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Control of lipid digestion and nutraceutical bioaccessibility using starch-based filled hydrogels: Influence of starch and surfactant type

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ABSTRACT

The aim of this study was to prepare starch-based filled hydrogels fortified with a model lipophilic nutraceutical (β-carotene) and determine the influence of starch type (mung bean or rice starch) and surfactant type (whey protein isolate (WPI) or Tween 20 (T20)) on lipid digestibility and β-carotene bioaccessibility. Microstructure, lipid digestion, and β-carotene bioaccessibility were measured as the emulsions and filled hydrogels were passed through a simulated gastrointestinal tract that included oral, gastric, and intestinal phases. The bioaccessibility was defined as the fraction of β-carotene solubilized within the mixed micelle phase after lipid digestion. Non-encapsulated T20-stabilized emulsions had better aggregation stability than WPI-stabilized emulsions under simulated gastrointestinal conditions, which led to a higher β-carotene bioaccessibility. Both Tween 20- and WPI-stabilized emulsions incorporated in starch-based filled hydrogels led to high lipid digestion and β-carotene bioaccessibility. The release behavior of free fatty acids during lipase digestion depended on the type of starch used as a hydrogel matrix, which was attributed to differences in starch structure. The information obtained in this study may be useful for the fortification of starch-based gelled products with lipophilic nutraceuticals.

1. Introduction

Growing concern about high incidences of chronic diseases (such as diabetes, hypertension and cancer) in both developed and developing nations has encouraged many food companies to fortify their products with bioactive ingredients, such as vitamins, minerals, and nutraceuticals (Chaudhry & Castle, 2011; Cushen, Kerry, Morris, Cruz-Romero, & Cummins, 2012). Nevertheless, there are often considerable challenges that must be overcome before many of these bioactive agents can be successfully incorporated into functional food and beverage products (McClements, Decker, & Park, 2009). For example, many highly lipophilic bioactive molecules are difficult to incorporate into foods because of their chemical instability, poor water-solubility, and/or low oral bioavailability (Jingling Tang & Zhong-Gui, 2007; Liang, Shoemaker, Yang, Zhong, & Huang, 2013; McClements, 2013; Reboul, 2013). Thus, there is a need for food-grade delivery systems to encapsulate, protect, and release this type of bioactive agent, which are suitable for utilization in a wide range of commercial food products.

Emulsion-based delivery systems are one of the most convenient methods for incorporating lipophilic bioactive agents into foods (Qian, Decker, Xiao, & McClements, 2013; Singh, Ye, & Horne, 2009; Yi, Li, Zhong, & Yokoyama, 2014). There have been numerous studies on the factors influencing the formation, stability, and bioaccessibility of β-carotene enriched emulsion-based delivery systems. The bioaccessibility of carotenoids can be greatly improved by co-ingesting them with digestible lipids, such as triacylglycerols (Hou et al., 2012; Thakkar, Maziya-Dixon, Dixon, & Failla, 2007). When the triacylglycerols are digested by gastric and pancreatic lipases they form free fatty acids and monoacylglycerols. These lipid digestion products are then incorporated along with bile acids and phospholipids into mixed micelles that solubilize and transport the carotenoids to the epithelium cells (Qian, Decker, Xiao, & McClements, 2012; Wang, Liu, Mei, Nakajima, & Yin, 2012; Yonekura & Nagao, 2007).

Many commonly consumed food products have gel-like properties, such as sauces, dips, dressings, and desserts. These products

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are often prepared using food-grade proteins and/or polysaccharides that form a three-dimensional polymer or particle network in water, thereby providing some viscoelastic properties to the system. There is therefore a need for emulsion-based delivery systems that can be incorporated into these types of food-grade hydrogels to fortify them with lipophilic nutraceuticals. In addition, there is a need to understand how encapsulation of bioactive components within hydrogels alters their bioaccessibility, *i.e.,* their release from the food matrix and incorporation into mixed micelles under gastrointestinal conditions.

Starch is the most commonly consumed polysaccharide in the human diet and is present in appreciable amounts in many staple foods, including potato, wheat, corn, rice, and barley (Bharath Kumar & Prabhasankar, 2014; Gunaratne & Corke, 2007; Lee, Woo, Lim, Kim, & Lim, 2005). The fact that starch is abundant, cheap, safe, and readily forms hydrogels upon heating means that it is particularly suitable for fabricating food-grade filled hydrogels. In addition, there are other advantages of using starch as a gelling material in foods. First, the rheological and textural properties of filled hydrogels can be controlled by varying the characteristics of the starch used, *i.e.,* amylose content, molecular structure, and concentration (Lu et al., 2009; Xie et al., 2009; Zhang et al., 2013). Second, the rate and extent of starch digestion within the human gastrointestinal tract (GIT) can also be controlled by selecting different types of starch (Syahariza, Sar, Hasjim, Tizzotti, & Gilbert, 2013; You, Lim, Lee, & Chung, 2014; Zhu, Liu, Wilson, Gu, & Shi, 2011). As a result, lipid digestion and bioactive release within the GIT may be controlled by encapsulating bioactive-fortified lipid droplets within hydrogels containing different starch types.

Rice is the most widely consumed staple food for a large part of the human population and rice-based gel products are particularly popular in Asia, and are finding increasing acceptance in other cultures (Ronda, Villanueva, & Collar, 2014; Rosell, Barro, Sousa, & Mena, 2014). In this study, we used two kinds of starch to form the hydrogels (rice and mung bean starch) because they have different molecular, physicochemical, and biological properties (Ahmed, 2012; Hoover, Li, Hynes, & Senanayake, 1997; Kim, Lee, Baik, Joo, & Yoo, 2007), which may impact their influence of the gastrointestinal fate of encapsulated lipid droplets. Mung beans are seeds of legumes, which have high levels of starch and protein, and are able to form strong starch gels (Hoover et al., 1997). We also used two different kinds of lipid droplets to fortify the hydrogels with β-carotene: droplets coated by either a protein or a non-ionic surfactant. The influence of starch and emulsifier type on the microstructure, lipid digestibility, and β-carotene bioaccessibility of the filled hydrogels was examined using a simulated GIT. This carotenoid was used as an example of a highly lipophilic bioactive component that might be used to fortify functional foods (Rao & Rao, 2007; Tanumihardjo, 2013).

The information obtained from this study should be useful for the development of starch-based functional food products fortified with lipophilic bioactive components that may improve human health and wellness.

