1 **Influence of methylcellulose on attributes of β -carotene fortified**
2 **starch-based filled hydrogels: Optical, rheological, structural,**
3 **digestibility, and bioaccessibility properties**

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ABSTRACT

There is considerable interest in controlling the gastrointestinal fate of nutraceuticals to improve their efficacy. In this study, the influence of methylcellulose (an indigestible polysaccharide) on lipid digestion and β -carotene bioaccessibility was determined. The carotenoids were encapsulated within lipid droplets that were then loaded into rice starch hydrogels containing different methylcellulose levels. Incorporation of 0 to 0.2% of methylcellulose had little impact on the dynamic shear rheology of the starch hydrogels, which may be important for formulating functional foods with desirable textural attributes. The microstructure, lipid digestion, and β -carotene bioaccessibility of the filled hydrogels were measured as the samples were passed through simulated oral, gastric, and small intestinal phases. The lipid digestion rate and carotenoid bioaccessibility decreased with increasing methylcellulose. This effect was attributed to the ability of the methylcellulose to inhibit molecular diffusion, promote droplet flocculation, or bind gastrointestinal components thereby inhibiting triacylglycerol hydrolysis at the lipid droplet surfaces. This information may be useful for rationally designing functional foods with improved nutritional benefits.

Keywords: methylcellulose; β -carotene; lipid digestion; emulsions; nanoemulsions; hydrogels; gastrointestinal tract; starch

1. Introduction

The food industry is attempting to fortify many types of food and beverage products with nutraceuticals. Nutraceuticals are bioactive agents found in foods that are not required for normal human functioning (unlike nutrients, minerals, and vitamins), but that may improve human health, wellbeing, and performance through their biological effects (McClements, 2013). Nutraceuticals include food components such as ω -3 oils, carotenoids, polyphenols, phytosterols, and curcumin. Many of these nutraceuticals cannot simply be introduced into functional foods in their pure form because of physicochemical constraints, such as limited solubility, chemical instability, and poor bioavailability (Tang & Zhong-Gui, 2007; Liang, Shoemaker, Yang, Zhong, & Huang, 2013; McClements, 2013; Reboul, 2013). These challenges can often be overcome using food-grade colloidal delivery systems, such as emulsions, nanoemulsions, microemulsions, solid lipid nanoparticles, and filled hydrogels (Tokle, Lesmes, Decker, & McClements, 2012; Nik, Langmaid, & Wright, 2012; Verrijssen, Balduyck, Christiaens, Loey, Buggenhout, & Hendrickx, 2014; Mun, Kim, Shin, & McClements, 2015; Zhang, Xu, Jin, Shah, Li, & Li, 2015). Lipophilic nutraceuticals can be encapsulated within lipid droplets that can readily be dispersed in water, that can protect the bioactives from degradation, and that are digested in the gastrointestinal tract (GIT) to form mixed micelles that enhance bioaccessibility (McClements, 2013).

Many commonly consumed food products have gel-like properties, such as some desserts, confectionary, and meat-substitutes. These products are often prepared using food-grade biopolymers that form a three-dimensional network that traps water and leads to a material with viscoelastic properties. In a previous study, our group showed that β -carotene could be encapsulated in lipid droplets and then dispersed in macroscopic starch hydrogels (Mun, Kim, Shin, & McClements, 2015; Mun, Kim, & McClements, 2015). This research showed that β -carotene bioaccessibility was higher in filled hydrogels (lipid droplets dispersed in hydrogels), than in lipid droplets or in hydrogels alone. This effect was attributed to the ability of the starch hydrogels to prevent the lipid droplets from aggregating in the gastrointestinal tract, which increased the accessibility of the lipase to the emulsified lipid phase.

In the current study, the influence of the addition of methylcellulose on the microstructure, lipid digestibility, and β -carotene bioaccessibility of the filled starch hydrogels was examined

