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Chi Yan

Xiaolin Wu

Yi Wang

Shengfeng Peng

Jun Chen

See next page for additional authors

Authors

Chi Yan, Xiaolin Wu, Yi Wang, Shengfeng Peng, Jun Chen, Liqiang Zou, David Julian McClements, and Wei Liu

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Utilization of polysaccharide-based high internal phase emulsion for nutraceutical encapsulation: Enhancement of carotenoid loading capacity and stability

Chi Yan [a], Xiaolin Wu [a], Yi Wang [d], Shengfeng Peng [a,b,*], Jun Chen [a], Liqiang Zou [a], David Julian McClements [e], Wei Liu [a,c]

[a] State Key Laboratory of Food Science and Technology, Nanchang University, No. 235 Nanjing East Road, Nanchang 330047, Jiangxi, China
[b] School of Life Sciences, Nanchang University, Nanchang 330031, Jiangxi, PR China
[c] National R&D Center for Freshwater Fish Processing, Jiangxi Normal University, Nanchang, Jiangxi 330022, China
[d] Meilu Biotechnology Co., Ltd., Yining Town, Xiushui Country No.35, Baihui Street, Jiujiang 332005, Jiangxi, China
[e] Biopolymers and Colloids Laboratory, Department of Food Science, University of Massachusetts, Amherst, MA 01003, USA

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ABSTRACT

The main goal of the present work was to access the ability of high internal phase emulsions (HIPEs) to encapsulate β-carotene. The carotenoid loading capacity of the HIPEs was around 20-fold higher when OSA-starch/chitosan complexes were used than when only OSA-starch was used. This impact could be mainly assigned to the capacity of the former HIPEs to trap carotenoid caystals in a stable form. The OSA-starch/chitosan complexes were shown to absorb on the oil droplets interface and form a 3D network in the aqueous phase, which helped to prevent droplet coalescence induced by β-carotene crystal. The incorporation of β-carotene within the oil droplets enhanced its resistance to chemical degradation when exposed to heat, ultraviolet radiation, or gastrointestinal conditions. Our results provide information that may aid the design and development of edible soft solids containing high carotenoid levels, which may be applied in food and pharmaceutical industry.

1. Introduction

Vitamin A (retinol) is involved within a broad spectrum of biological functions in humans (Melendez-Martinez, 2019). In particular, it is essential for the proper development and functioning of human vision, immunity, reproduction, and other processes (Grune et al., 2010). However, preformed vitamin A is only present in animal food (such as liver and eggs), which may not be commonly consumed by all members of a population. Consequently, the intake of vitamin A for people living in many developing countries, as well as for vegetarians and vegans who do not eat some or all animal foods, is often lower than the amount required to ensure good health and performance. Indeed, vitamin A deficiency has been declared as a major public health problem throughout the world, with almost one-third of children under five being impacted (Awasthi & Awasthi, 2020; Duran-Cabral, Fernandez-Jalao, Estevez-Santiago, & Olmedilla Alonso, 2017; Gupta, Arora, Sharma, & Sharma, 2019). The deficiency of vitamin A is the main cause of preventable blindness in children, has adverse influences on fetal growth, and increases the susceptibility to infections and death (Awasthi & Awasthi, 2020; Duran-Cabral et al., 2017; Ribeiro, Shahgol, Estevinho, & Rocha, 2020). β-carotene ($C_{40}H_{56}$) is a member of the carotenoid group of nutraceuticals, which has been reported to be an important precursor for vitamin A (Liu, Wang, McClements, & Zou, 2018). Humans can therefore meet their demands for vitamin A by consuming β-carotene-rich foods in their diets. After ingestion, the β-carotene is transformed into retinol catalyzed by β-carotene 15,15′-monooxygenase (Gul et al., 2015). The toxicity often observed when high doses of vitamin A are administered orally are not observed when high doses of β-carotene are used, which can be attributed to feedback mechanisms that regulate β-carotene production in human systems (Grune et al., 2010). In addition to its pro-vitamin A activity, β-carotene also have other potential health benefits, such as the ability to reduce cancers, cardiovascular disease, and infections (Ha, Park, Kim, & Lee, 2012; Kaulmann & Bohn, 2014; Manfred & Adrian, 2018). Moreover, studies have reported that supplementation of β-carotene (50 mg per day) for a long time can provide cognitive benefits to a healthy population (Grodstein, Kang,

* Corresponding author at: State Key Laboratory of Food Science and Technology, Nanchang University, Nanchang 330047, Jiangxi, China.
E-mail address: pengsf@ncu.edu.cn (S. Peng).

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Glynn, Cook, & Gaziano, 2007; Melendez-Martinez, 2019). As an efficient natural antioxidant with the ability to quench nearly 1000 free radicals per molecule, β-carotene may also help prevent oxidative cellular damage.

The incorporation of β-carotene into commercial food products is often challenging. First, the poor solubility of β-carotene in water and extremely high melting point lead it difficult to incorporate with other aqueous-based food matrixes (Qian, Decker, Xiao, & Mcclements, 2012; Yan, McClements, Zou, & Liu, 2019). Second, the bioavailability of β-carotene, especially obtained from natural food, has been proved to be relatively low (<10%), which is mainly because the carotenoids are trapped inside plant cell structures and because of the poor solubility in gastrointestinal fluids (Gul et al., 2015; Wang, Liu, Mei, Nakajima, & Yin, 2012). Third, β-carotene contains multiple conjugated double bonds, which makes it highly sensitive to oxidation (Fang, Zhao, Liu, Liang, & Yang, 2019). Numerous encapsulation technologies have been explored for their potential to improve the solubility, chemical stability, and oral bioavailability of β-carotene, including oil-in-water emulsions (Chen, Yokoyama, Liang, & Zhong, 2020; Liu et al., 2020), powders (Fang et al., 2019), liposomes (Tanaka et al., 2020), and macromolecule complexes (Jain, Thakur, Ghoshal, Katare, & Shivhare, 2016). Emulsion-based systems are one of the most suitable for this purpose due to their relative ease of preparation and incorporation into food products (Lin, Liang, Williams, & Zhong, 2018; McClements, 2010). However, the loading capacity of the β-carotene in conventional oil-in-water emulsions is typically relatively low (<0.1 wt%) due to the limited solubility of the carotenoids in oil (Chen, Li, Li, McClements, & Xiao, 2017; Lin, Liang, Zhong, Ye, & Singh, 2018; Yi et al., 2020). The consumption of low concentration β-carotene emulsions to meet the daily required β-carotene may lead a lot of oil phase intake and the financial burden is extremely huge for consumers in developing countries. These points limit the potentially beneficial bioactivety of β-carotene. Researchers have reported that at least 6 mg/d of β-carotene should be ingested to reach the recommended intake of vitamin A (Grune et al., 2010). However, ingestion of at least 12 mg/d β-carotene over an extended period has been reported to protect humans from UV-induced skin diseases (Heinrich et al., 2003). To meet this level, a large volume of β-carotene-loaded emulsions would have to be regularly consumed, which may limit their intake. It would therefore be desirable to fabricate emulsions containing high concentrations of β-carotene. Previous studies have shown that high levels of β-carotene can be incorporated into emulsions containing high amounts of synthetic surfactants, but these systems may not be suitable for commercial food applications (Li et al., 2019). There is therefore still a need for emulsion-based systems that are fabricated from natural ingredients using simple and economical methods that can incorporate high levels β-carotene.

