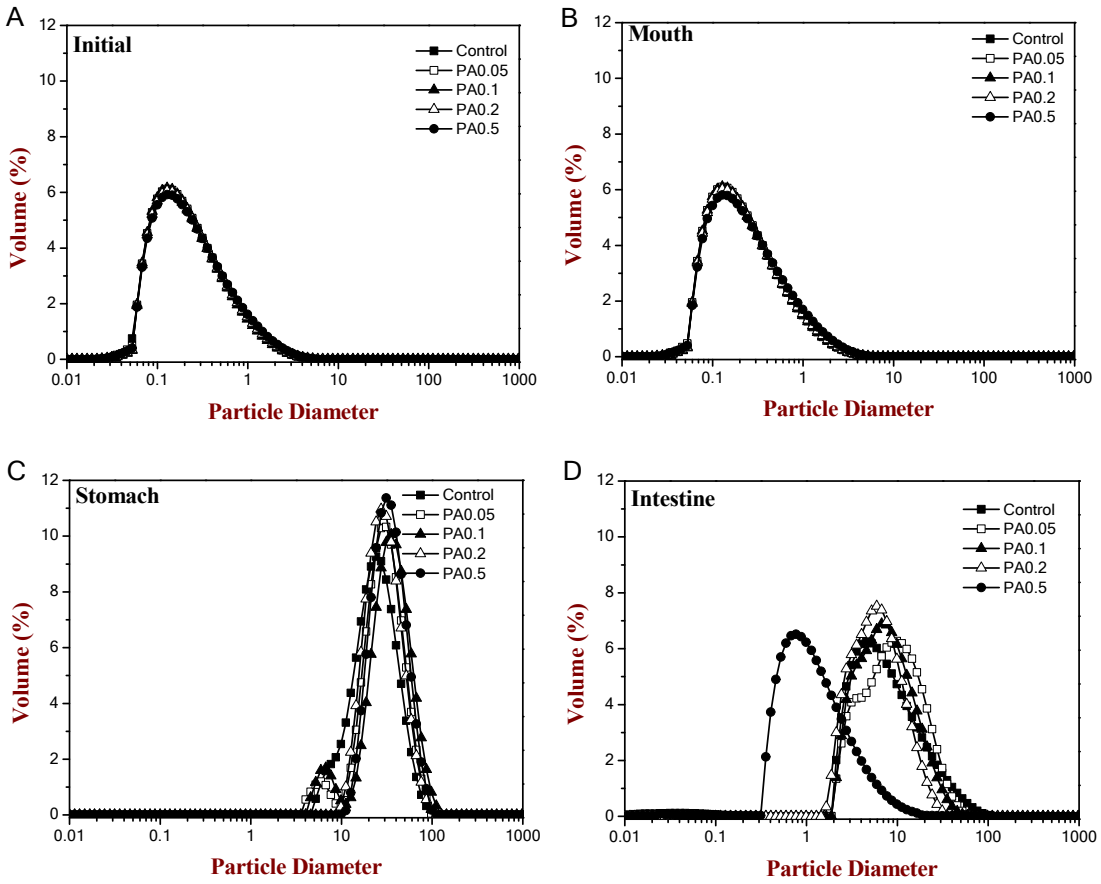

Figure 2. Particle size distributions of emulsion with different phytic acid level after exposure to different GIT phases: (A)initial phase, (B) mouth phase, (C) stomach phase and (D) small intestine phase.

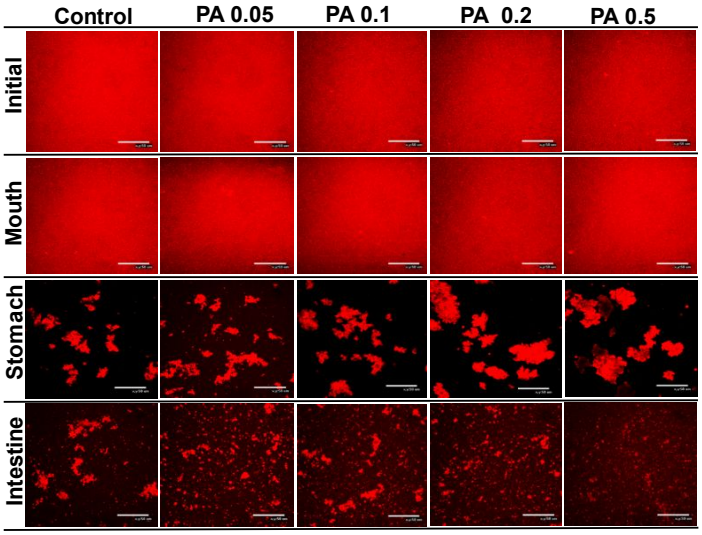

Figure 3. Microstructure of curcumin-emulsion containing different phytic acid level after exposure to different GIT phases. Scale bar is 50 μm.