2. Material and methods

2.1. Materials

The rice starch used for this study was isolated from native rice (Ilmi byeo, Korea) in a laboratory using a traditional alkaline method (Lumdubwong & Seib, 2000). Mung bean starch, isolated from dehulled and swollen grains using an alkaline steeping method, was kindly provided by Dr. Malshick Shin (Carbohydrate Biomaterial Laboratory, Chonnam National University, South Korea). The rice and mung bean starch used in this study contained around 0.04 and 0.3% protein and around 14 and 40% amylose, respectively. β-carotene, Tween 20, pancreatin (from porcine pancreas) and bile extract (porcine) were purchased from Sigma Aldrich (St. Louis, MO). Whey protein isolate (WPI) was kindly provided by Davisco Foods International Inc. (BiPro, Le Sueur, MN, USA). Corn oil was purchased from a local supermarket. All other chemicals used were of analytical grade. Double-distilled water was used to prepare all solution and emulsions.

2.2. Measurement of dynamic viscoelasticity of starch

The gel properties of native rice and mung bean starch were determined using a dynamic shear rheometer (AR1500ex Rheometer, TA Instruments Ltd, New Castle, DE, USA) operating in oscillatory mode with parallel plate geometry (20 mm diameter, 1 mm gap). A starch dispersion was prepared by mixing powdered starch (5 g) with distilled water (100 g) and then heating at 95 °C in a water bath for 30 min. At the end of the heating period, the resulting hot starch paste was immediately loaded between the parallel plates of the rheometer previously equilibrated at 4 °C, and a thin layer of paraffin oil was applied to the outer edges of the sample to prevent evaporation during measurement. The change in shear modulus was then measured over time at a strain of 0.5% and an oscillation frequency of 1 Hz. Subsequently, a dynamic frequency sweep test was conducted by applying a constant strain of 0.5%, which was within the linear viscoelastic region, over a frequency range between 0.63 and 63 rad/s.

2.3. Emulsion preparation

An oil phase was prepared by dispersing β-carotene (0.3%, w/w) in corn oil using a sonicating water bath (Model 250, Ultrasonic cleaner, E/MC RAI research, Long Island, New York) for 5 min and then heating (≈60 °C for 30 min) to ensure complete dissolution. The influence of emulsifier type was examined by preparing emulsions using either a protein (WPI) or a non-ionic surfactant (Tween 20). Different oil-to-emulsifier ratios had to be used to prepare emulsions containing lipid droplets with similar mean diameters using the two types of emulsifier. For WPI-stabilized emulsions, the ratio of oil-to-emulsifier was 6:1 while for Tween-20-stabilized emulsions it was 10:1. Stock WPI-stabilized emulsions were therefore prepared by homogenizing 6 wt % corn oil and 94 wt % emulsifier solution (1.06 wt% WPI, pH 7.0), while stock Tween 20-stabilized emulsions were prepared by homogenizing 10 wt% corn oil and 90 wt% emulsifier solution (1.1 wt% Tween 20, pH 7.0). Course emulsions were formed by blending oil and aqueous phases together using a high shear mixer for 2 min (M133/1281-0, Biospec Products, Inc., ESGC, Switzerland) and then fine emulsions were formed by passing the coarse emulsions through a microfluidizer four times at 10 kpsi (Model 110L, Microfluidics, Newton, MA).

2.4. Preparation of filled hydrogels

The influence of starch type was examined by forming filled hydrogels from either rice starch or mung bean starch. A stock oil-in-water emulsion was diluted with 10 mM phosphate buffer solution (pH 7.0) to form emulsions containing 4 wt% lipid, and then the desired amount of starch (10% w/w) was dispersed in the diluted emulsion. The resulting mixtures of emulsion, starch, and buffer solution were then heated at 90 °C for 10 min to gelatinize the starch. The heated mixtures were loaded into a flat, cylindrically shaped vessel where the gel was allowed to set, and then the samples were stored at 4 °C overnight. Filled hydrogel samples were cut into 0.5 cm height × 0.5 cm diameter cylinders prior to use.

2.5. Particle size characterization

The mean particle diameter and particle size distribution of the samples were measured at various stages in the simulated gastrointestinal process. The particle size distribution was measured using static light scattering (Mastersizer 2000, Malvern Instruments Ltd., Worcestershire, UK). The samples were diluted in 10 mM phosphate buffer to avoid multiple scattering effects. The particle size was reported as either the surface-weighted mean diameter (d_{43}) or the volume-weighted mean diameter (d_{32}) (McClements, 2005).

2.6. Simulated gastrointestinal tract model

A simulated gastrointestinal tract (GIT) consisting of oral, gastric, and intestinal phases was used to simulate the biological fate of ingested samples.

2.6.1. Oral phase

Simulated saliva fluid (SSF), containing mucin and various salts, was prepared according to a previous study (Sarkar, Goh, & Singh, 2009). The samples (emulsion or filled hydrogels) were mixed with SSF at a 50:50 ratio and the mixture was then adjusted to pH 6.8. The mixture was incubated at 37 °C for 10 min with continuous agitation at 100 rpm. This incubation time was used to standardize the procedure, but it is considerably longer than the time a food may actually spend in the mouth during mastication.

2.6.2. Gastric phase

Simulated gastric fluid (SGF) was prepared using a method reported previously (Sarkar, Goh, Singh, & Singh, 2009) by dissolving 2 g of NaCl, and 7 mL of HCl (37%) in 1 L of water and then adding 3.2 g of pepsin. Afterwards, the pH was adjusted to 1.2 using 1.0 HCl. The sample from the oral phase was mixed with SGF at a 50:50 ratio and the pH of the sample was adjusted to 2.5 using NaOH (1 M). The sample was then incubated at 37 °C for 2 h with continuous agitation at 100 rpm.

2.6.3. Small intestinal phase

A pH-stat automatic titration unit (Metrohm USA Inc., Riverview, FL) was used to simulate the conditions in the small intestinal phase of the GIT (Salvia-Trujillo, Qian, Martín-Belloso, & McClements, 2013b). An aliquot (30 mL) of sample from the gastric phase was placed in a temperature-controlled (37 °C) chamber and the pH was set at 7.0. Then 3.5 mL of bile extract solution (187.5 mg/3.5 mL) and 1.5 mL of salt solution (10 mM of calcium chloride and 150 mM of sodium chloride) were added to the sample and the mixture was adjusted to pH 7.0. Afterwards, 2.5 mL of freshly prepared pancreatin suspension (187.5 mg/2.5 mL) dissolved in phosphate buffer was added to the mixture. The pH of the mixture was monitored and the volume of 0.25 M NaOH (mL) necessary to neutralize the free fatty acids (FFA) released from the lipid digestion was recorded over two hours. The percentage of FFA released was calculated using the following equation:

$$\%\ \mathrm{FFA} = 100 \times \frac{V_{\mathrm{NaOH}} \times m_{\mathrm{NaOH}} \times M_{\mathrm{lipid}}}{w_{\mathrm{lipid}} \times 2}$$

where V_{NaOH} is the volume of titrant in liters, m_{NaOH} is the molarity of sodium hydroxide, M_{lipid} is the molecular weight of corn oil (872 g/mol), and w_{lipid} is the weight of oil in the digestion system in grams. Blanks (samples without oil) were run, and the volume of titrant used for these samples was subtracted from the corresponding samples that contained oil.