The use of high internal phase emulsion (HIPEs) in a wide range of application has been explored because of their specific characteristics, such as gel-like properties and high resistance to creaming (Hu et al., 2017; Kim et al., 2017; Wijaya, Van der Meeren, Wijaya, & Patel, 2017). The high internal phase volume fraction in HIPEs (>74%) means that the droplets are dense and deformed. Moreover, in the case of oil-in-water emulsions, it means that there is a relatively large volume of oil available to incorporate hydrophobic bioactive molecules. Due to their unique structure and physicochemical characteristics, HIPEs have already been employed as drug carrier systems (Tan et al., 2017), templates for the creation of porous materials (Silverstein, 2014), and as filter membranes (Kim et al., 2017). The gel-like structure and relatively good microbiological stability (due to their low water activity) are also beneficial for many food applications (Tan, Sun, Lin, Mu, & Ngai, 2014). Previous reports have shown that HIPE can be used to encapsulate liposoluble ingredients, which improve their stability, bioavailability, and release characteristics (Yan, McClements, Zhu et al., 2019). For instance, the retention and bioaccessibility of β-carotene could be enhanced via coating the oil droplets in HIPEs with whey protein microgels (Liu et al., 2019). Moreover, the viability of *Lactobacillus plantarum*, a probiotic, during pasteurization has been improved by encapsulating it within HIPEs (Su et al., 2018).

In our previous research, surfactant-free HIPEs stabilized by chitosan/OSA-starch electrostatic complexes were developed (Yan, McClements, Zhu et al., 2019). In the present study, our main aim was to establish whether these HIPEs could be used to encapsulate relatively high levels of β-carotene. Initially, the influence of β-carotene concentration on the formation, texture, stability, and microstructure of these HIPEs was investigated. Then, the effect of pH adjustment on the properties of β-carotene-loaded HIPEs was studied as a simple means of modifying their textural and functional attributed. Finally, the stability and oral bioaccessibility of β-carotene in the HIPEs system were evaluated using a simulated gastrointestinal model.

2. Materials and methods

2.1. Materials

OSA-starch (PURITY GUM 2000) was donated by Ingredion company (Songjiang, Shanghai, China). β-carotene (≥96%) and chitosan (C105802, 200–400 mPa.s) were purchased from Aladdin Biochemical Technology Co., Ltd (Shanghai, China). Corn oil was purchased from Yi Haikerry Grain and Oil Food Company (Nanchang, China). Mucin (M2378), pepsin (P7125, ≥400 U/mg^{-1}), lipase (L3126, 100–500 U/mg^{-1}), pancreatin (P1750), Nile Blue A and Nile Red were obtained from the Sigma Chemical Company (St. Louis, MO). All other reagents were of analytical grade.

2.2. Preparation of OSA-starch/chitosan electrostatic complexes solution

The polysaccharide complexes were prepared according to a previous method (Yan, McClements, Zhu et al., 2019). A stock chitosan solution was prepared via dissolving chitosan powder in acetic acid solution (1%). A stock OSA-starch solution was obtained by adding the powdered ingredient in water. Then, chitosan and OSA-starch solution were mixed together to prepare a solution containing both OSA-starch and chitosan, the concentration was 1.0 wt% and 0.2 wt%. Finally, the pH values were adjusted by adding HCl or NaOH solutions.

2.3. Fabrication of polysaccharide-based HIPEs loading with β-carotene

The oil phase was prepared by adding the β-carotene to the corn oil at different ratio and stirred 1 h at 90 °C. Corn oil (15 g) including different amounts of β-carotene and OSA-starch/chitosan complexes solution (5 g) were sheared by high-shear blender (ULTRA TURRAX T18 digital, IKA, Staufen, Germany) at 15,000 rpm for 2 min. The concentrations of OSA-starch and chitosan in the final HIPE systems produced were 0.25 wt% and 0.05 wt%, respectively, while the β-carotene concentration was varied from 0 to 2 wt%.

2.4. Centrifugation stability

The stability of emulsions was investigated by centrifugation, which is according to a reported method (Zhou et al., 2018). 20 g of samples were weighted and then centrifuged at 8000*g* for 30 min. The appearance of samples after treatment was recorded by a digital camera.

2.5. Rheological properties

The rheological properties of samples were quantified by Rotary Rheometer (MCR302, Anton Paar, Germany). A plate-and-plate geometry measurement cell was applied (pp-50, 50 mm diameter). A frequency sweep (0.1–100 rad/s) and shear sweep (shear rate 0.01–100 s^{-1}) were applied to investigate the rheological properties of HIPEs. The other conditions were maintained constant: test temperature = 25 °C; gap between plates = 1 mm; strain = 0.5% (which was within the linear

viscoelastic region).

2.6. Characterization of the microstructure

Confocal laser scanning microscope (Carl Zeiss LSM710, Jena, Germany) was applied to observe the microstructure of samples. Nile Red and Nile Blue A were added as dyes before the preparation of HIPEs, and the dyed HIPEs were added into a glass-bottom cell culture dish prior to observation.

A cryo-scanning electron microscope (Cryo-SEM) was also employed to detect the microstructure (Quanta 450, FEI, USA) based on a method described previously (Liu et al., 2020). The samples were rapidly frozen by liquid nitrogen and then observed by the Cryo-SEM.

2.7. Stability of β-carotene encapsulated in HIPEs

For these studies, the composition of polysaccharide complexes and the β-carotene concentration (2.0 wt%) in these systems were fixed, and the pH was varied from 4.0 to 6.0. The β-carotene embeded in HIPEs constructed by only OSA-starch (pH 5.0, 5%) was also investigated as a control group.

UV light stability: The UV light stability study was mearsured according to method in our previous study with some modifications (Liu et al., 2020). The samples (15 g) were poured into a transparent glass culture dish (60 mm diameter × 10 mm height) and then the dishes were placed on a container with 4 UV-lamps (312 nm) at room temperature.

Thermal stability: the thermal stability of the carotenoids was assessed by incubating sealed samples at 60 °C in the dark. At 2 day intervals, the concentration of β-carotene remaining was determined and calculated.

2.8. In vitro bioavailability of β-carotene

The *in vitro* bioavailability was determined using a static simulated gastrointestinal tract (Lin, Liang, Ye, Singh, & Zhong, 2017; Yan, McClements, Zou et al., 2019). HIPEs stabilized by polysaccharide complexes with different pH value (4.0–6.0) were evaluated since they have different structures. The concentration of β-carotene was fixed at 2.0 wt% in these systems. Traditional β-carotene-loaded HIPEs stabilized by 5 wt% OSA-starch (pH 5.0) was also studied as a control group. All of the enzymes and solutions were dissolved at least two hours in advance, and heated at 37 °C in a water bath before utilization.

Mouth stage: Simulated saliva fluid with mucin, α-amylase, and salts was obtained based on previously reported methods (Lin et al., 2017; Lin, Liang, Zhong et al., 2018). Then, 7.5 ml of samples (diluted by buffer solution) were mixed with an equal volumes of simulated saliva fluid and incubated in a shaker (100 rpm for 10 min) after adjusting to pH 6.8.

Gastric stage: Simulated gastric fluid composed with pepsin, sodium chloride, and hydrochloric acid was obtained based on the previous study (Chen et al., 2018). Then, 15 ml of simulated gastric fluid was mixed with the samples collected from the mouth stage and incubated in a shaker (100 rpm for 2 h) after being adjusted to pH 2.5.

Intestinal stage: The pH of processed samples were modulated to pH 7.0, and added to simulated intestinal fluid containing lipase, pancreatin, bile solution, and amyloglucosidase. The value of the samples were measured and adjusted to 7.0 by adding sodium hydroxide using a pH-stat (Metrohm, China Inc). The volume of consumed sodium hydroxide solution could be used to figure up the percentage of oil droplets digested.