using a simulated GIT. Methylcellulose is an indigestible water-soluble polysaccharide that is widely used in the food industry for its physicochemical and physiological properties (Chawla & Patil, 2010). Methylcellulose is commonly used as a thickener and emulsifier in foods, as well as to prevent constipation in supplements (Li & Nie, 2016). Previous studies have shown that the microstructure of starch hydrogels can be appreciably altered by the addition of indigestible polysaccharides such as guar gum, xanthan gum, and cellulose derivatives (Techawipharat, Suphantharika, & BeMiller, 2008; Ptaszek, Beriski, Ptaszek, Witczak, Repelewicz, & Grzesik, 2009; Gładkowska-Balewicz, Norton, & Hamilton, 2014). Other studies have shown that interactions between starch and indigestible polysaccharides may appreciably alter the functional properties of starch hydrogels, such as their appearance, rheology, and stability (Baranowska, Sikora, Krystyjan, & Tomasik, 2011; Samutsri & Suphantharika, 2012; Sikoral, Tomasik, & Krystyjan, 2010; Krystyjan, Adamczyk, Sikora, & Tomasik, 2013). We therefore hypothesized that the addition of methylcellulose to filled starch hydrogels would alter their microstructure, rheology, and gastrointestinal fate, which could alter the bioaccessibility of any encapsulated nutraceuticals. This information may be useful in the design of functional foods with enhanced health benefits, such as controlled lipid digestion or nutraceutical bioaccessibility.

2. Materials 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). 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 were of analytical grade. Double-distilled water was used to prepare all solution and emulsions. Most concentrations are reported as wt%, *i.e.*, g of specified component per 100 g of sample.

2.2. Viscoelastic behavior

The dynamic viscoelastic properties of the samples were determined using a dynamic

shear rheometer (AR 1500 ex, TA instruments Ltd, New Castle, DE, USA) operating in oscillatory mode with parallel plate geometry (20 mm diameter, 1 mm gap). Starch pastes were prepared by dispersing rice starch powder in distilled water (10 wt% starch) and then heating at 90 °C for 10 min in the absence and presence of emulsion and methyl cellulose. After thermal treatment, the resulting hot paste was loaded between the parallel plates of the rheometer that had previously been 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. A dynamic frequency sweep test was conducted by applying a constant strain of 0.5%, which was within the linear viscoelastic region (established in a preliminary test), over a frequency range between 0.63 and 63 rad/s. The storage (G') and loss (G'') moduli of selected samples were then reported over a range of frequencies, and the moduli of all samples were reported at a constant frequency of 10 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. An aqueous emulsifier solution was prepared by dispersing WPI (1.06 wt%) in 10 mM phosphate buffer (pH 7.0). A stock emulsion was prepared by homogenizing 6 wt% corn oil and 94 wt% emulsifier solution (1.06 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).

2.4. Preparation of filled hydrogels containing methylcellulose

Initially, the desired amount of methylcellulose was dissolved in 10 mM phosphate buffer (pH 7.0) and then stock WPI-stabilized emulsion and rice starch were added into the methylcellulose solution. The final concentrations of lipid, rice starch, and methylcellulose contained in the mixtures were 4 wt% lipid, 10 wt% of rice starch, and 0, 0.05 wt%, 0.1 wt%, or 0.2 wt% of methylcellulose. **These levels of methylcellulose were selected so as to cover a range of values that might be used in commercial products to modify their properties (Dar and Light, 2014).** These mixtures were then heated at 90 °C for 10 min to gelatinize the starch granules. The heated mixtures were loaded into a flat, cylindrically shaped vessel,

and then stored at 4 °C overnight to allow the hydrogels to set.

2.5. Simulated gastrointestinal tract model

Samples were passed through an *in vitro* GIT model consisting of mouth, stomach, and small intestine phases, which has been described in detail in previous studies (Salvia-Trujillo, Qian, Martin-Belloso, & McClements, 2013a,b; Mun, Kim, & McClements, 2015; Lopez-Pena, Zheng, Sela, Decker, Xiao, & McClements, 2016). Hence, the method is only briefly described here. This method has been widely used by our laboratory in previous studies, and is fairly similar to the standardized method proposed recently (Minekus et al 2014).

For the mouth phase, the samples were mixed with simulated saliva fluid (SSF) containing mucin and various salts (Sarkar, Goh, & Singh, 2009) at a 50:50 vol/vol ratio. The pH of the mixture was then adjusted to pH 6.8 and then incubated at 37 °C for 10 min with continuous agitation. This incubation period is longer than the time a food would typically spend in the mouth, but was used to ensure consistency from sample to sample. For the stomach phase, the sample was mixed with simulated gastric fluid (SGF), which was prepared by dissolving 2 g NaCl, and 7 mL of HCl (37%) in 1l of water and then adding 3.2 g of pepsin (Sarkar, Goh, Singh, & Singh, 2009). The sample was then adjusted to pH 2.5 and incubated at 37 °C for 2 h with continuous agitation at 100 rpm. For the small intestine phase, 30 mL of samples from the gastric phase was placed in a temperature-controlled (37 °C) chamber and the system was set at pH 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 samples 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 and incubated at 37 °C for 2 h. The pH of the sample was monitored using a pH-stat automatic titration unit (Metrohm USA Inc., Riverview, FL) and the solution was maintained at pH 7.0 by adding 0.25 M NaOH to neutralize any free fatty acids (FFA) released due to lipid digestion. The percentage of FFAs released was calculated from the volume of alkaline solution required to neutralize the samples using the following equation:

$$\frac{V_{\text{NaOH}} \times m_{\text{NaOH}} \times M_{\text{lipid}}}{\text{ }}$$

$$\% \text{ FFA} = 100 \times \frac{\text{w}_{\text{lipid}} \times 2}{\text{V}_{\text{NaOH}} \times \text{M}_{\text{lipid}}}$$

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 blank samples was subtracted from the corresponding test samples that contained oil.

2.6. Microstructure

To determine structural changes that occurred within different phases of the GIT model, confocal scanning laser microscopy with a 60× objective lens and 10× eyepiece were used (Nikon D-Eclipse C1 80i, Nikon, Melville, NY). Samples were dyed by adding 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 IMA 1010BOS, 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)

2.7. β -carotene bioaccessibility

After the small intestinal stage, the bioaccessibility of β -carotene was determined using a method described previously (Qian, Decker, Xiao, & McClements, 2012; Salvia-Trujillo, Qian, Martin-Belloso, & McClements, 2013a). Digesta were collected and centrifuged at 2647g for 40 min at 25 °C (CL10 centrifuge, Thermo Scientific, Pittsburgh, PA, USA). This process led to the generation of tubes that contained a sediment at the bottom and a supernatant above. The supernatant was collected and assumed to be the “micelle” phase containing the bioactive component. Aliquots of 5 mL of the digesta or the micelle phase were mixed with 5 mL of chloroform, vortexed and then centrifuged at 1158g for 10 min at 25 °C. The bottom layer containing the solubilized β -carotene was collected, while the top layer was mixed with an additional 5 mL of chloroform and the same procedure was repeated. The bottom chloroform layer was added to the previous one and analyzed

spectrophotometrically (Ultraspec 3000 pro, GE Health Sciences, USA) at 450 nm. A cuvette containing pure chloroform was used as a reference cell.

The concentration of β -carotene extracted from a sample was determined from a calibration curve of absorbance versus β -carotene concentration in chloroform. The bioaccessibility was then calculated using the following equation:

$$\text{Bioaccessibility} = 100 \times (C_{\text{Micelle}}/C_{\text{Digesta}})$$

where C_{micelle} and C_{Digesta} are the concentration of β -carotene in the micelle fraction and in the raw digesta after the pH-stat experiment, respectively (Qian, Decker, Xiao, & McClements, 2012; Salvia-Trujillo et al., 2013 ab). It should be noted that this method does not take into account any chemical changes of the β -carotene, and so the calculated bioaccessibility is only based on the ratios of carotenoids that can be measured by UV-visible spectrometry in the micelle phase and digesta.

2.8. Statistical analysis

All experiments were performed in at least triplicate using freshly prepared samples. Means and standard deviations were calculated from these data.

3. Results and discussion

3.1. Influence of methylcellulose on rheological and optical properties

The rheological properties of filled starch hydrogels were characterized in the absence and presence of methylcellulose. The storage modulus (G') of all the samples was much larger than the loss modulus (G'') across the entire frequency range tested (**Fig. 1a**), which indicated that the samples were predominantly elastic rather than fluid. Both G' and G'' increased with increasing measurement frequency indicating that the gels became stiffer at higher frequencies, which was presumably because they had a characteristic relaxation time within the frequency range used in our measurements. The influence of methylcellulose concentration on the rheological properties of the filled starch hydrogels was determined at a

fixed frequency (10 rad/s) (**Fig. 1b**). There was little change in the storage modulus (G') of the hydrogels with increasing methylcellulose concentration, but there was a slight increase in the loss modulus (G'') from 30.0 Pa (0% MC) to 39.4 Pa (0.2% MC). These results suggest that addition of relatively low levels of methylcellulose to the hydrogels only had a modest impact on their dynamic shear rheology, which may be important when designing functional foods fortified with methylcellulose. The measured rheological properties of the unfilled starch hydrogels containing methylcellulose were similar to those of the filled starch hydrogels (data not shown), indicating that the fat droplets also did not make a major contribution to the overall rheology of the filled hydrogels. It should be noted that the small-strain oscillatory measurements used in this study do not provide information about the rheological response of the starch hydrogels under conditions that they may experience in practice, such as during food manufacturing, food preparation, or mastication.

