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Control of β-carotene bioaccessibility using starch-based filled hydrogels

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ABSTRACT

β-Carotene was incorporated into three types of delivery system: (i) "emulsions": protein-coated fat droplets dispersed in water; (ii) "hydrogels": rice starch gels; and (iii) "filled hydrogels": protein-coated fat droplets dispersed in rice starch gels. Fat droplets in filled hydrogels were stable in simulated mouth and stomach conditions, but aggregated under small intestinal conditions. Fat droplets in emulsions aggregated under oral, gastric, and intestinal conditions. β-Carotene bioaccessibility was higher when encapsulated in filled hydrogels than in emulsions or hydrogels, which was attributed to increased aggregation stability of the fat droplets leading to a larger exposed lipid surface area. β-Carotene bioaccessibility in starch hydrogels containing no fat was very low (≈1%) due to its crystalline nature and lack of mixed micelles to solubilise it. The information presented may be useful for the design of rice-starch based gel products fortified with lipophilic nutraceuticals.

1. Introduction

Rice is an important staple food in Asia and has been used to prepare many different types of food such as cooked rice, rice noodles, rice cake, and rice papers (Cao, Wen, Li, & Gu, 2009; USDA report, 2010). 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). Increasing consumer concern about health and wellness, has encouraged the food industry to fortify various types of food products with bioactive ingredients, such as vitamins, minerals, and nutraceuticals (Chaudhry & Castle, 2011; Cushen, Kerry, Morris, Cruz-Romero, & Cummins, 2012). The incorporation of these bioactive materials into rice-based gel products that are routinely consumed could lead to improvements in human health in Asia and other countries.

Nevertheless, there are challenges to incorporating many types of bioactive agents into food products (McClements, 2013). For example, many bioactive components are highly hydrophobic molecules that have a low solubility in aqueous environments, are incompatible with hydrophilic gels, and have relatively poor or variable oral bioavailability (Jingling Tang & Zhong-Gui, 2007; Liang, Shoemaker, Yang, Zhong, & Huang, 2013; McClements, 2013). Consequently they are difficult to incorporate into aqueous-based food products and their potential health benefits are not fully realised because they are poorly absorbed by the human body. To overcome these limitations, a useful method is to entrap the components into appropriate delivery systems, such as emulsions, nanoemulsions, micelles, microemulsions or solid lipid nanoparticles (Matalanis, Jones, & McClements, 2011; Matalanis & McClement, 2012; McClements, Decker, & Park, 2009; Qian, Decker, Xiao, & McClements, 2013; Singh, Ye, & Horne, 2009; Troncoso, Aguilera, & McClements, 2012a). Previous studies have shown that the bioaccessibility of highly lipophilic bioactive components (such as carotenoids) can be greatly increased by co-ingesting them with 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 that are incorporated along with bile acids and phospholipids into mixed micelles that can solubilise and transport hydrophobic materials to epithelial cells (Hernell, Staggers, & Carey, 1990; Porter, Trevaskis, & Charman, 2007).

Carotenoids are a class of natural pigments found in many fruits and vegetables, as well as some animal products (Johnson, 2002). In particular, β-carotene is one of the most important dietary carotenoids because of its relative abundance and high pro-vitamin A activity. Consumption of sufficiently high levels of carotenoids

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has been associated with a decreased risk of various chronic diseases in humans, and therefore they are a strong candidate for fortification of functional foods (Gutierrez et al., 2013; Handelman, 2001; Johnson, 2002).

The aim of the current study was to examine the influence of incorporating β-carotene into rice starch hydrogels and filled hydrogels on its bioaccessibility using a simulated gastrointestinal tract (GIT). In particular, we examined the influence of fat content on the bioaccessibility of encapsulated β-carotene, since previous studies have shown that the amount of lipid present is important (Porter et al., 2007; Salvia-Trujillo, Qian, Martín-Belloso, & McClements, 2013a). The fat was incorporated into the rice starch hydrogels in the form of oil-in-water emulsions. The information obtained in this study should be useful for the development of rice-based functional food products fortified with lipophilic bioactive components that may improve human health and wellness.