Retention and bioaccessibility: After the three-stage digestion, the processed samples were centrifuged. The β-carotene in middle mixed micelles phase was extracted by n-hexane (Park, Mun, & Kim, 2018). Then, the absorbance of the β-carotene in the solvent at 450 nm was recorded using a UV–Vis spectrophotometer (U-T1810, Yipu, China). The retention (R) and bioaccessibility (B) could be calculated using following equations:

$$B(\%) = 100^{*}C_M/C_D$$

$$S(\%) = 100^{*}C_D/C_I$$

where C_M, C_D, and C_I are the concentration of β-carotene in the micelle phase, in the digesta, and in the HIPEs before digestion (accounting for dilution effects).

2.9. Statistical analysis

All of the experiments were carried out at least three times, and the mean and standard deviation were obtained from these values by analysis software (Statistical Product and Service Solutions, 21.0).

3. Results and discussion

3.1. Preparation of HIPEs loaded with high contents β-carotene

Our initial objective was to determine whether a high amount of β-carotene could be successfully loaded into HIPEs constructed with OSA-starch or OSA-starch/chitosan complexes (pH 5.0). The maximum solubility of β-carotene in corn oil was mearsured by dispersing an excess amount of β-carotene at 95 °C. Then the oil was centrifuged at room termperature and the amount of dissolved β-carotene was determined. The solubility of β-carotene in the corn oil is 0.41% ± 0.01%, which means the solubility of β-carotene in the conventional HIPEs is around 0.31% (75 wt% oil phase).

Fig. 1a shows that HIPEs remained gel-like as the concentration of β-carotene increased from 0 to 2 wt% in the final systems, as demonstrated by the tilting the samples. As the β-carotene concentration was increased, the color of the HIPEs changed from white to orange to deep red, which is due to increased light absorption by the carotenoid molecules. The fact that much more than 0.31% β-carotene could be included in these systems, suggested that any carotenoid crystals formed were trapped within the semi-solid matrix of these HIPEs. In contrast, the HIPEs constructed with only OSA-starch were unable to encapsulate large amount of β-carotene, and the oil droplets coalesced almost immediately after dispersion. This effect suggested that the OSA-starch alone was unable to protect the droplets from coming close and merging together.

Centrifugation experiments were also carried out to investigated the stability of the β-carotene-loaded HIPEs (Fig. 1b). The HIPE constructed by OSA-starch/chitosan complexes stayed stable, regardless of the carotenoid concentration. In contrast, the HIPEs prepared using only OSA-starch were unstable when the β-carotene content was 0.5 wt% or higher. This effect may have been because β-carotene crystals were formed when the carotenoid concentration exceeded the maximum solubility (0.31%). These results indicated that HIPEs stabilized by OSA-starch/chitosan complex could encapsulate β-carotene at a concentration greater than the solubility limit, whereas HIPEs stabilized by OSA-starch could not.

In general, β-carotene crystals would deposit in the bottom of the oil droplets due to its higher density. A previous report has also proved that the β-carotene crystal could distribute at the oil droplets surface and the HIPEs compaction degree of the oil droplet decreased with the increasing loaded β-carotene amount (Li et al., 2019). Our result indicate that the presence of β-carotene crystals at the oil droplet surfaces promoted their destabilization in the case of OSA-starch, but not in the case of OSA-starch/chitosan. Thus, the presence of this kind of complex could improve the stability of HIPEs containing high levels of β-carotene crystals.

3.2. Characterization of HIPEs loaded with high-content β-carotene

Confocal microscopy was used to observe the effects of high

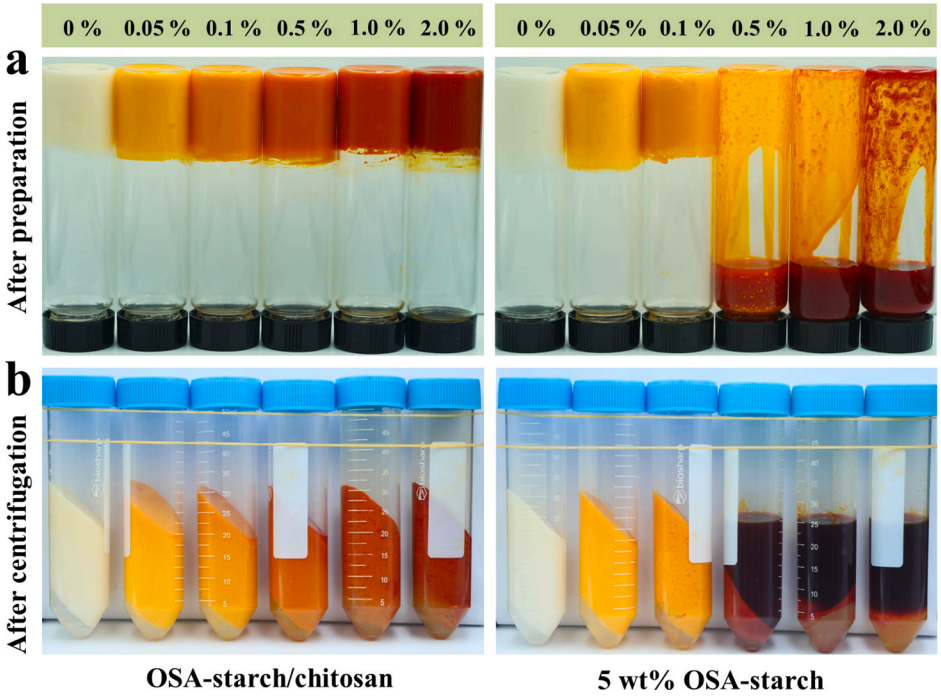

Fig. 1. Photographs of HIPEs containing different amounts of β-carotene stabilized by OSA-starch/chitosan complexes and 5 wt% OSA-starch at pH 5: (a) immediately after preparation; (b) after centrifugation.

concentration β-carotene on the microstructure of HIPEs (Fig. 2). Interestingly, there were no significant changes in oil droplet size and compaction degree with the increase in β-carotene concentration. The oil phase and polysaccharides were dyed to observed the size of oil droplets and the distribution of polysaccharide molecules in the HIPEs. As expected, the oil droplets were crowded and the OSA-starch/chitosan complexes were present at the droplet surface (red fluorescence), which was similar to our previous research (Yan, McClements, Zhu et al., 2019). The assistance of a polysaccharide-rich coating around the oil droplet leads to the construction of protective films that generate electrostatic and steric repulsive forces that help prevent droplet coalescence. The presence of a polysaccharide coating around the oil droplets could not be observed in the HIPEs prepared using only OSA-starch, which may have been because it only formed a very thin layer.

Cryo-SEM was carried out to further observe the microstructure of samples (Fig. 3a). The HIPEs formed using only OSA-starch contained large spherical particles with smooth surfaces, which were presumably the OSA-starch packed the droplet. In contrast, HIPEs formed using OSA-starch/chitosan complexes contained smaller particles with rougher surfaces that were separated by a honey comb-like structure (Fig. 3b), which were presumably polysaccharide-coated oil droplets surrounded by a network of aggregated polysaccharide molecules (Mao & McClements, 2012). Combining the results from the visual appearance, centrifugation stability, and microstructure analysis, it could deduce that the HIPE formed with these electrostatic complexes could be used as a carrier to encapsulate a large amount of β-carotene. And the high stability of OSA-starch/chitosan can be mainly due to the changes of complexes interfacial behavior and water phase structure. First, our previous studies have proved that the emulsifiers properties of OSA-starch can be improved via the fabrication of OSA-starch/chitosan complexes, the interfacial adsorption of polysaccharides and the thickness of oil interface coating also increased which have been further proved by CLSM images (Fig. 2) (Yan, McClements, Zhu et al., 2019). The thicker coating can improve the stability of emulsion systems via provide electrostatic and steric repulsive forces. Moreover, the 3D-network of OSA-starch/chitosan in the water phase can prevent the move of individual oil droplets and resist the aggregation of oil droplets.