The formation of starch hydrogels involves the swelling and bursting of starch granules during heating followed by the release of amylose and amylopectin molecules (Krystyjan, Adamczyk, Sikora, & Tomasik, 2013). The released starch molecules may then form a three-dimensional network with some elastic-like properties due to cross-linking of the amylose molecules through hydrogen bonding of helical regions (Eidam, Kulicke, Kuhn, & Stute, 1995; Yang, Irudayaraj, Otgonchimeg, & Walsh, 2004; Samutsri & Supphantharika, 2012). Previous studies have reported that indigestible polysaccharides may disrupt the formation of starch hydrogels by interfering with the formation of these cross-links (Eidam, Kulicke, Kuhn, & Stute, 1995). However, we did not observe this effect in our study, which may have been because the methylcellulose concentration used was relatively low or because the methylcellulose remained in the aqueous phase between the starch molecule cross-links.

The β -carotene enriched emulsions and filled starch hydrogels had a bright yellowish-orange color, which can be attributed to the selective absorption of light by the carotenoids (Mun, Kim, Shin, & McClements, 2015; Mun, Kim, & McClements, 2015). Carotenoids contain numerous conjugated double bonds that enable them to strongly absorb visible light. Visual observation of the samples indicated that they had a uniform appearance throughout the study (data not shown), suggesting that the β -carotene did not form crystals that sediment and that the lipid droplets did not cream.

3.2. Influence of methylcellulose on microstructure in GIT

The initial mean particle diameter ($d_{4,3}$) of the WPI-stabilized emulsions used to prepare the filled hydrogels was 0.26 μm , and the initial particle size distribution was monomodal (data not shown). The changes in the particle size and microstructure of these emulsions as they passed through the simulated GIT were similar to those observed using a similar system in our previous studies (Mun, Kim, Shin, & McClements, 2015; Mun, Kim, & McClements, 2015), and so are not described in detail here. Briefly, extensive aggregation of the fat droplets was observed in the oral and gastric phases (**Fig. 2a**), which can be attributed to a number of effects: depletion and/or bridging flocculation by mucin in the simulated saliva; changes in pH and ionic strength in the different GIT regions; and hydrolysis of adsorbed proteins by pepsin in the gastric phase. In the small intestine phase the fat droplets were digested by lipase and therefore largely disappeared from the confocal images (**Fig. 2a**).

The semi-solid structure of all the filled hydrogels remained intact after exposure to the oral and gastric phases, so it was not possible to measure the particle size distribution of these samples using light scattering. Consequently, only confocal microscopy was used to monitor changes in the microstructure of these samples as they passed through the simulated GIT. The microstructural changes in the filled starch hydrogels in the absence of methylcellulose are shown in **Figure 2b**. Initially, fine fat droplets were fairly evenly dispersed throughout the starch hydrogels. Upon exposure to simulated oral and gastric fluids, the fat droplets remained relatively small and were still fairly evenly dispersed throughout the hydrogels, which is in contrast to the extensive fat droplet aggregation observed in the emulsions (**Fig. 2a**). This suggests that the presence of the starch hydrogel inhibited the aggregation of the fat droplets, presumably by forming a highly viscous aqueous phase that retarded droplet movement.

The incorporation of 0.05% methylcellulose into the filled starch hydrogels did not affect their initial microstructure. Again, the fat droplets remained relatively small and evenly distributed throughout the hydrogel when the samples were exposed to simulated oral and gastric conditions (**Fig. 2c**). However, when 0.2% methylcellulose was present in the filled hydrogels, there appeared to be some fat droplet aggregation in the initial samples (**Fig. 2d**), which may have been due to thermodynamic incompatibility of the biopolymers or due to

depletion flocculation of the fat droplets. Nevertheless, the fat droplets still appeared to be fairly evenly dispersed within the mouth and stomach phases, suggesting that they were still trapped in a hydrogel network.

After exposure to small intestinal conditions, the hydrogel structure disintegrated and the samples became fluid-like, which may have been due to dilution effects and/or amylase activity (Mun, Kim, Shin, & McClements, 2015). Dilution will have decreased the overall biopolymer concentration, whereas amylase activity will have led to hydrolysis of some of the starch molecules, thereby leading to breakdown of the hydrogel network. Interestingly, after exposure to the small intestine phase there appeared to be an increase in the number of large lipid-rich particles within the samples as the methylcellulose level increased (**Fig. 2d**).