2. Materials and methods

2.1. Materials

The starch used for this study was isolated from native rice (Ilmi byeo, Korea) in a laboratory using a traditional alkaline method (Lumdubwong & Seib, 2000). β-Carotene, 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. Emulsion preparation

An oil phase was prepared by dispersing β-carotene (0.3%, w/w) in corn oil by sonicating (5 min) and heating (≈60 °C for 30 min) to ensure complete dissolution. An aqueous emulsifier solution was prepared by dispersing WPI (2.5 wt%) in 10 mM phosphate buffer (pH 7.0). A stock emulsion was prepared by homogenising 20 wt% corn oil and 80 wt% emulsifier solution (2.5 wt% WPI, pH 7.0) using a high speed blender for 2 min (M133/1281-0, Biospec Products, Inc., ESGC, Switzerland) and then passing through a microfluidizer four times at 10 kpsi (Model 110L, Microfluidics, Newton, MA).

2.3. Preparation of filled hydrogels

A stock oil-in-water emulsion (20% (w/w) oil) was diluted with 10 mM phosphate buffer solution (pH 7.0) to form O/W emulsions with varying oil concentration (2, 4, 6, and 8 wt%) and then the desired amount (10% w/w) of rice starch was dispersed into the diluted emulsion. The resulting mixtures of emulsion, rice 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 for overnight.

2.4. Emulsion characterisation

The mean particle diameter, particle size distribution, and electrical charge of the samples were measured before and during 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). The electrical charge of samples before and during the simulated gastrointestinal conditions was determined by dynamic light scattering (Zetasizer NanoZS, Malvern Instruments Ltd., Worcestershire, UK).

2.5. Simulated gastrointestinal tract model

A relatively simple simulated gastrointestinal tract (GIT) model consisting of oral, gastric, and intestinal phases was used to simulate the biological fate of ingested samples. In the case of filled hydrogel, samples were cut into 0.5 × 0.5 cm size pieces before samples were exposed to a simulated mouth system.

2.5.1. Oral phase

Simulated saliva fluid (SSF), containing mucin and various salts, was prepared according to a previous study (Sarkar, Goh, & Singh, 2009). The samples (hydrogel, emulsion, and filled hydrogel) 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.

2.5.2. 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 M 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.

2.5.3. 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 of 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 into the mixture. The pH of the mixture was monitored and the volume of 0.25 M NaOH (ml) necessary to neutralise the free fatty acids (FFA) released from the lipid digestion was recorded during two hours. The percentage of FFA released was calculated using the following equation, which assumes that two free fatty acids and one monoacylglycerol are released per triacylglycerol molecule:

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

where V_{NaOH} is the volume of titrant in litres, 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.

It should be noted that the simple GIT model used in this study cannot accurately account for the complex events occurring in the human body. In particular, this model does not include gastric lipase (which normally promotes some initial lipid digestion in the stomach) and it uses highly acidic simulated gastric fluids (which are more representative of the fasted rather than fed

stomach). Ingested filled hydrogels would encounter highly acidic conditions in the stomach if they were consumed without food, *e.g.*, if they were taken as nutraceutical-fortified pastels or gummies. Conversely, if they were consumed with foods, then the GIT model would have to be modified, which may lead to different results than those found here.

In addition, we did not include amylase in the oral phase of the GIT model used to obtain the results reported in this study. In a preliminary test, alpha-amylase (5 Units/ml) was incorporated into the oral phase. However, we did not observe any major differences in microstructure, FFA release, or bioaccessibility in the absence or presence of amylase, and so this enzyme was omitted from the tests of the emulsions and filled hydrogels. The impact of amylase is likely to depend on the type and structural organisation of the starch hydrogels used, and therefore we recommend that it should be included in future studies.

2.6. Confocal fluorescence microscopy

Confocal fluorescence microscopy images were taken to determine structural changes that occurred within different phases of the GIT model. Initial samples and the 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. A Nikon Confocal Microscope (Nikon D-Eclipse C1 80i, Nikon, Melville, NY) with a 60× oil immersion objective lens was used to capture the confocal images. All images were taken and processed using the instrument software program (EZ-CS1 version 3.8, Nikon, Melville, NY).

2.7. β-Carotene bioaccessibility

The bioaccessibility of β-carotene was determined after the samples had been subjected to the full simulated gastrointestinal (GI) model using the method described previously (Qian, Decker, Xiao, & McClements, 2012; Salvia-Trujillo et al., 2013a). After the small intestinal stage of the simulated gastrointestinal model, digesta were collected and centrifuged. The supernatant was collected and was assumed to be the "micelle" fraction, in which bioactive component is solubilised. The measurement procedure used in this study was the same with that described previously (Qian et al., 2012; Salvia-Trujillo et al., 2013a).