2.7. Microstructure

To determine structural changes that occurred within different phases of the GIT model, optical microscopy and/or confocal scanning laser microscopy with a 60 × objective lens and 10 × eyepiece were used (Nikon D-Eclipse C1 80i, Nikon, Melville, NY). A small amount of aliquot of each sample was placed on a microscope slide and covered with a cover slip prior to analysis. For confocal microscopy measurements, initial samples and samples taken from the GIT model were dyed with Nile red, a fat-soluble fluorescent dye that was previously dissolved at 0.1% (w/v) in ethanol. An air-cooled argon ion laser (Model IMA1010BOS, Melles Griot, Carlsbad, CA) was used to excite Nile red at 488 nm. All images were taken and processed using the instrument software program (EZ-CS1 version 3.8, Nikon, Melville, NY).

2.8. β-carotene bioaccessibility

The bioaccessibility of β-carotene was determined after the samples had been subjected to the full simulated GIT model using the method described previously (Qian et al., 2012; Salvia-Trujillo, Qian, Martin-Belloso & McClements, 2013a). After the small intestinal stage, digesta were collected and centrifuged, which resulted in samples that contained a sediment at the bottom with a supernatant above. The supernatant was collected and assumed to be the "micelle" fraction, in which the bioactive component was solubilized. The bioaccessibility was calculated from the concentrations of β-carotene determined in the micelle fraction and supernatant using a procedure described previously (Qian et al., 2012; Salvia-Trujillo et al., 2013a).

2.9. Statistical analysis

All experiments were carried out in at least duplicate using freshly prepared samples. Means and standard deviations were calculated from these data.

3. Results and discussion

3.1. Preparation of β-carotene-loaded emulsion, and filled hydrogel

All the emulsions and filled hydrogels had a strong and bright yellowish-orange color after preparation, which can be attributed to selective absorption of light by the encapsulated β-carotene. We did not observe phase separation of the β-carotene from any of the emulsions or filled hydrogels, which suggested that β-carotene remained dissolved within the lipid droplets (rather than crystallizing), and also that the lipid droplets remained evenly dispersed throughout the emulsions and filled hydrogels.

3.2. Rheological properties of starches

The rheological properties of the rice and mung bean starches used in this study were characterized to provide insights into the differences in their hydrogel properties (Fig. 1). Initially, changes in gel strength were measured over time after starch hydrogels were placed in the rheometer measurement cell. For both starches, the elastic modulus (G′) was higher than the viscous modulus (G″) throughout the entire incubation period, indicating that they exhibited predominantly elastic-like characteristics (Fig. 1a). For rice starch, the moduli did not change over time indicating that full gelation had already occurred before placing the hydrogel in the rheometer. On the other hand, for mung bean starch, there was an initial increase in modulus until a relatively constant value was obtained after 200 min, suggesting that gel formation occurred

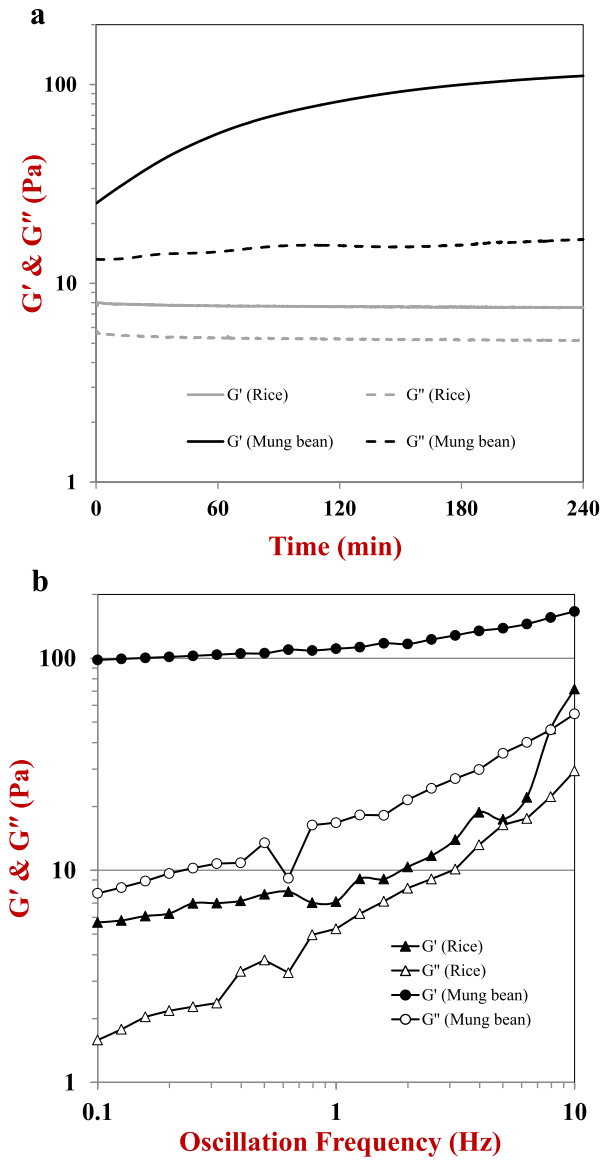

Fig. 1. a). Changes in the rheological properties (G′ and G″) of 5% rice starch and 5% mung bean starch pastes over time when stored at 4 °C measured by oscillatory viscoelastic analysis. b). Changes in the rheological properties (G′ and G″) of 5% rice starch and 5% mung bean starch pastes during a frequency sweep at 4 °C measured by oscillatory viscoelastic analysis.

more slowly. The mung bean starch hydrogel had a much higher G′ and a greater difference between G′ and G″ than the rice starch hydrogel indicating that it formed stronger and more elastic gels. This result is attributed to the higher amylose and protein content of mung bean starch compared to rice starch. Proteins may have participated in the formation of a biopolymer network within the hydrogels, and therefore increased gel strength. Starch gelation is the result of water absorption and swelling of starch granules, followed by leaching of linear amylose chains, and close packing of swollen starch granules. The final gel structure typically consists of swollen starch granules surrounded by a continuous amylose phase, which is responsible for the overall rheological properties of starch pastes and gels (Hermansson & Svegmark, 1996; Hongsprabhas & Israkarn, 2008; Miles, Morris, Orford, & Ring, 1985). In addition, there are differences in the size of the starch granules from different natural sources of starch. Consequently, differences in both the composition and the structure of the two starches may account for the observed differences in their rheological properties.