The rheological properties of the carotenoid-loaded HIPEs are also measured because they are important in many food industries (Fig. 3). Compared with loss modulus (G″), the storage modulus (G′) of all samples was significantly large throughout frequencies tested, which indicates that the HIPEs exhibited predominantly elastic behavior. The value of two kind of modulus rised steadily with increasing frequency, the result suggests that the noncovalent interaction was dominated responsible for gel-like structures (Dai et al., 2018). And the value of them rised as β-carotene amount in the systems increased, these phenomena are contrary to a previous report (Li et al., 2019). The viscosity of the pure oil phase at 50 s^{-1} measured increased from 11.6 ± 0.5 to 52.9 ± 8.2 mPa.s when the β-carotene concentration rised. It has been reported that the oil droplets composed of higher viscosity oil, as active fills and main components of the HIPEs gel network, would become less deformable during rheological measurements and strengthen the gel strength (Zou, Thijssen, Yang, & Scholten, 2019). The apparent viscosity of HIPEs also increased with increasing β-carotene concentration (Fig. 3d). Moreover, the apparent viscosity exhibited shear-thinning behavior, which is typical of materials where there is progressive disruption of a gel network with increasing shear rat (Li et al., 2019).

In summary, our rheological measurements indicated that the addition of β-carotene enhanced the gel strength of HIPE systems, which may be an advantage for cetain applications.

3.3. *Effect of pH values on HIPEs loading β-carotene*

The impact of pH on the performance of the HIPE loaded with a high amount of β-carotene was examined because foods vary widely in their pH conditions. Moreover, it may be possible to tune the properties of HIPEs by manipulating their pH. In this section, the β-carotene concentration trapped in the HIPEs was fixed at 2.0 wt% and the pH value was adjusted to a range varying from 3.0 to 7.0.

As shown in Fig. 4a, fluid HIPEs were formed at pH 3.0, which flowed in the containers when they were inverted. Conversely, the gel HIPE could be constructed when pH higher than 3, as demonstrated by the fact that they stayed where them were in the containers. These same phenomenon has also been observed in our previous research on related

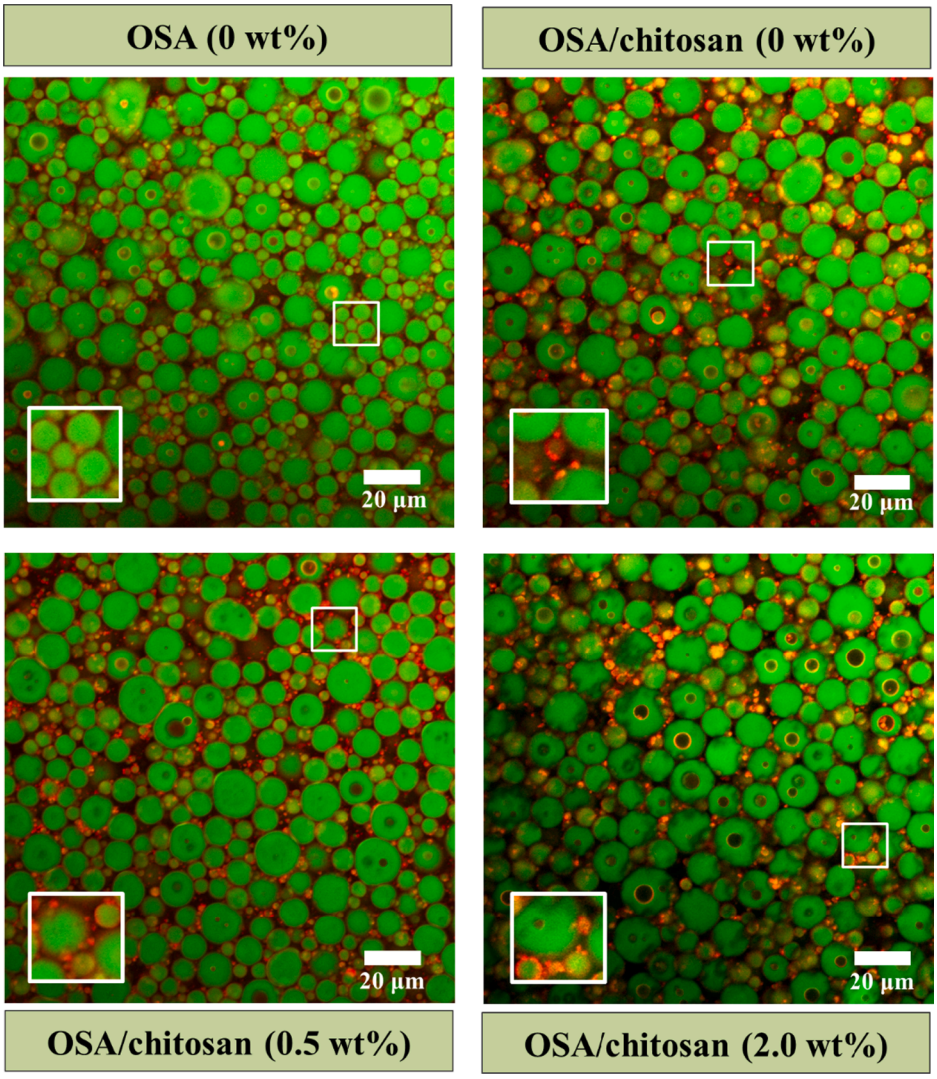

Fig. 2. CLSM images of HIPEs loading different amount of β-carotene (0 wt%, 0.5 wt%, and 2.0 wt%) stabilized by OSA-starch/chitosan complexes and 5 wt% OSA-starch at pH 5. (Red: polysaccharides, green: oil phase). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

systems (Yan, McClements, Zhu et al., 2019). The HIPE which exhibits like lipuid under most acidic conditions could mainly be attributed to a dissociation of the electrostatic complex. The anionic OSA-starch molecules lose some of their negative charge at pH 3 and therefore do not interact as strongly with the cationic chitosan molecules.

The effects of pH on the carotenoid-loaded HIPE centrifugation stability were also evaluated (Fig. 4b). At pH 3.0 and 7.0, the HIPEs were unstable with a layer of oil phase observed at the top of samples. Centrifugation increases the disruptive forces acting on the oil droplets, which can promote their coalescence. These phenomena indicate that polysaccharide coatings formed at extreme pH are less effective at protecting the droplets against coalescence. As mentioned earlier, at pH 3.0, this may be due to dissociation of the complexes because the OSA-starch loses some of its negative charge. Conversely, at pH 7.0, the complexes may dissociate because the chitosan loses some of its positive charge and precipitate from solution. Therefore, relatively stable carotenoid-loaded HIPEs could only be formed from pH 4.0 to 6.0.