All experiments were performed in at least triplicate. Unless otherwise specified, data are expressed as the mean ± standard deviation from ⩾3 data samples.

3. Results and discussion

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

The overall objective of this study was to develop nutraceutical-fortified rice-based gels by combining emulsions and rice starch gels. Three different delivery systems containing β-carotene were prepared: "emulsions" containing protein-coated fat droplets dispersed in water; (ii) "hydrogels" consisting of rice starch gels; and (iii) "filled hydrogels" consisting of protein-coated fat droplets dispersed within rice starch gels (Fig. 1). The general appearance and properties of the three delivery systems was quite different: the hydrogel was a cloudy whitish colour with a paste-like texture; the emulsion had a cloudy yellowish-orange colour and was a low viscosity fluid; and, the filled hydrogel was a cloudy yellowish-orange colour with a paste-like texture. The lack of a strong colour in the hydrogel can be attributed to the fact that β-carotene has a very low water solubility and was probably present as small crystals dispersed throughout the starch hydrogel matrix. In contrast, the β-carotene was fully dissolved within the oil phase of the emulsions and filled hydrogels and therefore the overall system had a strong and bright yellowish-orange colour. We did not observe any phase separation of the β-carotene from the emulsions or filled hydrogels, suggesting that it remained fully dissolved.

3.2. Influence of delivery system structure on behaviour in simulated GIT

The purpose of this set of experiments was to gain a better understanding of the influence of initial delivery system composition and structure on their behaviour within a simulated gastrointestinal tract. Emulsions and filled hydrogels containing β-carotene were therefore passed through each step of the gastrointestinal model and changes in their microstructure, particle size, and charge were measured.

3.2.1. Microstructure

The changes in the microstructure of the emulsions and filled hydrogels were examined by confocal microscopy (Fig. 2). Initially, small lipid droplets were evenly dispersed throughout the aqueous phase of the emulsions or throughout the starch hydrogel matrix of the filled hydrogels. When the emulsion was exposed to simulated oral conditions, some larger sized lipid particles were observed, but most of the fat droplets maintained their initial size. According to previous studies, interactions of fat droplets with mucin within the mouth can cause depletion and/or bridging flocculation, resulting in droplet aggregation under simulated oral conditions (Salvia-Trujillo et al., 2013b; Sarkar, Goh, & Singh, 2009; Silletti, Vingerhoeds, Norde, & van Aken, 2007). Mucin is a complex biopolymer molecule that contains both negative and positive groups at neutral pH. Consequently, some bridging flocculation may have occurred between the negatively charged protein-coated fat droplets and the mucin molecules in the artificial saliva. In addition, some depletion flocculation may have occurred due to the presence of a relatively high concentration of non-adsorbed biopolymer molecules in the aqueous phase. When the emulsions moved from the oral to the gastric stage, large clumps were observed (Fig. 2) and the measured particle size increased significantly (Fig. 3). The increased aggregation of the fat droplets in the gastric phase may have happened for a number of reasons. The change in pH and ionic strength that occurs when fat droplets move from the oral to the gastric phase may have weakened the electrostatic repulsion between droplets, or promoted bridging flocculation. In addition, the hydrolysis of proteins present at the fat droplet surfaces by pepsin may have increased their instability to aggregation. Previous studies have also reported that emulsions stabilized by various food proteins, such as whey protein isolate, sodium caseinate, β-lactoglobulin, or β-casein, are prone to flocculation and/or coalescence under simulated gastric conditions, which has been attributed to interactions with mucin, protein hydrolysis, or changes in electrostatic interactions (Sarkar, Goh, Singh et al., 2009; Singh & Ye, 2013). However, other studies suggest that the stability of oil droplets under gastric conditions also depends on the amount of non-adsorbed protein present within the aqueous phase, with excess free protein improving aggregation stability within the stomach phase (Nik, Wright, & Corredig, 2010). The microstructure of the emulsion samples changed again in the simulated small intestine phase with evidence of some large irregular shaped particles surrounded by smaller particles (Fig. 2). These changes may be attributed to alternations in dilution, pH, ionic strength, and digestive enzymes when the fat droplets move from the stomach phase into the intestinal phase (Li, Ye, Lee, &