The formation of a solid-like gel structure was also confirmed by a frequency sweep test conducted immediately after the time sweep test (Fig. 1b). These experiments clearly indicated a higher rigidity of mung bean starch gels compared with rice starch gels. We hypothesized that these differences in the textural characteristics of the starch hydrogels might influence their subsequent gastrointestinal fate, and therefore we examined their behavior within a simulated GIT.

3.3. Impact of delivery system structure and composition on behavior in simulated GIT

Emulsions and filled hydrogels containing β-carotene-fortified lipid droplets were passed through each phase of the GIT model and changes in their particle size (Fig. 2) and microstructure (Fig. 3 and Fig. 4) were measured.

3.3.1. Emulsions

The initial mean particle diameters ($d_{4,3}$) of β-carotene-fortified emulsions (4 wt% fat) were 0.26 μm and 0.24 μm for WPI-stabilized and Tween 20-stabilized systems, respectively (Fig. 2b). The initial particle size distributions (PSD) were roughly monomodal for both types of emulsifiers (Fig. 2a) and the lipid droplets were evenly distributed throughout the emulsions (Fig. 3). These results indicated that stable emulsions containing similar sized droplets could be produced using the two different emulsifiers. This was to be expected since both Tween 20 and WPI are food-grade emulsifiers that can stabilize oil droplets from aggregation through a combination of steric and electrostatic repulsion (McClements, 2005).

Upon exposure to simulated oral conditions, the PSD of the two kinds of β-carotene-loaded emulsions became bimodal, with a population of particles similar in size to those in the original sample, and another population of much larger particles (Fig. 2a). As a result there was an appreciable increase in the measured mean particle diameter after exposure to the oral conditions (Fig. 2b). The optical microscopy images confirmed that extensive droplet aggregation occurred under simulated oral conditions (Fig. 3). Presumably, these aggregates were the result of depletion and/or bridging flocculation of the lipid droplets by mucin in the simulated saliva fluid (Salvia-Trujillo et al., 2013b; Sarkar, Goh, & Singh, 2009). Mucin is a complex mixture of biopolymers that contain cationic, anionic, and hydrophobic groups. Consequently, bridging flocculation may have occurred between charged groups on the lipid droplet surfaces and oppositely charged groups on the mucin molecules in the artificial saliva. In addition, depletion flocculation may have occurred due to the presence of non-adsorbed biopolymer molecules in the aqueous phase. The increase in mean particle diameter ($d_{4,3}$) of the Tween 20-stabilized emulsion was smaller than that of the WPI-stabilized emulsion after exposure to the oral phase, which suggested that the lipid droplets coated by non-ionic surfactant were less prone to flocculation than those coated by protein. The most likely reason for this phenomenon is that the Tween 20-coated droplets have less regions on their surfaces that the mucin molecules can attach to and cause bridging flocculation. The ability of droplets coated by non-ionic surfactants to resist flocculation under simulated oral conditions has also been reported in a number of previous studies (Salvia-Trujillo et al., 2013b; Silletti, Vingerhoeds, Norde, & van Aken, 2007; Vingerhoeds, Blijdenstein, Zoet, & van Aken, 2005).

After exposure to stomach and small intestine conditions, emulsions prepared from different emulsifiers showed distinctly different behavior. When the WPI-stabilized emulsion moved from