The visual images of HIPEs throughout the pH loaded with a high amount of β-carotene was also recorded by camera. After removal from the containers, the ability of the HIPEs to maintain their shape rised with the pH rised to 6.0 but then decreased when the pH was further raised to 7.0 (Fig. 5a). As discussed earlier, the more fluid-like behavior at the pH extremes can be attributed to dissociation of the electrostatic polysaccharide complexes in the HIPEs. Interestingly, the β-carotene loaded HIPEs fromed at pH 6.0 could be cut into a cube using a knife. The ability to create different textures at different pH values may be valuable for certain food applications. For instance, in some cases it may be advantageous to have a soft flowable material (e.g., a dressing), whereas in other applications a firmer structure is required (e.g., a dessert).

To further investigate the pH-responsive of the HIPEs, rheological measurements were carried out (Fig. 5b and c). The G′ values of the test samples were higher than the G″ values at the same frequency, which indicated the HIPEs were predominantly semi-solid elastic materials (Yan, McClements, Zhu et al., 2019). The G′ of HIPEs rised as pH values was raised to 6.0 then reduced when it was further raised to pH 7.0, which was consistent with the visual images. The apparent viscosity shown as Non-newtonian systems and raised as the pH increased from 3.0 to 6.0, while it decreased at pH 7.0. Taken together, the phenomenon indicated that the gel-like HIPE containing high levels of β-carotene could be fabricated over a certain pH range.

The microstructural organizations of the HIPEs at varied pH were observed by CLSM (Fig. 6), with oil phase (green) and polysaccharides (red) being stained. The dimension of the droplet reduced and their compaction rised as the pH increased from 3.0 to 6.0 but then decreased

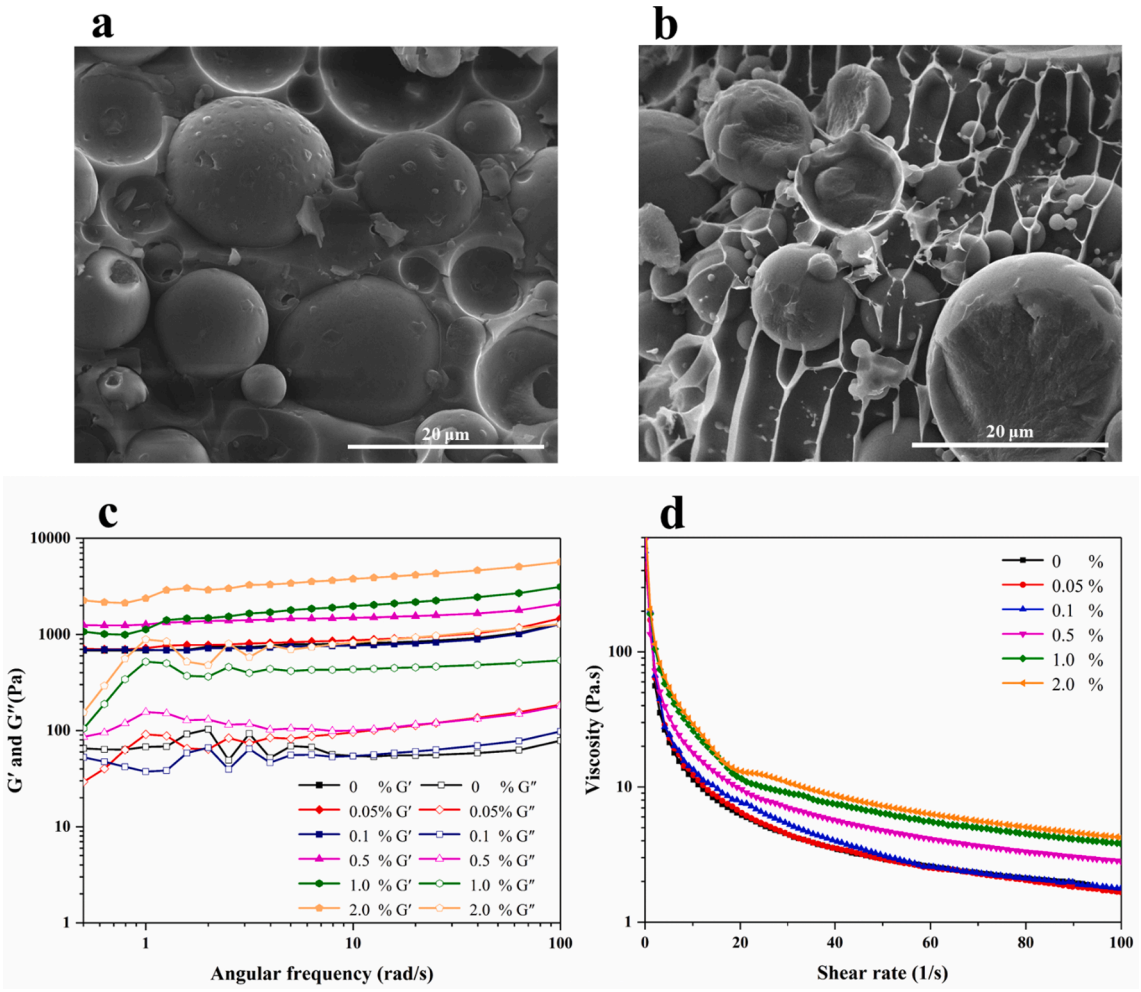

Fig. 3. Cryo-SEM images of HIPEs stabilized by (a) 5 wt% OSA-starch and (b) OSA-starch/chitosan complexes at pH 5.0; Rheological properties of HIPEs containing different amount of β-carotene stabilized by OSA-starch/chitosan (c) oscillatory frequency sweep curves; and (d) apparent viscosity versus shear rate.

when it was raised further to 7.0. Meanwhile, the distribution of polysaccharides (red) in the HIPEs also depended on pH values. The amount of polysaccharides at the oil droplet surfaces increased from pH 3.0 to 6.0, while decreased at pH 7.0. These phenomenon have also been observed in our previous studies (Yan, McClements, Zhu et al., 2019), and the increased of polysaccharide at the oil–water interface with the increase of pH value from 3 to 6 can be mainly due to the changes of the polysaccharide complexes emulsifying properties with the variation of pH values. And the large number of free polysaccharides in the water phase at pH 7.0 is mainly due to the presence of chitosan interpolymer ($pK_a \sim 6.5$). There was also evidence of polysaccharide in the spaces between the oil droplets, which is consistent with the cryo-SEM images (Fig. 3b). These results support the rheology measurements – the viscosity and shear modulus of the samples may have increased at intermediate pH because of the formation of a 3D network of polysaccharide complexes that provided mechanical rigidity. These complexes formed a protection layer in the oil droplet interface and network between droplets, which would have strengthed the gels formed and protected the droplets from coalescence. The dissociation of the OSA-starch/chitosan complexes at low and high pH values reduced the gel strength and stability of the HIPEs.

In these studies, the rheological, texture and stability of HIPEs could be manipulated by altering the pH value of water phase, and the pH-responsive properties of these systems can be mainly due to the changes of electrostatic force between polysaccharides. Although previous also reported some pH-responsive HIPEs, most of them were fabricated by some materials which are not allow to be employed in food field (Chen et al., 2013; Sun et al., 2014). And the regulation of pH mainly influenced the demulsification of HIPEs and interface behaviors of emulsifiers (Jia et al., 2021). Another interesting HIPE system has also been prepared with cross-linking/electrostatic deposition of polysaccharides via complex processes (including successive ultrasonication and centrifugation), and the pH modulating could only control the release of lipid nutrients (Huang, Wang, & Tan, 2021). Compared with previous pH-responsive HIPEs, the OSA-starch/chitosan HIPEs may be more suitable for creating a range of edible soft materials that are suitable for different food applications.