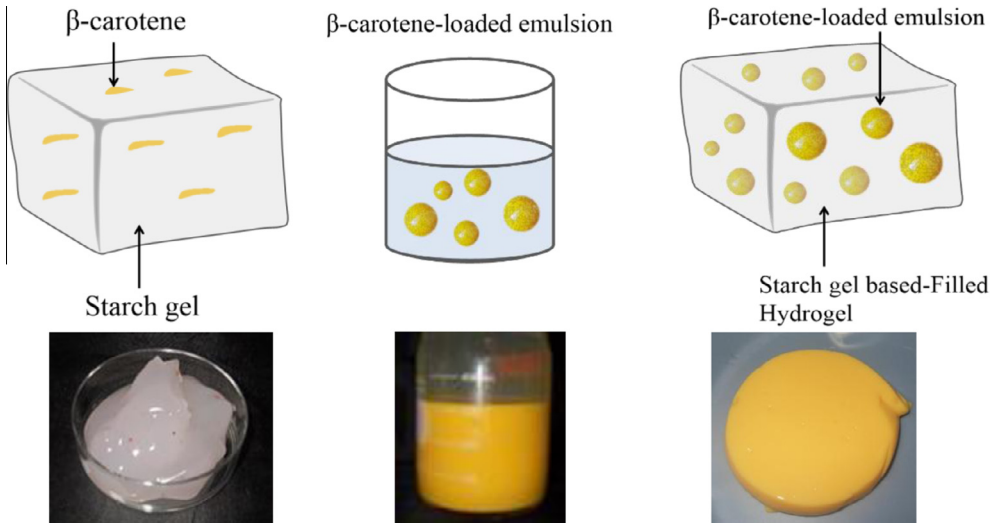

Fig. 1. Schematic representation and visual appearances of starch hydrogels, oil-in-water emulsions, and filled hydrogels prepared in this study. All the samples contained the same amount of β-carotene.

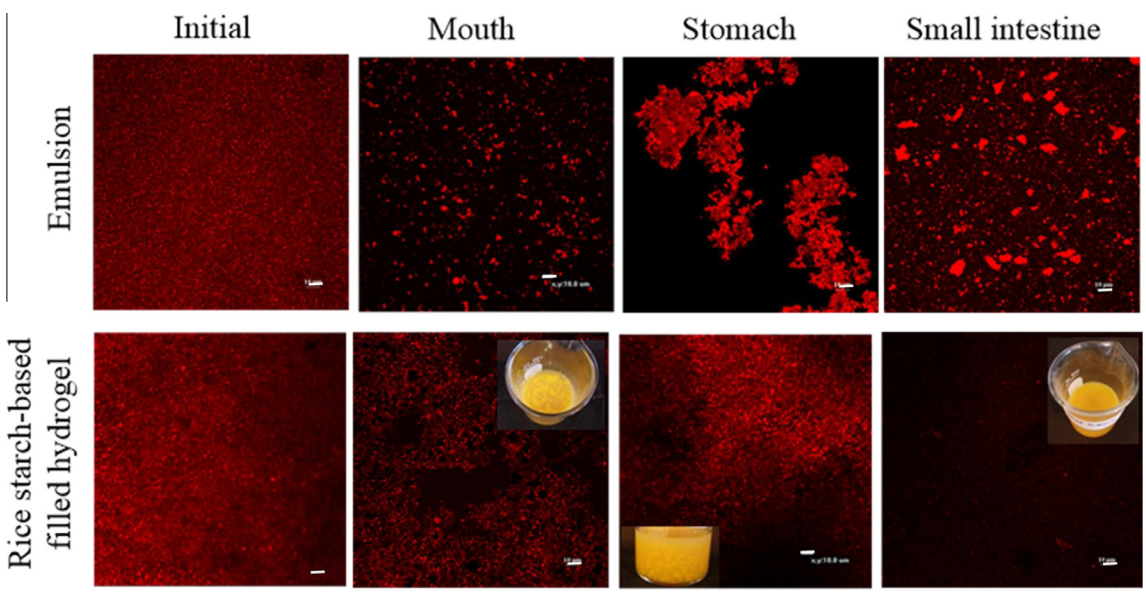

Fig. 2. Influence of simulated gastrointestinal conditions on microstructures of oil-in-water emulsions (containing 4 wt% fat stabilized by WPI) and rice starch-based filled hydrogels (also containing 4 wt% fat stabilized by WPI). Inserted photographs indicate the appearance of the samples following digestion in the mouth, stomach and small intestine phases. The scale bars represent a length of 10 μm, and the red regions represent lipids.

Singh, 2013; Lindahl, Ungell, Knutson, & Lennernas, 1997; Singh & Ye, 2013).