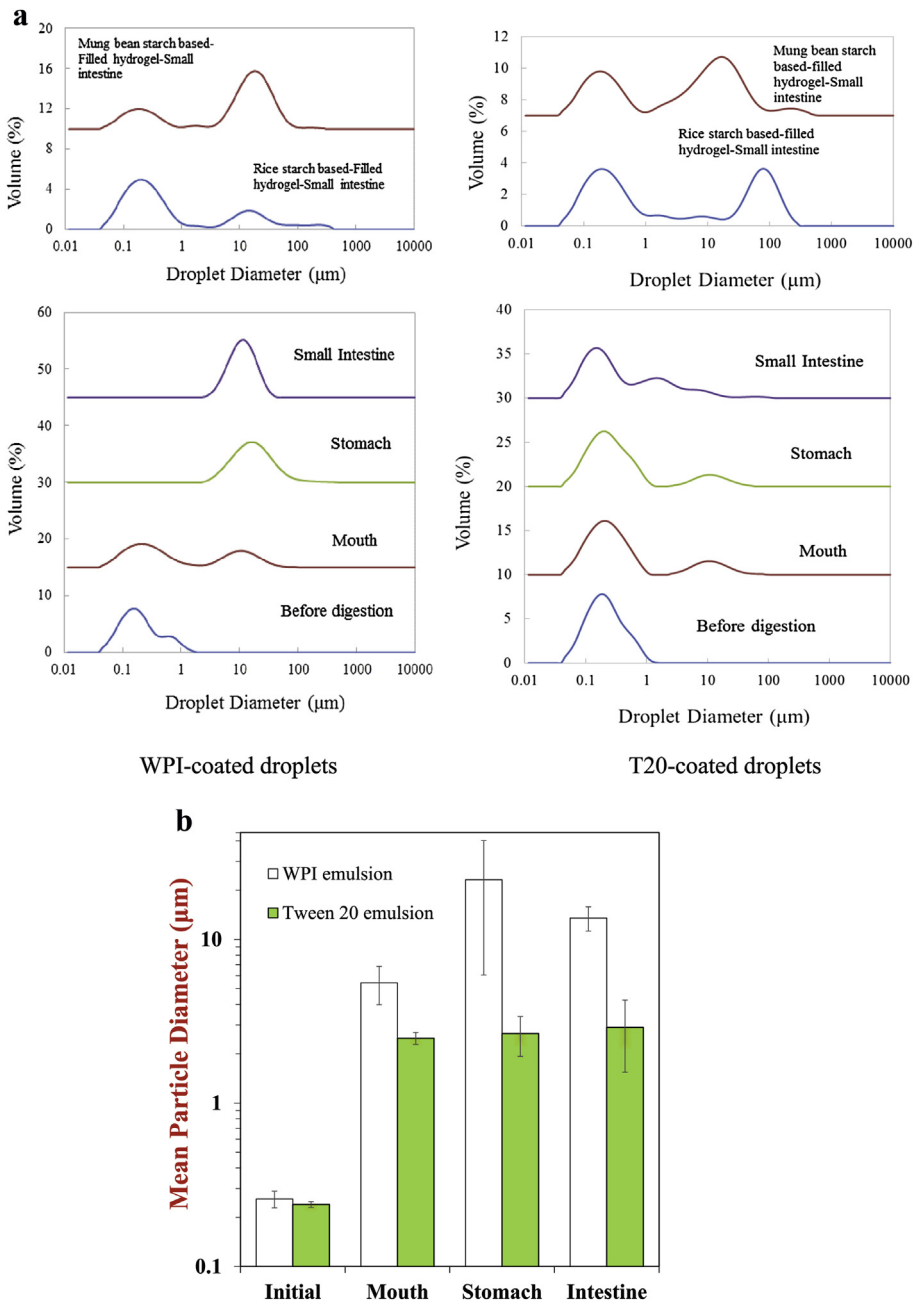

Fig. 2. a). Influence of simulated gastrointestinal conditions on the particle size distributions of samples initially containing β-carotene-fortified lipid droplets (stabilized by WPI or Tween 20) present in emulsions or starch-based filled hydrogels. b). Influence of simulated gastrointestinal conditions on the mean particle diameters ($d_{4,3}$) of corn oil-in-water emulsion stabilized by WPI and Tween 20.

the oral to the gastric stage, visible phase separation occurred, large clumps were observed under the microscope (Fig. 3), and the particle size increased (Fig. 2a and b). Lipid droplets with dimensions similar to those in the initial emulsions disappeared from the particle size distributions after exposure to gastric conditions (Fig. 2a). The increased aggregation of the lipid droplets in the WPI-stabilized emulsions in the gastric phase may have occurred for a number of reasons (Singh et al., 2009). When WPI-coated lipid droplets move from the oral to the gastric phase they are exposed to changes in pH and ionic strength that weaken the electrostatic repulsion between droplets thereby promoting flocculation. Gastric fluids also contain digestive enzymes (pepsin) that hydrolyze adsorbed proteins, which can also promote droplet aggregation. Conversely, when Tween 20-stabilized emulsions moved from the oral to the gastric stage, large clumps were not detected by optical microscopy (Fig. 3) and the particle size distribution was fairly similar to that observed in the initial emulsions (Fig. 2a). These results suggest that the aggregates formed by the Tween 20-coated lipid droplets in the oral phase dissociated when they were exposed to gastric conditions. This phenomenon is consistent with a reduction in the depletion attraction between emulsion droplets after dilution (McClements, 2005). Examination of the full PSDs of the Tween 20-stabilized emulsions after exposure to the gastric phase indicated that a large fraction of the lipid droplets had similar sizes to the initial emulsions. Our results suggest that Tween 20 was more efficient at protecting the lipid droplets from aggregation

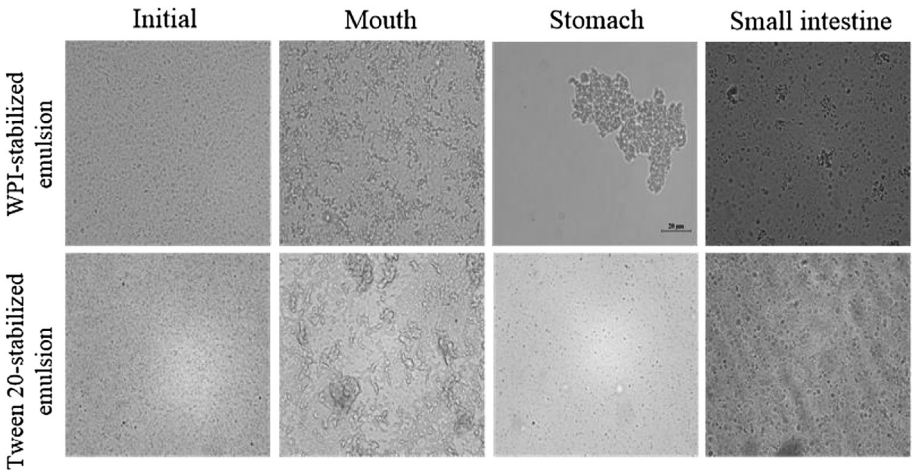

Fig. 3. Influence of simulated gastrointestinal conditions on microstructures of O/W emulsions (initially containing fat 4 wt%) stabilized by WPI or Tween 20 measuring using optical microscopy.

under gastric conditions than WPI. Previous researchers have also reported that lipid droplets coated by certain types of non-ionic surfactant are stable to gastric conditions (Golding et al., 2011; Van Aken, Bomhof, Zoet, Verbeek, & Oosterveld, 2011).

3.3.2. *Filled hydrogels*

In the case of the filled hydrogels, the semi-solid structure of the hydrogels was maintained even after exposure to the oral and gastric phases, and so it was not possible to measure the PSDs of the initial samples or the samples obtained after exposure to mouth and stomach conditions. Nevertheless, particle size information could be obtained on the samples after they were exposed to the small intestine phase because the hydrogel disintegrated under these conditions. A bimodal particle size distribution was obtained with a population of relatively small particles around 0.3 μm and another population of relatively large particles around 20 μm (Fig. 2a). Numerous kinds of particles may have been present within the intestinal fluids after digestion, including undigested fat droplets, micelles, and vesicles, and it is difficult to establish their nature using light scattering techniques (McClements et al., 2009; Singh et al., 2009). We therefore used conventional and confocal fluorescence microscopy methods to analyze the change in the