Results from previous studies suggest that these lipid-rich particles could be undigested fat droplets, micelles, and/or vesicles (McClements, Decker, & Park, 2009; Singh, Ye, & Horne, 2009). Usually, fat droplets are hydrolyzed by lipase in the small intestine phase. Lipase molecules adsorb to the fat droplet surfaces and hydrolyze the triacylglycerols (TAGs) into free fatty acids (FFAs) and monoacylglycerols (MAGs). The resulting FFAs and MAGs are then solubilized by bile salts or precipitated by calcium ions, which helps remove them from the fat droplet surfaces during lipid digestion. Our results suggest that the presence of the methylcellulose may have interfered with this process, thereby inhibiting the digestion of the emulsified TAGS. The methylcellulose is an indigestible polysaccharide that may have inhibited the diffusion of lipase molecules to the fat droplet surfaces, or that inhibited the removal of FFAs and MAGs from the lipid droplet surfaces. Alternatively, the methylcellulose may have altered the structure of the colloidal particles in the mixed micelle phase by binding to FFAs, MAGs, or bile salts. These interactions may have promoted the formation of large vesicles rather than small micelles. Clearly, further research is required to establish the precise molecular and physicochemical origin of the ability of methylcellulose to alter the gastrointestinal fate of filled hydrogels.

3.3. Influence of methylcellulose on lipid digestibility

In this series of experiments the impact of incorporating methylcellulose into the filled starch hydrogels on the rate and extent of lipid digestion was measured under simulated small intestinal conditions (**Fig. 3**). At the end of 2 h digestion, the overall extent of lipid digestion

was fairly similar in the presence and absence of methylcellulose, regardless of its concentration. However, the rate of lipid digestion appeared to be somewhat slower for the filled hydrogels containing 0.2% methylcellulose than for the ones containing lower levels. These results suggested that sufficiently high levels of methylcellulose were able to delay lipid digestion, possibly by restricting the access of lipase to the droplet surfaces or by inhibiting the removal of FFAs and MAGs from the droplet surfaces as mentioned previously.

Tokle et al. (2012) examined the influence of dietary fiber on the digestion of fat droplets under simulated small intestinal conditions. They also reported that the dietary fiber did not prevent lipid digestion, but that it did slow down the rate of lipid digestion. In their study, anionic dietary fibers (pectin) were used, which may have interacted with cationic calcium ions and formed a gel-network that inhibited lipase diffusion or prevented calcium soaps of FFAs being formed. Other studies have suggested that dietary fibers may impact lipid digestion by binding to various components in the GIT, such as calcium ions, bile salts, free fatty acids, and lipase (Palafox-Carlos, Ayala-Zavala, & Gonzalez-Aguilar, 2011; Verrijssen, Balduyck, Christiaens, Loey, Buggenhout, & Hendrickx, 2014; Qiu, Zhao, Decker, & McClements, 2015). A differential scanning calorimetry study of cellulose ester - bile salt mixtures showed that the bile salts affected the thermal gelation of the cellulose derivatives, which suggested that there was some kind of binding interaction (Torcello-Gómez, & Foster, 2014). Consequently, methylcellulose may have interfered with the normal role of bile salts in the lipid digestion process, *i.e.*, displacing other surface-active molecules from the fat droplet surfaces or solubilizing lipolysis products such as FFAs and MAGs (Bellesi, Ruiz-Henestrosa, & Pilosof, 2014; Euston, Baird, Campbell, & Kuhns, 2013; Li, Hu, & McClements, 2011; Mun, Decker, & McClements, 2007; Nik, Wright, & Corredig, 2011). Thus, the retardation of lipid digestion observed at high methylcellulose levels may have been partially due to binding of bile salts. Alternatively, it may have been due to the ability of the methylcellulose to increase the viscosity of the aqueous phase around the lipid droplets, thereby slowing down the diffusion of reactants, catalysts, and products involved in the lipid digestion process (Palafox-Carlos, Ayala-Zavala, & Gonzalez-Aguilar, 2011; Tokle, Lesmes, Decker, & McClements, 2012; Verrijssen, Balduyck, Christiaens, Loey, Buggenhout, & Hendrickx, 2014).