The filled hydrogels behaved quite differently to the emulsions within the simulated gastrointestinal tract. Exposure of the filled hydrogels 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 system. 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 maintained (Fig. 2, insets). However, the structure of the filled hydrogel disintegrated after exposure to simulated small intestinal conditions, and the sample appeared fluid (rather than paste-like). The breakdown of the starch hydrogel structure within the small intestine may have been due to dilution, shearing, or amylase activity from the pancreatin.

3.2.2. Particle size

The initial mean particle diameter ($d_{4,3}$) of β-carotene-loaded emulsions (4 wt% fat) was 0.26 μm and the particle size distribution (PSD) was monomodal (Fig. 3B). It was not possible to measure the particle size of β-carotene-loaded hydrogels or filled hydrogels because these samples were semi-solid. Upon exposure to simulated oral conditions, the PSD of the 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. Presumably, these larger particles were aggregates formed due to depletion or bridging flocculation of fat droplets by mucin. After exposure to gastric stomach and small intestinal conditions, the fat droplets with sizes similar to those in the initial

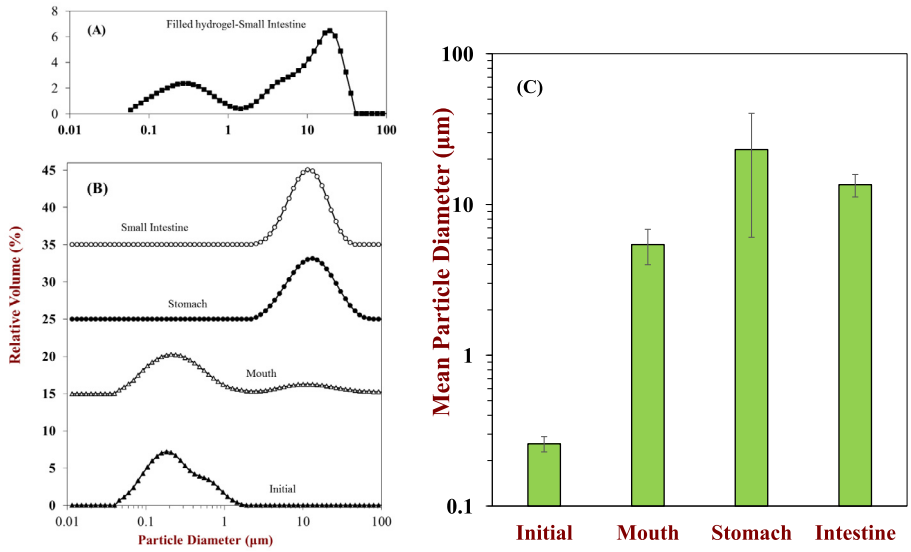

Fig. 3. Influence of simulated gastrointestinal conditions on the particle size distributions of filled hydrogels (A) and oil-in-water emulsions (B), and on the mean particle diameter ($d_{4,3}$) of the emulsions (C).

emulsions disappeared, and only relatively large particles were detected. As mentioned earlier, the formation of large particles in the gastric and small intestinal phases might have been due to aggregation of the fat droplets caused by the pH, ionic strength, or enzyme activity in the stomach and small intestinal fluids.

In the case of the filled hydrogel, the overall semi-solid structure of the hydrogel was maintained even after exposure to the oral and gastric phases (Fig. 2 – inset pictures), and so it was not possible to measure the particle of the initial sample or the samples obtained after exposure to mouth and stomach conditions. Nevertheless, particle size information could be obtained on the sample after it was 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 a population of relatively large particles around 20 μm (Fig. 3A). The confocal microscopy images show a fairly even distribution of small fat particles surrounded by a few larger ones (Fig. 2). We believe that these fat particles are the remnants of the fat droplets after lipid digestion, such as micelles and liposomes containing fatty acids, monoacyglycerols, bile salts and phospholipids.

The mean particle diameter and PSD of emulsions and filled hydrogels containing different fat levels (2, 6, and 8 wt%) were also measured. All of the results were similar to those obtained from the samples containing 4 wt% oil and therefore this data was not shown.