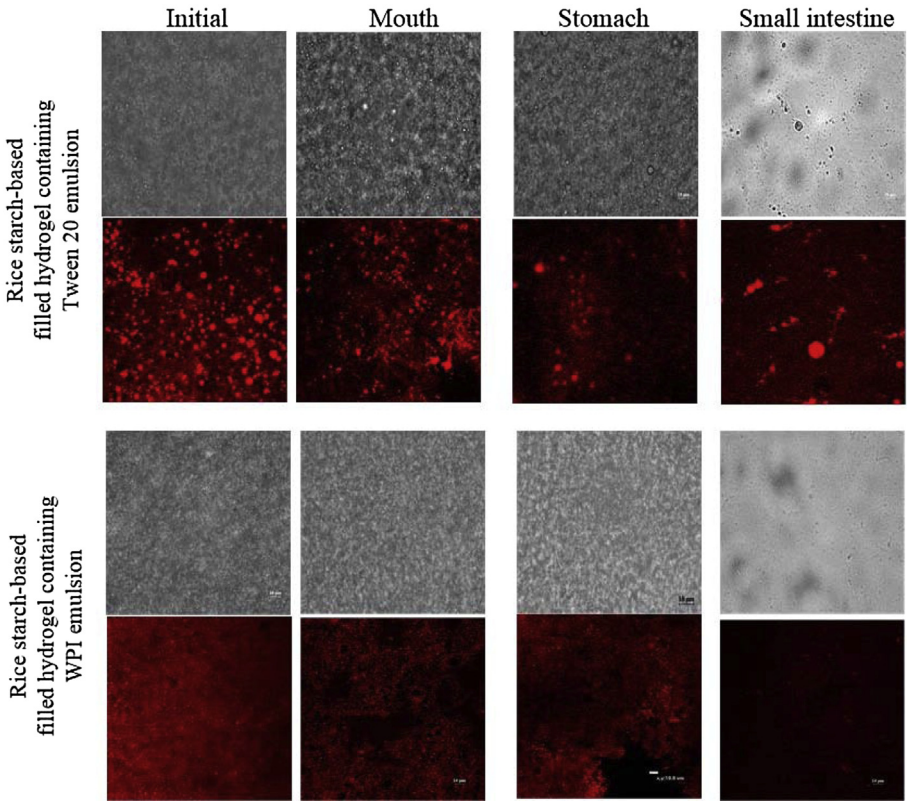

Fig. 4. Influence of simulated gastrointestinal conditions on microstructures of rice starch based filled hydrogels (initially containing 4 wt% lipid) measured using optical and confocal microscopy.

microstructure of the filled hydrogels as they moved through the simulated GIT.

The filled hydrogels behaved differently than the emulsions within the simulated GIT, and their behavior depended on the type of surfactant used to prepare them. In the case of WPI-stabilized emulsions, small lipid droplets were evenly distributed throughout the gel matrix in the initial hydrogels (Fig. 4). In the case of Tween 20-stabilized emulsions, the lipid droplets were also evenly distributed throughout the hydrogels, but the dimensions of the lipid droplets was noticeably bigger, which suggested that some droplet coalescence had occurred (Fig. 4). There are a number of possible reasons for this effect. First, dissolved starch molecules may have promoted depletion flocculation of the lipid droplets, which led to coalescence because the droplets were forced together (McClements, 2005). Second, the filled hydrogel preparation method may have promoted droplet coalescence, *e.g.,* due to heating or stirring. Third, a complex may have formed between Tween 20 and starch molecules (Gunning et al., 2003), where the aliphatic chains of the fatty acids sat inside the amylose helices, whilst the polar head groups of the fatty acids were excluded (Godet, Tran, Colonna, Buleon, & Pezolet, 1995). The non-polar hydrocarbon chain of the Tween 20 molecule might be incorporated into the amylose helix and the polar head-group might be protruding, thus affecting the stability of lipid droplets coated with Tween 20. Further studies are therefore needed to identify the precise physicochemical origin of the observed effects.

Exposure of the rice starch-based filled hydrogels containing WPI-stabilized emulsion to both simulated oral and gastric conditions did not have a major impact on their overall microstructure, with the fat droplets remaining relatively small and evenly distributed throughout the hydrogel. This result suggests that the starch hydrogel matrices surrounding the fat droplets protected them from extensive aggregation within the mouth and stomach. Indeed, visual observation of the samples after the oral and gastric phases indicated that the filled hydrogel structure was partially maintained (data not shown). Exposure of the starch-based filled hydrogels containing Tween 20-stabilized emulsion to both simulated oral and gastric conditions also did not have a major impact on their overall microstructure with the lipid droplets remaining trapped within the hydrogel matrices. However, the filled hydrogel disintegrated after exposure to small intestinal conditions, and the sample appeared fluid (rather than paste-like), regardless of sample type. The breakdown of the starch hydrogel structure within the small intestine may have been due to dilution, shearing, or amylase activity within the simulated small intestinal fluids. Dilution decreases the biopolymer and particle concentration in the system, which may lead to breakdown of any biopolymer or particle networks. The application of mechanical forces, such as shearing, may disrupt any weak bonds holding biopolymer or particle networks together. The presence of amylase in the intestinal fluids may lead to hydrolysis of starch molecules, which would also cause the breakdown of any starch hydrogel networks that surrounded the lipid droplets.

Changes in the microstructure of mung bean starch-based filled hydrogels containing lipid droplets coated by either WPI or Tween 20 were also measured. However, the observed microstructural changes for this system were fairly similar to those obtained for the rice starch-based filled hydrogels and therefore this data is not shown for the sake of brevity.

3.4. Influence of delivery system structure and composition on lipid digestibility

Fig. 5 shows the percentage of FFAs released from β-carotene-fortified emulsions and filled hydrogels prepared with different surfactants and starches during two hours digestion. The amount of FFA released from β-carotene-loaded starch gels containing no fat was also examined as a control, and this value was subtracted from those of the filled hydrogels. Fig. 6 shows the initial rate of lipid digestion (slope of FFA *versus* time in the first 10 min) and the final extent of lipid digestion (the amount of FFA released after 120 min). In general, there was a steep increase in the amount of FFA released during the first few minutes of digestion, followed by a more gradual increase at later times (Fig. 5a and b). However, the shape of the FFA *versus* digestion time profiles depended on the nature of starch and emulsifier used.

3.4.1. Emulsions

Initially, we compared the initial digestion rates and final amounts of FFA released for the emulsions stabilized by different