3.4. Influence of methylcellulose on β -carotene bioaccessibility

Finally, the impact of the composition of the hydrogel matrix on β -carotene bioaccessibility was measured (**Fig. 4**). In general, *bioaccessibility* is the fraction of an ingested compound that is solubilized in the gastrointestinal fluids in a form available for absorption (Hedrén, Diaz, & Svanberg, 2002). In the current study, the bioaccessibility was taken to be the fraction of the β -carotene that was released from the hydrogels and solubilized within the mixed micelles formed after lipid digestion.

In our previous study, the bioaccessibility of β -carotene in protein-coated lipid droplets was found to be higher when they were incorporated into a starch hydrogel than when they were in a free state, which was attributed to the ability of the hydrogel to inhibit extensive droplet flocculation in the GIT (Mun, Kim, Shin, & McClements, 2015; Mun, Kim, & McClements, 2015). As a result the lipase molecules could more easily adsorb to the lipid droplet surfaces and convert the TAGs to MAGs and FFAs that could form mixed micelles capable of solubilizing the carotenoids. A similar finding was observed in the current study: the bioaccessibility of β -carotene was 16% and 66% in emulsions and filled hydrogels containing no methylcellulose, respectively (**Fig. 4**). These values are in good agreement with those found in our previous studies (Mun, Kim, Shin, & McClements, 2015; Mun, Kim, & McClements, 2015). Interestingly, the bioaccessibility of β -carotene decreased as the methylcellulose concentration in the filled hydrogels increased (**Fig. 4**), despite the fact that the final extent of lipid digestion did not appear to be strongly affected by methylcellulose level (Fig 3). There are a number of possible reasons for this phenomenon.

First, methylcellulose molecules might have altered the nature of the mixed micelles formed by lipid digestion, *i.e.*, by interacting with bile salts, FFAs, or MAGs. Indeed, the confocal microscopy images of the samples indicated that there were more large lipid-rich particles formed after lipid digestion in the presence of high levels of methylcellulose (**Fig. 2d**). These lipid-rich particles may have been large liposomes, rather than small micelles, which could have sedimented to the bottom of the samples during the centrifugation step carried out as part of the bioaccessibility analysis. Second, the methylcellulose may have directly bound to the released β -carotene molecules and formed a dense molecular complex that sedimented during the centrifugation step. Further study is clearly needed to determine

the physicochemical origin of the ability of methylcellulose to reduce the bioaccessibility of β -carotene in filled starch hydrogels.

4. Conclusions

The impact of the composition of filled hydrogels on lipid digestion and β -carotene-bioaccessibility was determined. The rate of lipid digestion was reduced at higher methylcellulose concentrations, however, the final extent of lipid digestion was unaffected. The bioaccessibility of β -carotene was lower in protein-coated lipid droplets when they were in a free state than when they were encapsulated in filled hydrogels, which was attributed to the ability of the starch hydrogel to inhibit extensive lipid droplet flocculation. The incorporation of methylcellulose into the filled starch hydrogels reduced β -carotene bioaccessibility, which could be due to the ability of this dietary fiber to interact with specific components in the GIT or alter their diffusion. The information obtained from this study might be helpful for designing functional foods that perform in a specific way within the gastrointestinal tract.

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515

Figure Captions

Figure 1a. Influence of methylcellulose addition and measurement frequency on the dynamic shear properties of filled hydrogels measured at 4 °C using oscillatory viscoelastic analysis. The samples contained either 0 or 0.2% methylcellulose. The black points represent the storage modulus (G') and the white points represent the loss modulus (G'').

Figure 1b. Influence of methylcellulose level on the storage and loss components of the shear modulus of filled hydrogels measured at 4 °C using oscillatory viscoelastic analysis (10 rads/sec).

Figure 2. Confocal micrographs showing microstructural changes during passage through simulated oral, gastric, and intestinal phases; (A) emulsions; (B) filled starch hydrogels without methylcellulose, (C) filled starch hydrogels containing 0.05% methylcellulose; and (D) filled starch hydrogels containing 0.2% methylcellulose

Figure 3. Calculated FFA released from rice starch-based filled hydrogels containing different concentration of methylcellulose. Digestion was retarded in the presence of the highest methylcellulose level

Figure 4. Bioaccessibility (%) of β -carotene incorporated in WPI-stabilized emulsion and rice starch-based filled hydrogels containing methylcellulose (MC) after in vitro digestion.

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Manuscript title: Influence of methylcellulose on bioaccessibility of β -carotene incorporated within starch-based filled hydrogels, by Mun et al

Graphical Abstract

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