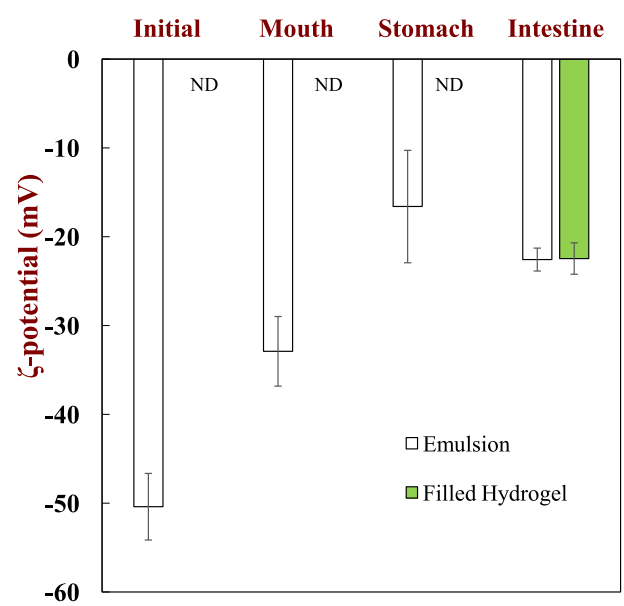

Fig. 4. Influence of simulated gastrointestinal conditions on the electrical charge of oil-in-water emulsions and filled hydrogels (both containing 4 wt% fat initially stabilized by WPI).

3.2.3. Electrical charge

The electrical charge (ζ-potential) of the fat droplets in the initial emulsions was around −50 mV (Fig. 4), which can be attributed to the fact that the adsorbed proteins (WPI) were well above their isoelectric point (around pH 5). The ζ-potential of the fat droplets in the emulsion became less negative after exposure to the oral, gastric, and small intestine phases, with the magnitude of the ζ-potential being smallest in the gastric phase. These variations in particle charge can be attributed to a number of factors: pH changes cause alterations in the sign and magnitude of the charge on the protein molecules; increases in ionic strength will decrease the ζ-potential; the binding of counter-ions to droplet surfaces alters their charge; the displacement of proteins by other molecules alters droplet charge; protein hydrolysis will alter droplet charge. It was not possible to measure the ζ-potential of the fat droplets trapped within the filled hydrogels in the initial sample or the samples exposed to the mouth and stomach phases because they had semi-solid structures. However, the charge could be measured in the sample exposed to small intestinal conditions because the hydrogel structure was disintegrated. In this case, the electrical charge was fairly similar to that obtained from the emulsion that was exposed to the small intestinal phase (≈−40 mV).

3.3. Influence of delivery system structure on lipid digestibility

In this series of experiments, the digestibility of the lipid droplets in emulsions and filled hydrogels was compared in systems that contained the same initial fat content (4%). 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. 5 shows the percentage of FFA released from the β-carotene-loaded emulsion and filled hydrogel samples during two hours digestion.

In general, there was a steep increase in the amount of FFA released during the first 10 min of digestion, followed by a more gradual increase at later times. The initial rate and final extent of lipid digestion both appeared to be higher for the filled hydrogel than for the emulsion (Fig. 5). We postulate that the observed differences in lipid digestion are related to differences in the structural organisation of the fat droplets within the two systems. There are several possible reasons why the rate and extent of lipid digestion in the filled hydrogels was faster than that in the emulsions. As discussed earlier, the fat droplets in the emulsions were unstable so aggregated in simulated gastric conditions, whereas those in the filled hydrogels remained evenly dispersed through the system without aggregating (Fig. 2). Once the filled hydrogels were exposed to simulated small intestinal condition, the gel matrix disintegrated and the fat droplets were released. As a consequence, there was a large specific surface area of fat exposed to the digestive enzymes (lipase), which led to rapid digestion. On the other hand, the fat droplets in the emulsions became highly aggregated after exposure to gastric conditions, which may have reduced the surface area of lipids exposed to the lipase. A number of *in vitro* studies have shown that the rate of lipid hydrolysis increases with an increase in surface area, *i.e.*, decrease in mean droplet diameter (Armand et al., 1999; Golding et al., 2011; Troncoso, Aguilera, & McClements, 2012b).

The influence of fat content on the rate and extent of lipid digestion was also examined. A series of emulsions and filled hydrogels containing different amounts of lipid phase (2–8%), but with the same β-carotene concentration (0.3 wt%) in the lipid phase. The total amount of FFA released after two hours digestion is shown for all of the samples in Fig. 5. There was a slight decrease in the amount of FFA produced as the total lipid concentration increased from 2 to 8 wt%. Our results agree with other *in vitro* studies which investigated the influence of fat content and this effect may be attributed to a number of physicochemical mechanisms: (i) as the lipid concentration increased, the amount of lipase per unit surface area of oil droplets decreased, (ii) the amount of bile salts present may have been insufficient to solubilise all of the lipid digestion products produced at higher lipid concentrations; (iii) at the higher lipid concentration, the amount of calcium present may have been insufficient to precipitate all the FFAs produced by digestion and remove them from the droplet surfaces (Ahmed, Li, McClements, & Xiao, 2012; Li, Hu, & McClements, 2011; Ye, Cui, Zhu, & Singh, 2013).