3.4. Chemical stability of β-carotene in HIPEs

β-carotene is extremely susceptible to degradation when exposed to external harsh conditions, such as light, high temperature, and oxygen because the presence of a large amount of conjugated double bonds. For these reasons, the stability of β-carotene embedded into HIPE systems was investigated in this section.

The photostability of the β-carotene was assessed via measuring reduction in carotenoid concentration over time when the HIPEs were exposed to UV light (Fig. 7). The level of carotenoids embedded into the OSA-starch HIPEs decreased to around 30% of its initial vakue after 10 days of UV exposure. The reduction in β-carotene concentration in the OSA-starch/chitosan HIPEs was considerably slower than the control samples, which indicates that the complexes were more effective at

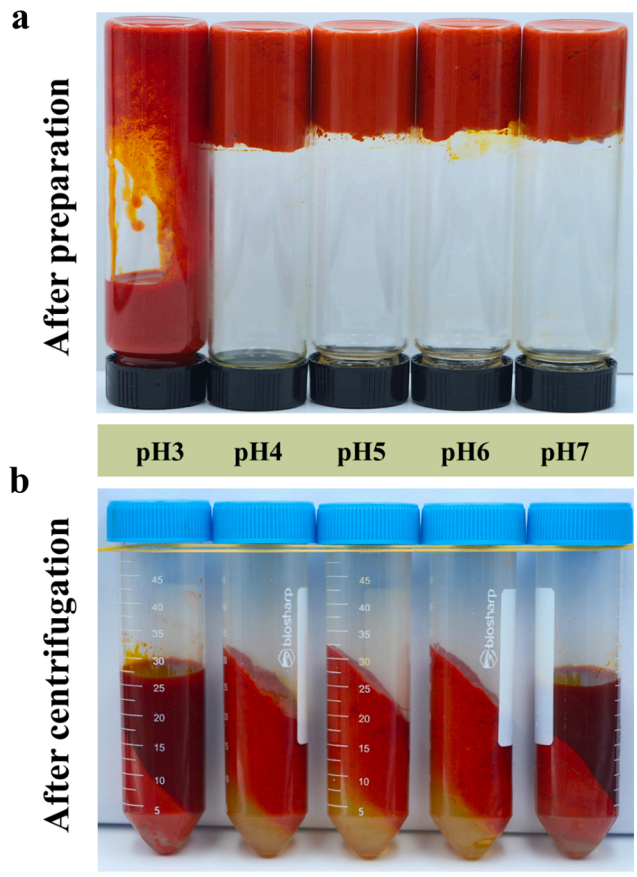

Fig. 4. Appearance of HIPEs containing 2 wt% β-carotene stabilized by OSA-starch/chitosan complexes at different pH values: (a) immediately after preparation; (b) after centrifugation.

protecting the carotenoids than the OSA-starch alone. The thermal stability of carotenoids was evaluated by storing HIPEs at 60 °C. The stability of carotenoids was considerably higher in OSA-starch/chitosan HIPEs than in the OSA-starch ones, with a higher concentration remaining after 4 days storage.

The enhancement of β-carotene stability in the OSA-starch/chitosan HIPEs can be attributed to several reasons. First, the positively charged film in the oil droplets interface and the network of polysaccharide in the water phase could prevent and decelerate the contact of oxygen and partial UV light with the internal β-carotene. Moreover, most β-carotene in these systems existed as crystal form which possesses better stability than dissolved form. In conclusion, the data shown that the stability could be greatly enhanced by encapsulated it in OSA-starch/chitosan HIPEs, thereby helping to minimize its loss during processing and storage.

3.5. Retention and bioaccessibility of β-carotene

Several factors can impact the biological activity of β-carotene after ingestion, including its bioaccessibility and chemical transformation in the gastrointestinal fluids. Indeed, previous researchers have reported that a portion of β-carotene experiences protonation and *cis–trans* isomerization, followed by chemical degradation under the acidic conditions (pH 2.5) found in a simulated gastric environment (Mortensen & Skibsted, 2000). Therefore, *in vitro* digestion systems were applied to investigate the digestion procedures of the HIPE constructed with electrostatic complexes and loaded with 2 wt% β-carotene at pH values ranging from 4.0 to 6.0. A HIPEs stabilized by only OSA-starch and loaded with 0.1 wt% β-carotene (pH 5.0) was used as a control.

The images of samples after *in vitro* stomach digestion are shown in Fig. 8a. The oil droplets in the control sample and the HIPEs at pH 4.0 were evenly dispersed in the gastric fluids. However, the HIPEs formed at pH 5.0 and 6.0 contained gel fragments that floated on the surface of the digestive juice, with the oil droplets being trapped inside the gel networks. These results can be attributed to the lack of digestive enzymes in the gastric phase to breakdown the polysaccharides used, as

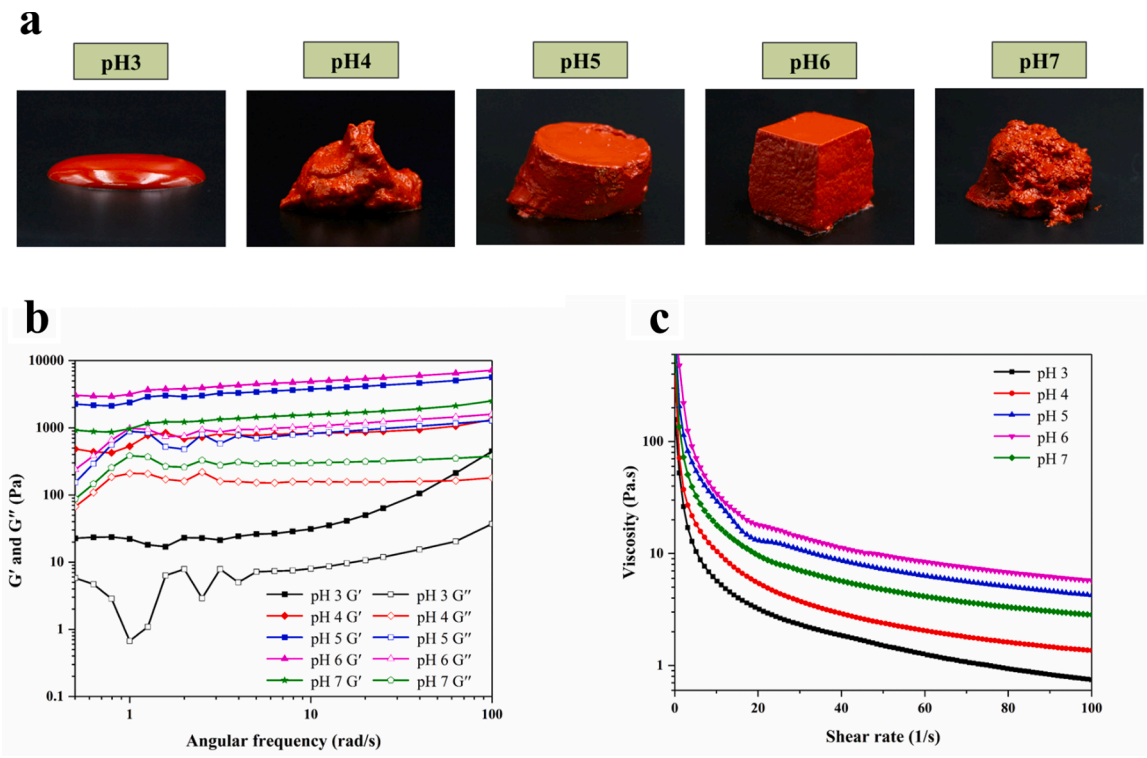

Fig. 5. Visual appearance (a) and rheological properties (b) of HIPEs containing 2 wt% β-carotene stabilized by OSA-starch/chitosan at different pH values.