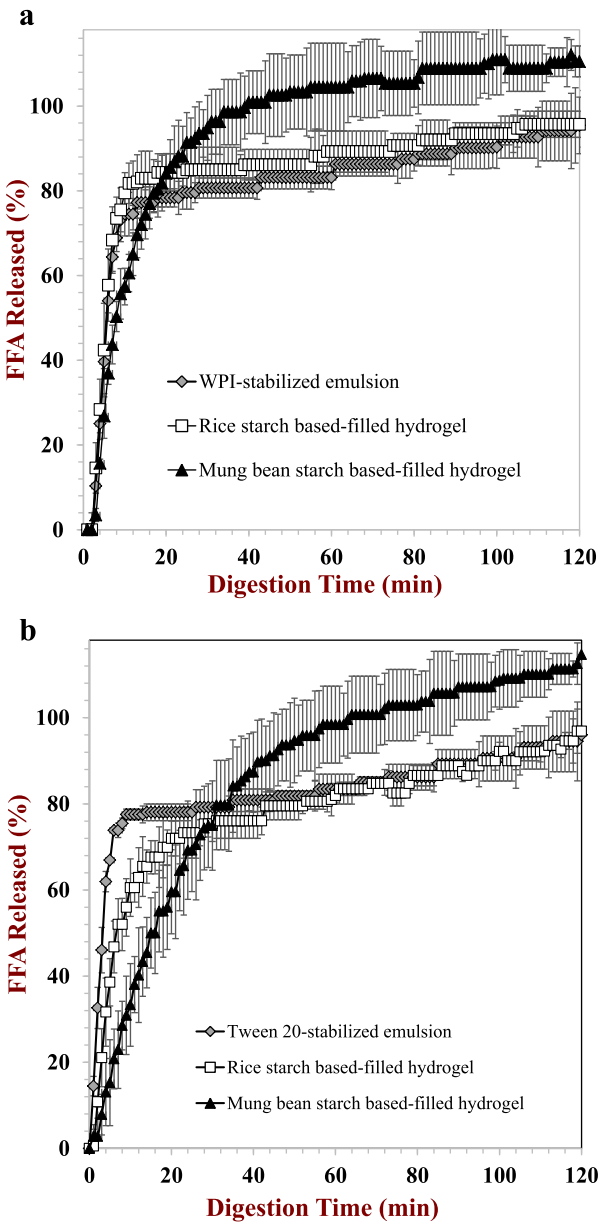

Fig. 5. a). Calculated FFA release of β-carotene-fortified lipid droplets (coated with WPI) dispersed in emulsions and filled hydrogels measured by automatic titration (pH Stat). b). Calculated FFA release of β-carotene-fortified lipid droplets (coated with Tween 20) dispersed in emulsions and filled hydrogels measured by automatic titration (pH Stat).

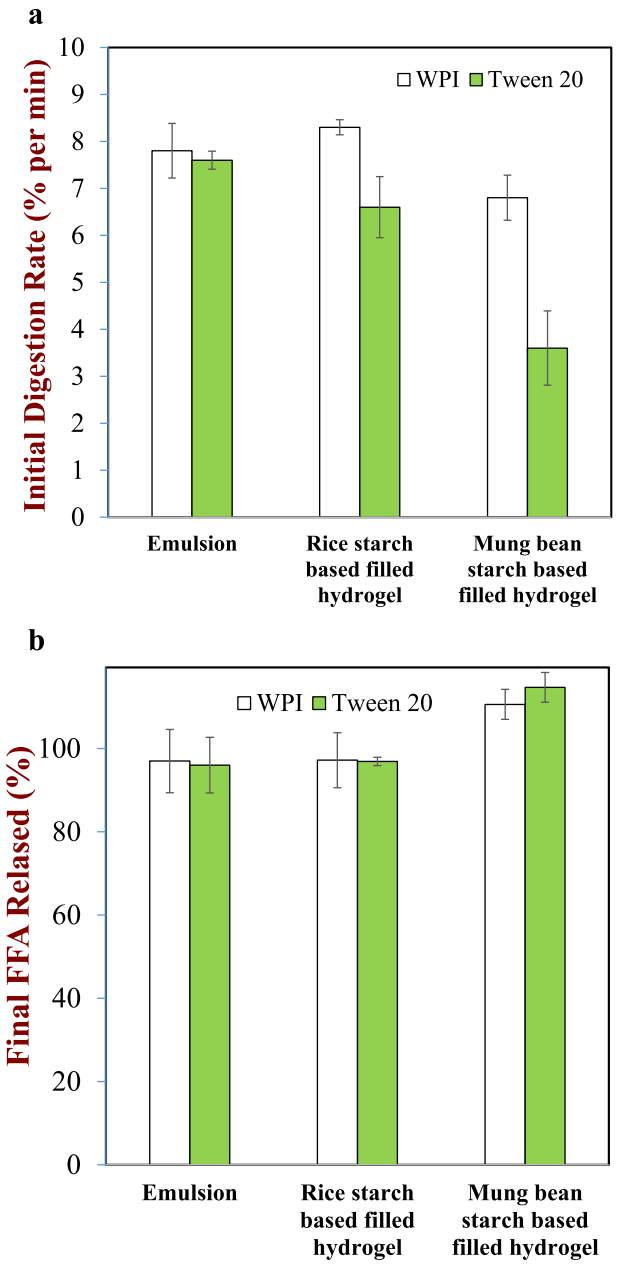

Fig. 6. a). Influence of delivery system structure and composition on initial digestion rate determined from pH-Stat titration curves (first 10 min). b). Influence of delivery system structure and composition on final amount of FFAs produced after 2 h digestion.

kinds of emulsifier (Fig. 6). These results suggest that initial emulsifier type had little influence on the rate or extent of lipid digestion in the emulsions. This is somewhat surprising since these two emulsions had quite different behaviors in the mouth and stomach phases, as seen in the particle size and microstructure measurements (Figs. 2 and 3). Presumably, the bile salts and phospholipids in the small intestinal fluids were still able to adsorb to the lipid droplet surfaces and displace the original emulsifiers, followed by lipase adsorption and digestion of the triacylglycerols.

3.4.2. Filled hydrogels

For the systems containing WPI-coated lipid droplets, the rate and extent of lipid digestion was fairly similar for the emulsion and the rice starch filled hydrogels (Fig 5a). However, the initial rate of lipid digestion was slower and the final amount of FFAs released was higher for the mung bean filled hydrogels. For the systems containing Tween 20-coated lipid droplets, the initial rate of lipid digestion was fastest in the emulsions, then the rice starch-based filled hydrogels, and then the mung bean-based filled hydrogels (Figs. 5b and 6a). The final amount of FFA released was similar for the emulsions and rice starch-based filled hydrogels, but considerably higher for the mung bean starch-based filled hydrogels. These results suggest that the mung bean starch was able to suppress the initial rate of lipid digestion in both types of emulsions.