3.4. Influence of delivery system structure on bioaccessibility

In this series of experiments, we measured the bioaccessibility of β-carotene in the different delivery systems: hydrogel; emulsion; filled hydrogel. This information is important to ensure that any encapsulated bioactive component is actually available for absorption after ingestion of a functional food product. The bioaccessibility of β-carotene clearly depended on the structure and composition of the delivery system (Fig. 6). The β-carotene had a very low bioaccessibility (≈1%) when it was directly incorporated into a rice starch hydrogel. It is well known that carotenoids need to be incorporated into mixed micelles within the small intestine prior to absorption (Nik, Wright, & Corredig, 2011; Porter et al., 2007). 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 solubilise certain types of lipophilic bioactive components but they tend to be much less efficient than complex mixed micelles containing free fatty acids and monoacyglycerols (Kaukonen, Boyd, Porter, & Charman, 2004; Pedersen, Mullertz, Brondsted, & Kristensen, 2000; Yao, Xiao, & McClements, 2014). It has been reported that in the absence of lipolysis, the fraction of bioactive materials transferred into simple mixed micelles is relatively low: ≈2% for β-carotene, ≈1.5% for Coenzyme Q, ≈24% for vitamin D, and: ≈31% for phytosterols (Yao et al., 2014). Our results suggest that the addition of β-carotene directly into a starch hydrogel should be avoided because the encapsulated components may not be bioavailable.

On the other hand, when the β-carotene was incorporated into protein-stabilized emulsions, the bioaccessibility of β-carotene increased to ≈23% (for the emulsion containing 6 wt% fat). This result can be attributed to the fact that the fat in the emulsion forms mixed micelles capable of solubilising lipophilic components under simulated small intestinal conditions. It has been reported that the presence of exogenous lipid in the duodenum stimulate the secretion of biliary lipids (bile slats, phospholipids, and cholesterol) which combine with lipid digestion products to generate a series of colloidal species including micelles, mixed micelles, vesicles, and emulsion droplets. These colloidal structures contain hydrophobic regions in which lipophilic bioactive agents can be

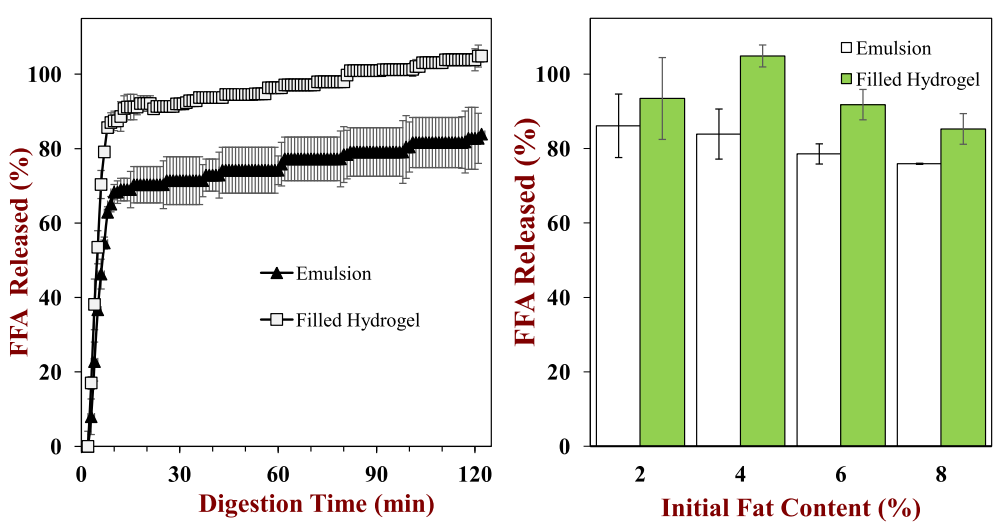

Fig. 5. Kinetics of free fatty acid release from oil-in-water emulsions and filled hydrogels (both initially containing 4 wt% fat) determined by pH-stat titration (left), and influence of initial fat content on the amount of FFA (%) released after two hours digestion (right).