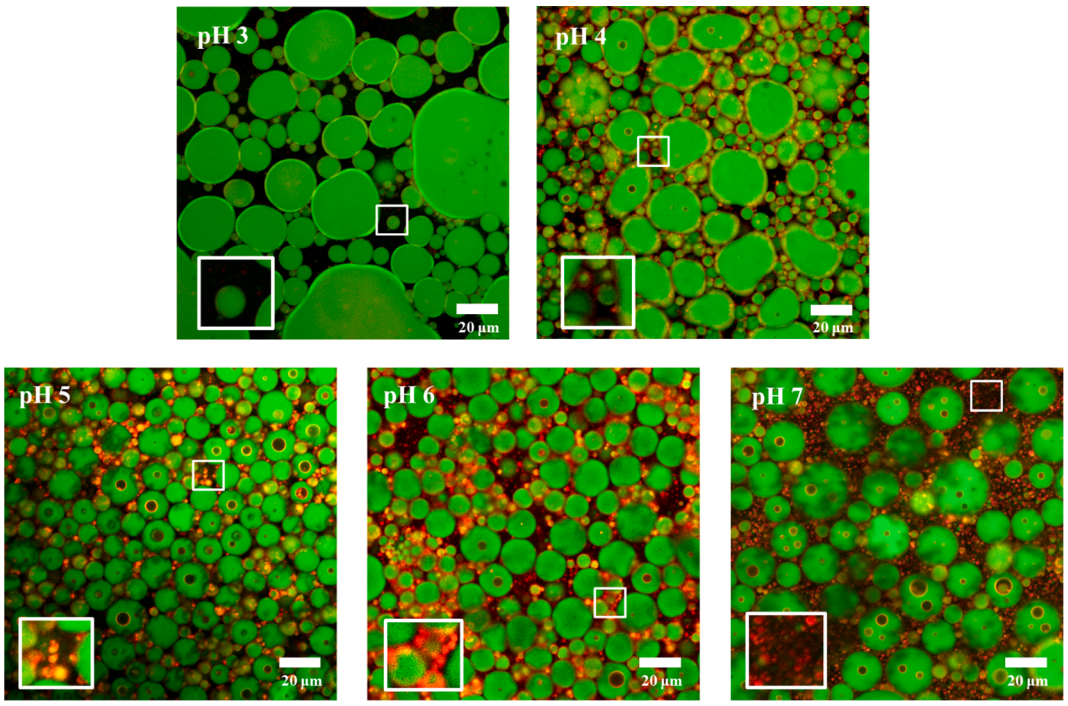

Fig. 6. CLSM of HIPEs containing 2 wt% *β*-carotene stabilized by OSA-starch/chitosan at different pH values.

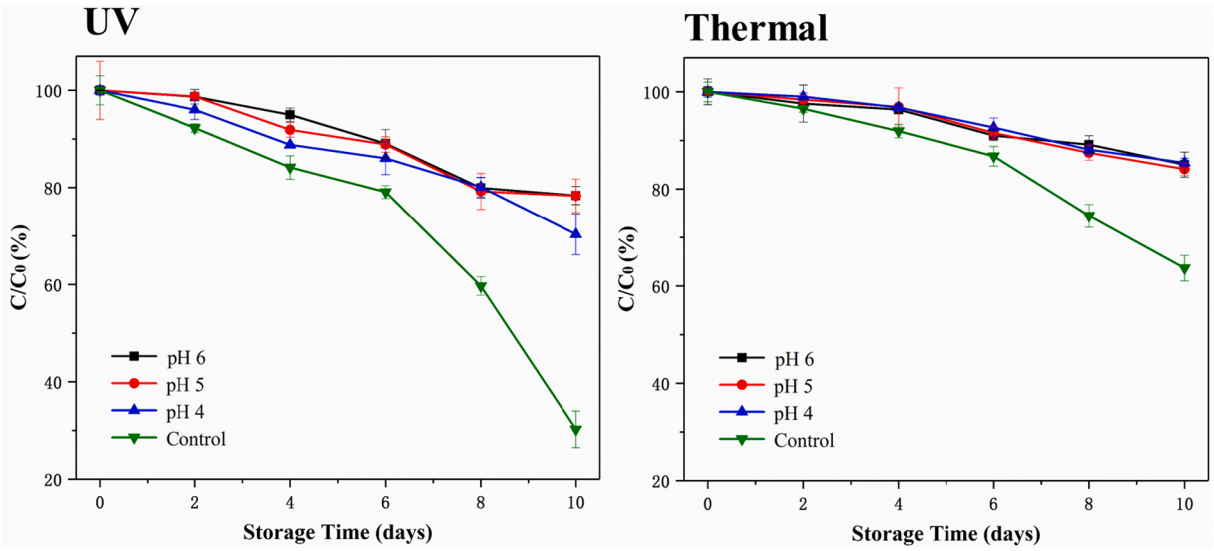

Fig. 7. Stability of *β*-carotene encapsulated within HIPEs stabilized by OSA-starch/chitosan at different pH values and 5 wt% OSA-starch. (Left) Degradation of *β*-carotene exposed to UV light; (Right) Degradation of *β*-carotene at 60 °C in dark.

well as to the construction of a firm polysaccharide network that prevented oil droplets from leaking out. In the intestinal stage, the extent of lipid digestion was measured by monitoring the release of free fatty acid over time (Fig. 8b). The initial digestion rate was faster for the control and HIPEs at pH 4.0 than for the HIPEs at pH 5.0 and 6.0. This effect can be due to the larger surface area of lipids contacting with lipase when the droplets were freely dispersed in the intestinal fluids. Conversely, when the oil droplets were trapped inside the gel fragments there were less lipase could contact with the lipids. This phenomenon of delayed digestion may be useful for creating functional foods designed to enhance satiety or for creating sustained release profiles of bioactive agents.

At the end of the simulated gastrointestinal model the retention of *β*-carotene was measured to provide insights into its chemical stability under gastrointestinal conditions (Fig. 8c). Compared with control samples, the retention was considerably higher for the *β*-carotene encapsulated in HIPEs constructed by complexes, which may arise to to several reasons. First, the cationic electrostatic complexes coating droplets may prevent contact of the encapsulated *β*-carotene with oxygen, pro-oxidants, and free radicals (Atarian, Rajaei, Tabatabaei, Mohsenifar, & Bodaghi, 2019). Second, the majority of *β*-carotene was in a crystalline form in the OSA-starch/chitosan HIPEs but a soluble form in the OSA-starch HIPEs. Typically, the crystalline form of a carotenoid will be more chemically stable because it is harder for pro-oxidants to access the double bonds. The retention of *β*-carotene increased slightly with increasing pH. This result is mainly attributed to the fact that the carotenoids are better protected in the gel fragments because of the increased pathlength that the pro-oxidants have to travel to reach the oil