As mentioned earlier, mung bean starch has a higher amylose content and forms stronger gels than rice starch (Fig. 1). After starch granules are gelatinized, starch undergoes a relatively slow re-association process ("retrogradation") upon cooling. During retrogradation, starch molecules re-associate and form tightly packed structures stabilized by hydrogen bonding, which are resistant to digestive amylases (Haralampu, 2000). It is therefore possible that the more rigid retrograded regions formed by mung bean starch after heating-cooling slowed down the rate of starch digestion by amylases, and therefore inhibited the release of lipid droplets into the intestinal fluids. The digestibility of lipid droplets trapped within filled hydrogels may therefore be controlled to a certain extent by selecting different types of starch. The reason that a higher final amount of FFAs were released after digestion for the systems containing mung bean starch may have been because it contained a relatively high level of proteins. Proteins may be digested by proteases in the simulated intestinal fluids, which would release peptides, amino acids, and protons (H^+) thereby reducing the solution pH. The NaOH required to neutralize the protons released due to protein digestion would be included in the calculated amount of FFAs released, thereby leading to an overestimate of the actual value of fatty acids released.

The initial rate of lipid digestion was higher for the filled hydrogels containing WPI-coated lipid droplets than for those containing Tween 20-coated lipid droplets for both types of starch (Fig. 6a). A potential reason for the fact that the Tween 20-coated droplets were digested more slowly was because they had a larger initial size (smaller surface area) than the WPI-coated droplets in the filled hydrogels (Fig. 4). In addition, the Tween 20 molecules may have interacted with the starch molecules (amylose), which may have inhibited the ability of the lipase to reach the lipid substrate.

3.5. Influence of delivery system structure and composition on β-carotene bioaccessibility

The bioaccessibility of β-carotene clearly depended on the structure and composition of the delivery system (Fig. 7). The β-carotene had a very low bioaccessibility (less than 1%) when it was directly incorporated into a rice and mung bean starch hydrogel in the form of crystals (data not shown). It is well known that carotenoids need to be incorporated into mixed micelles within the small intestine prior to absorption (Fernández-García et al., 2012; Rodríguez-Roque, Rojas-Graü, Elez-Martínez, & Martín-Belloso, 2014). In the absence of lipid digestion products, the bile salts and phospholipids in the intestinal fluids only form simple mixed micelles (Madenci & Egelhaaf, 2010). These simple mixed micelles can solubilize certain types of lipophilic bioactive components but they tend to be much less efficient than complex mixed micelles containing free fatty acids and monoacyglycerols (Yao, Xiao, & McClements, 2014). On the other hand, when the β-carotene was incorporated into WPI- and Tween 20-stabilized emulsions initially containing 4 wt% fat, the bioaccessibility of β-carotene increased to ≈14% and 54%, respectively. These bioaccessibility values are fairly similar to those reported previously for emulsions and

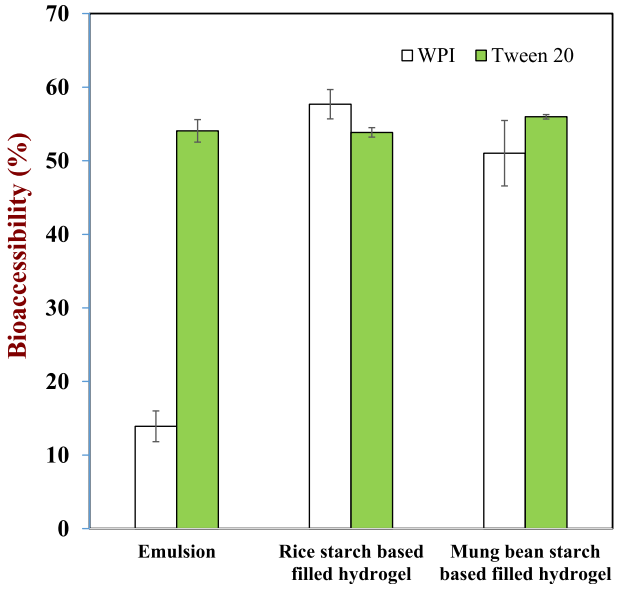

Fig. 7. Bioaccessibility (%) of β-carotene after in vitro digestion in filled hydrogels and corn oil-in-water emulsion stabilized by WPI and Tween-20.

nanoemulsions (Salvia-Trujillo et al., 2013b; Qian et al., 2012). This result suggests that the fat from the emulsions formed mixed micelles after digestion that were capable of solubilizing lipophilic components under simulated small intestine conditions. The difference in bioaccessibility between WPI-stabilized and Tween 20-stabilized emulsions might be caused by differences in the interactions of the emulsifiers with the mixed micelles formed after digestion (since the rate and extent of lipid digestion were fairly similar). For example, non-ionic surfactants may be incorporated into mixed micelles and increase their solubilization capacity, whereas partially digested protein molecules may promote aggregation and precipitation of mixed micelles. Indeed, it is known that β-carotene can interact with β-lactoglobulin (Reboul, 2013), which is the major protein fraction of WPI.

For the Tween 20-coated lipid droplets, the bioaccessibility of β-carotene did not change appreciably when the droplets were incorporated into either type of starch hydrogel (Fig. 7). On the other hand, for the WPI-coated lipid droplets, the bioaccessibility of β-carotene was appreciably higher in the hydrogels than in the emulsions (Fig. 7). This might have occurred because the hydrogels protected the fat droplets from extensive aggregation within the oral and gastric phases, but released them within the small intestine thereby enabling them to be readily digested. In addition, the starch hydrogels may have prevented the WPI from interacting with the mixed micelles and promoting their aggregation and precipitation. This effect was less important for the Tween 20-coated droplets because they were not highly flocculated after the gastric phase, and the Tween 20 would not be expected to aggregate or precipitate the mixed micelles.

4. Conclusions

β-carotene-loaded filled hydrogels were successfully prepared by combining oil-in-water emulsions stabilized by different emulsifiers with hydrogels prepared using different types of starch. The initial rate of lipid digestion measured using a simulated gastrointestinal model depended on emulsifier and starch type, whereas the final extent of lipid digestion was less dependent on these factors. Incorporation of the lipid droplets into mung bean starch hydrogels led to an appreciable decrease in the lipid digestion rate, which was attributed to the ability of this kind of starch to resist degradation due to its high amylose and protein contents. In simple oil-in-water emulsions, the bioaccessibility of β-carotene was lower in WPI-coated lipid droplets than in Tween 20-coated ones, which was attributed to the ability of the whey proteins to aggregate and precipitate mixed micelles. However, once the lipid droplets were incorporated in starch gels, the bioaccessibility of β-carotene became relatively high, regardless of emulsifier or starch type. The starch hydrogels prevented the lipid droplets from aggregating in the mouth and stomach, which may have increased the accessibility of the lipase to the lipid phase. In addition, the presence of starch may have prevented whey protein from aggregating and precipitating mixed micelles containing β-carotene. The information obtained in this study may be useful for the development of nutraceutical-fortified starch-based gels suitable for use as functional food products.

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