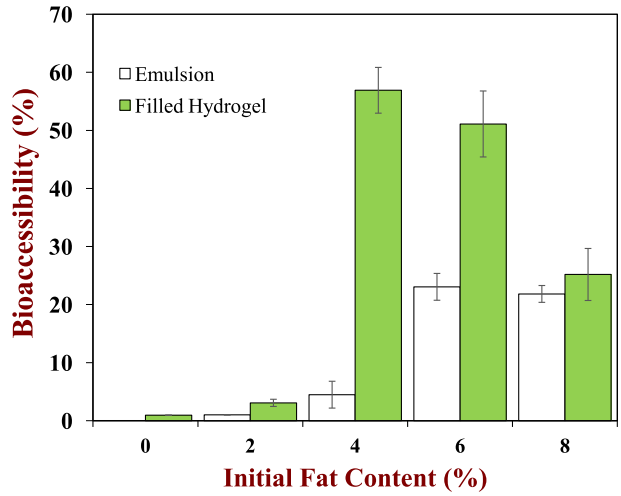

Fig. 6. Influence of initial fat content on the bioaccessibility (%) of β-carotene after *in vitro* digestion in filled hydrogel and emulsions.

solubilised and transported (Porter, Pouton, Cuine, & Charman, 2008; Porter et al., 2007).

For this system, the bioaccessibility of β-carotene tended to increase as the total fat content increased up to about 6%, due to the increase in β-carotene solubilisation capacity of mixed micelles (Porter et al., 2007, 2008). It is well known that the solubilisation capacity of the mixed micelles formed on digestion of lipid-based drug delivery systems is dependent on both the nature and quantity of the included lipid, the presence of additional solubilising excipients (such as surfactants, co-surfactants, and co-solvents) and the concentration of endogenous (bile salts and phospholipids) solubilising species present in the intestine (Porter et al., 2008; Yang & McClements, 2013a,b). In our study, adding more fat to the systems likely increased the solubilisation capacity of the mixed micelles by generating a greater amount of free fatty acids and monoacylglycerols. However, if too much fat is present, then there may be insufficient bile salts and phospholipids present in the intestinal fluids to form mixed micelles with the free fatty acids produced, which may decrease bioavailability (see later).

The bioaccessibility of β-carotene incorporated within WPI-stabilized emulsions ranged from around 1–23%, depending on the lipid concentration. However, these values are lower than those reported in some other studies. In a study using Tween 20-stabilized nanoemulsions, it was reported that the bioaccessibility of β-carotene was around 50% (Salvia-Trujillo et al., 2013b). The difference of bioaccessibility between WPI-stabilized emulsion prepared in this study and Tween 20-stabilized emulsion might be caused by the ability of the emulsifier to prevent lipid droplets from aggregating under simulated oral and gastric conditions.

The bioaccessibility of the β-carotene encapsulated within the filled hydrogel was higher than that obtained from the emulsion system, particularly at intermediate fat contents. This might have occurred because the hydrogel matrix protected the fat droplets from aggregation within the oral and gastric phases, as shown by confocal microscopy (Fig. 2). The fat droplets in the filled hydrogels remained evenly distributed throughout the system, whereas those in the emulsions aggregated. The starch hydrogel might also be able to affect the bioaccessibility of the β-carotene by other mechanisms, but further research is needed to establish this. The decrease in bioaccessibility at the high fat contents may have been because there were not enough bile salts and phospholipids present in the simulated intestinal fluids to solubilize all of the free fatty acids produced by lipid digestion.

4. Conclusions

We have shown that β-carotene-loaded filled hydrogels can be successfully fabricated by combining oil-in-water emulsions with rice starch hydrogels. A simulated gastrointestinal model was used to measure the rate and extent of lipid digestion, as well as the bioaccessibility of encapsulated β-carotene. Our study showed that lipid droplets trapped inside filled hydrogels are digested faster than those in conventional emulsions, and that β-carotene bioaccessibility was higher in filled hydrogels than in emulsions or hydrogels. These effects were attributed to differences in the microstructure of the delivery systems within the simulated gastrointestinal tract. In particular, the starch hydrogels appeared to prevent the fat droplets from aggregating in the mouth and stomach, which increased the accessibility of the lipase to the lipid phase. Consequently, more mixed micelles were available to solubilise the β-carotene released from the delivery systems. The information obtained in this study will be useful for the development of nutraceutical-fortified rice gels suitable for use as functional food products.

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