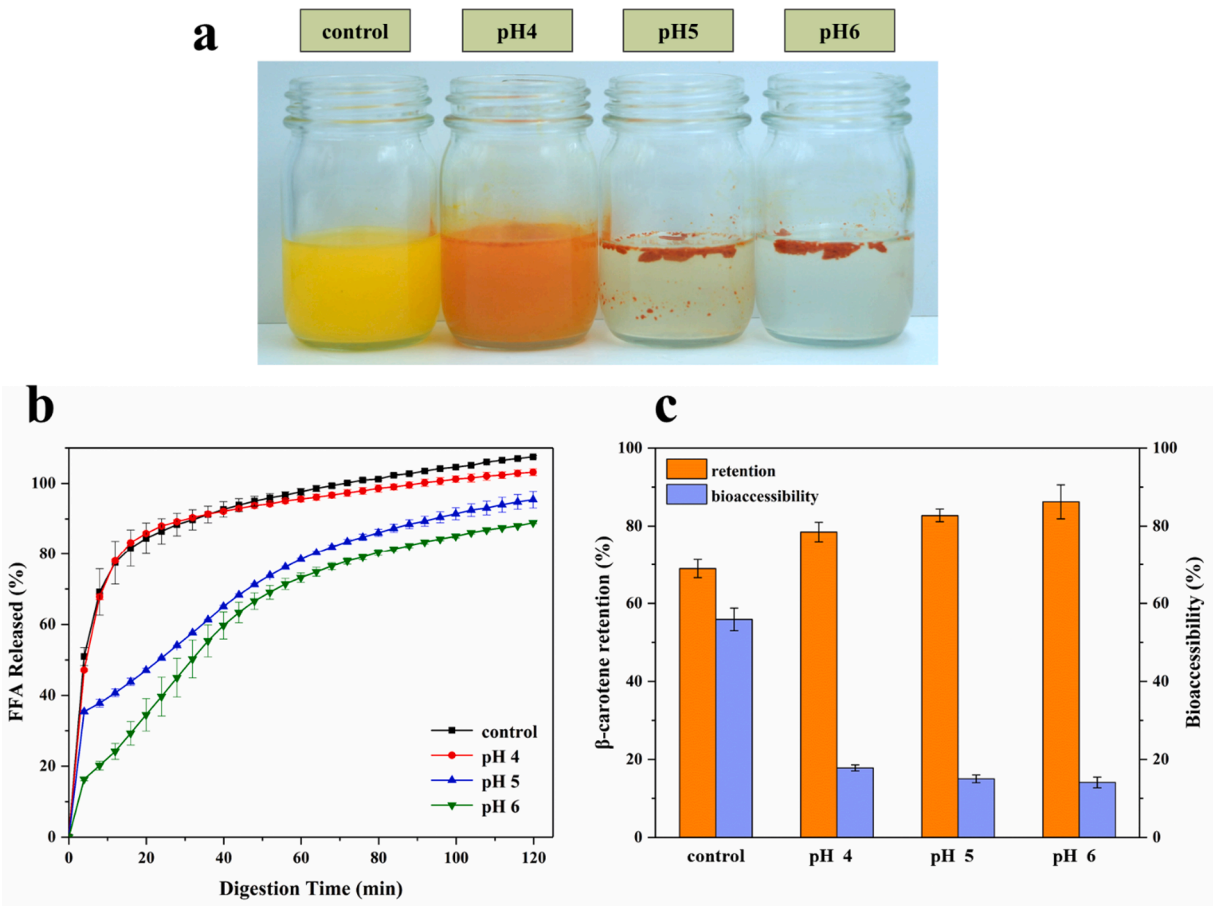

Fig. 8. (a) Appearance of samples before simulated *in vitro* small intestine digestion for HIPEs (pH 4.0–6.0) and control samples; (b) fatty acids released curve of HIPEs and control samples during digestion; (c) the *β*-carotene retention and bioaccessibility of HIPEs and control samples.

droplet surfaces. Moreover, the amount of polysaccharide absorbed to the droplet surfaces increased as the pH increased, as the pictures of CLSM. These mearsurements indicate that the HIPE constructed with electrostatic complexes are better to prevent the *β*-carotene from degradation during the digestion condition.

Finally, the bioaccessibility of protected *β*-carotene was investigated. The data was calculated by measuring *β*-carotene in the digesta and micelle phases after *in vitro* digestion. Mixed micelles, which are small colloidal particles composed of bile salts and free fatty acids, can solubilize any carotenoids released during digestion and then transport them to the enterocytes. The *β*-carotene bioaccessibility of control samples was higher than that of HIPEs stabilized by OSA-starch/chitosan. These effects could mainly be due to that crystalline carotenoids have a much lower bioaccessibility than solubilized ones. In addition, the surpression of lipid digestion in the OSA-starch/chitosan HIPEs leads to fewer mixed micelles being generated that can solubilize the *β*-carotene. The bioaccessibility decreased slightly with increasing pH, which could again be due to the decrease in lipid digestion in the HIPEs at higher pH values (Fig. 8b). Since the lower bioaccessibility of high loading contents HIPE carrier systems is mainly due to the low amount of lipid, it may be possible to overcome the low bioaccessibility of the carotenoids in the HIPEs by introducing another source of readily digestible fat, such as small lipid droplets. The fat in other food matrices can participate in the formation of micelles and solubilization of β-carotene and improve the bioaccessibility and absorption of β-carotene. Indeed, previous researchers reported that bioaccessibility of *β*-carotene in carrots could be enhanced by mixing with small lipid droplets (Zhang et al., 2016). Meanwhile, previous studies reported that only a small amount of fat (3–5 g) is sufficient for the release of *β*-carotene (Van het Hof, West, Weststrate, & Hautvast, 2000). Therefore, the low bioaccessibility of *β*-carotene in OSA-starch/chitosan HIPEs can be easily improved via the presence of other food matrixes. And these strategies are rational because most of the high oil content of food systems (such as HIPEs) always be used as additive or flavoring, such as mayonnaise, in other food matrices. In general, the high loading contents of *β*-carotene HIPEs stabilized by OSA-starch/chitosan could be used with other food systems to meet the daily nutritional demand of human being.

4. Conclusion

In summary, we have shown that HIPEs constructed with electrostatic complexes could be used to encapsulate high quantities of *β*-carotene. We also investigated the effects of *β*-carotene concentration and pH on the characterizes of the HIPEs, such as their stability, rheology, and microstructure. These results showed that is no obvious influence of *β*-carotene amount on the stability of the HIPEs. In fact, increasing the concentration of *β*-carotene presents actually enhanced gel strengthen of HIPE systems. Cryo-SEM results showed that an OSA-starch/chitosan network formed around and between the oil droplet. The rheological characterize of HIPEs could be adjusted via altering pH values of the water phase surrounding the oil droplets, which enabled us to produce soft materials with either fluid-like or solid-like structure. The stability of *β*-carotene could be enhanced by embedding into the HIPE at both storage and gastrointestinal conditions. However, the bioaccessibility of the carotenoids was reduced after encapsulation in the HIPEs stabilized with electrostatic complexes, which could attributed to surpression of oil droplets digestion and high levels of crystalline *β*-carotene. In future studies, the examine strategies should be

investigated to overcome this limitation. The HIPE constructued in this projetc could be applied as the food-grade soft solid material which can be employed in food, cosmetics, or pharmaceutical applications.

Ethics statement

The authors declare that there are no animal and human subjects/ experiments in this work.

CRediT authorship contribution statement

Chi Yan: Conceptualization, Methodology, Investigation, Writing - original draft. **Xiaolin Wu:** Methodology. **Yi Wang:** Formal analysis. **Shengfeng Peng:** Validation, Methodology, Investigation. **Jun Chen:** Writing - review & editing. **Liqiang Zou:** Writing - review & editing. **David Julian McClements:** Writing - review & editing. **Wei Liu:** Writing - review & editing, Conceptualization, Project administration, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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