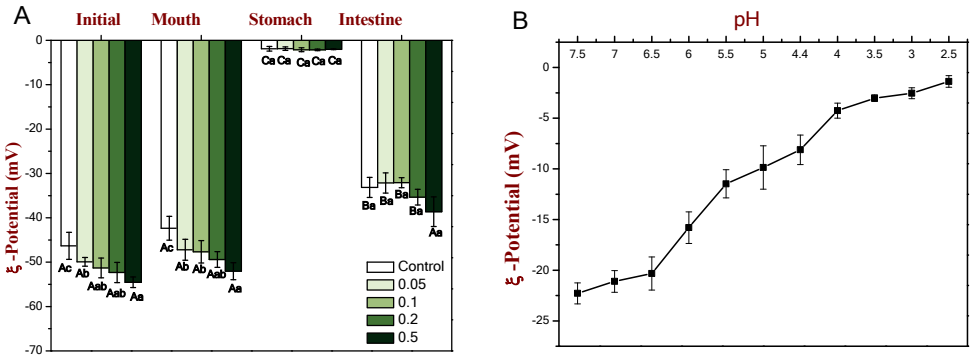

Figure 4. Influence of phytic acid concentrations on the ζ-potential of curcumin-emulsion at different simulated gastrointestinal phase (A). ζ-potential of phytic acid (PA) at different pH values (B). Different capital letters (A, B, C) mean significantly different (LSD, $p < 0.05$) between different GIT phases (same phytic acid level). Different lower-case letters (a, b, c) mean significantly different (LSD, $p < 0.05$) between different phytic acid levels (same GIT phase).

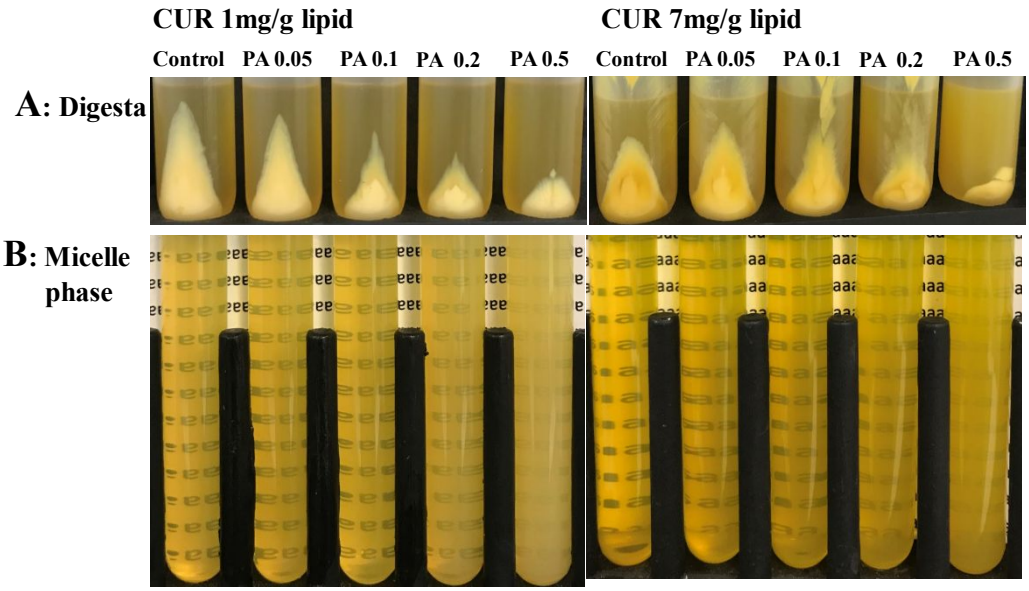

Figure 5. Visual appearance of A: digesta phase and B: micelle phase after digestion of curcumin loaded-emulsion containing different phytic acid level.

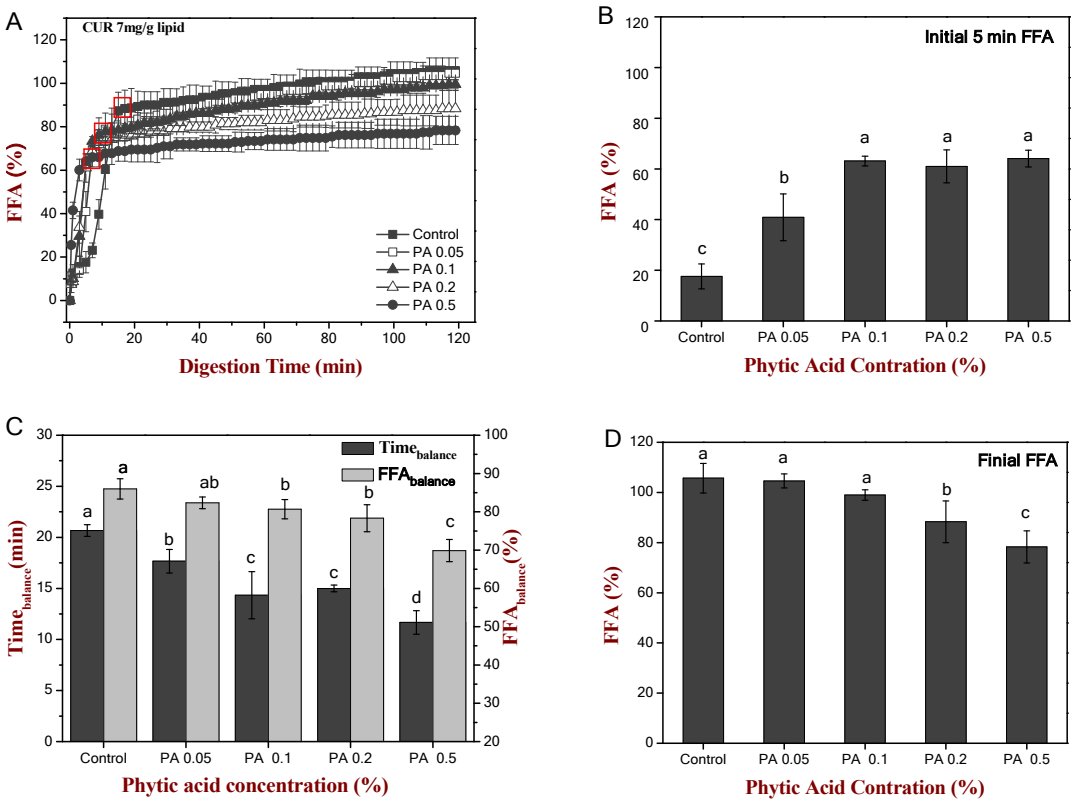

Figure 6. Lipid digestion results of emulsion containing different phytic acid level: (A) Total digestion curve; (B) the amount of FFAs released at initial 5 min; (C) the time and amount of FFAs released when fatty acid production reach to balance (D) the FFAs amount after 2 h digestion.

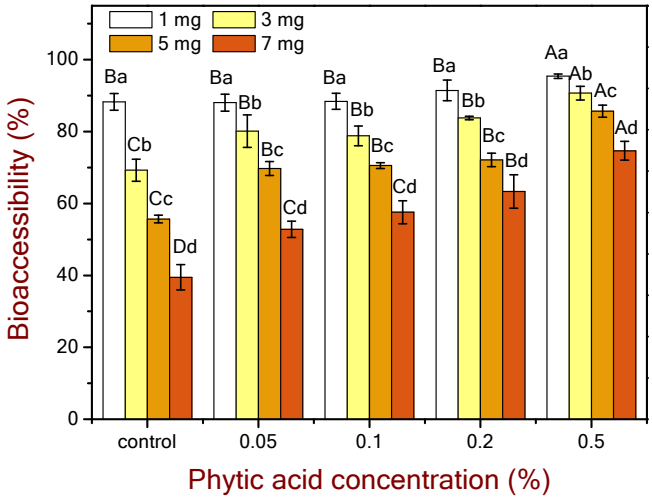

Figure 7. Influence of phytic acid concentration on bioaccessibility of curcumin in emulsion with different encapsulation levels (1, 3, 5, 7 mg Cur/g lipid).

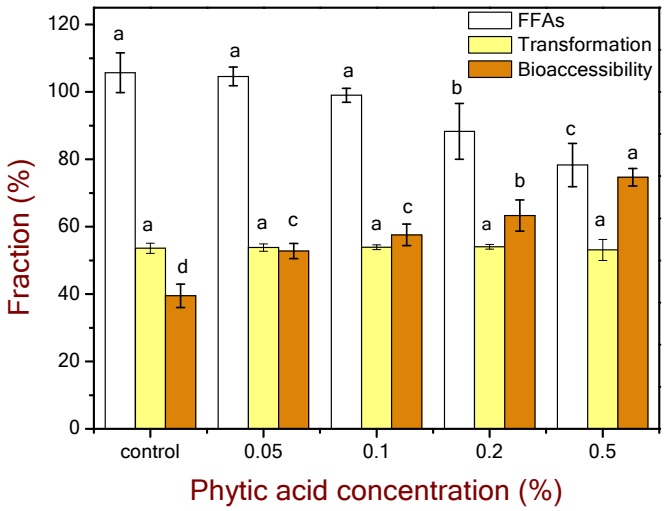

Figure 8. The FFAs amounts, bioaccessibility and transformation of curcumin dependence on phytic acid level, the concentration of loaded-curcumin was 7 mg Cur/g lipid.

Graphical abstract