Food Research International 105 (2018) 440–445

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Effect of xanthan gum on lipid digestion and bioaccessibility of β-carotene-loaded rice starch-based filled hydrogels

Shinjae Park, Saehun Mun*, Yong-Ro Kim*

Center for Food and Bioconvergence, and Department of Biosystems and Biomaterials Science and Engineering, Seoul National University, Seoul, 08826, Republic of Korea

ARTICLE INFO

Keywords:
β-Carotene
Filled hydrogel
Xanthan gum
Lipid digestion
Bioaccessibility

ABSTRACT

The aim of this study was to examine the effects of xanthan gum on the lipid digestibility, rheological properties, and β-carotene bioaccessibility of rice starch-based filled hydrogels. β-Carotene was solubilized within lipid droplets of emulsion that were then entrapped within rice starch hydrogels fabricated with different concentrations of xanthan gum. At a low concentration of xanthan gum (< 0.5 wt%), the viscous characteristics of the filled starch hydrogels increased. Furthermore, these hydrogels had a slower rate of lipid digestion than the β-carotene-loaded emulsion. As the concentration of xanthan gum was increased (to 1.0 wt% and 2.0 wt%), the filled starch hydrogels became more elastic gel-like than those without xanthan gum, and also had the fastest rate and highest final extent of lipid digestion. The addition of xanthan gum to the filled starch hydrogel lowered the bioaccessibility of β-carotene to varying degrees, depending on the xanthan gum concentration. The results obtained from this study can be useful in designing gel-like food products fortified with lipophilic nutraceuticals.

1. Introduction

β-Carotene is a carotenoid present in plants and fruits, especially in carrots and naturally pigmented vegetables. It is a precursor of vitamin A and several studies have reported that β-carotene plays an important role in lowering the risk of developing cancer and heart diseases due to its antioxidant activities (Maiani et al., 2009; Rao & Rao, 2007). Consequently, there has been a growing interest among consumers to manufacture functional foods and beverages containing β-carotene. However, there are several challenges in the incorporation of β-carotene into commercial food products due to its limited water solubility, chemical instability, and poor bioavailability in the human body. To overcome these challenges, many types of delivery systems have been developed, and studies have suggested that the absorption of dietary carotenoids can be enhanced when they are consumed with lipids or when they are co-ingested with lipid excipients (Matalanis, Jones, & McClements, 2011; Matalanis & McClement, 2012; Mun, Kim, Shin, & McClements, 2015; Qian, Decker, Xiao, & McClements, 2012; Salvia-Trujillo & McClements, 2016; Zhang & McClements, 2016).

In this context, one possible strategy to incorporate lipophilic bioactive material such as β-carotene into food products to improve its bioavailability involves the incorporation of β-carotene into delivery systems containing lipids, which are then incorporated into a food matrix. In previous studies, we fabricated starch-based filled hydrogels as β-carotene delivery systems, consisting of small lipid droplets containing β-carotene that were distributed within starch hydrogel matrix (Mun, Kim, & McClements, 2015; Mun, Park, Kim, & McClements, 2016). There are many food products having gel-like properties and particularly in Asia, rice-based gel-like food products are popular. Hence, we fabricated starch hydrogel as a model of a gel-based food product and incorporated a β-carotene-loaded emulsion into it. Changing the composition, structure, and properties of food matrices can also modulate the bioavailability of hydrophobic bioactive compound (Zhang & McClements, 2016). In a previous study, to investigate the effect of the composition of food matrix on β-carotene bioaccessibility, methylcellulose was added to a starch-based filled hydrogel; the results showed that β-carotene bioaccessibility of the filled starch hydrogels decreased upon addition of methylcellulose, which could be attributed to the interaction of methylcellulose with specific gastrointestinal tract (GIT) components or its ability to alter their diffusion (Mun et al., 2016). In addition to methylcellulose, the textural properties and stability of most starch-based products may be improved by the addition of hydrocolloids (Pongsawatmanit & Srijunthongsiri, 2008). In particular, rice starch-xanthan mixtures are widely used because of their beneficial effects on the texture and acceptability of food products (Viturawong, Achayuthakan, & Suphantharika, 2008).

Here, xanthan gum was used to evaluate the influence of the different filled hydrogel properties on lipid digestibility and β-carotene bioaccessibility. We hypothesized that the differences in the properties of added polysaccharide would alter the behavior of filled starch

* Corresponding authors.
E-mail addresses: saehun@snu.ac.kr (S. Mun), yongro@snu.ac.kr (Y.-R. Kim).

https://doi.org/10.1016/j.foodres.2017.11.039
Received 16 August 2017; Received in revised form 9 November 2017; Accepted 19 November 2017
Available online 21 November 2017
0963-9969/

hydrogels within the simulated GIT (*e.g.*, their susceptibility to enzymatic degradation, reaction with other materials, and their stability to ionic strength or pH changes).

Xanthan gum is the most extensively used polysaccharide because of its unique and useful properties. It is produced by *Xanthomonas campestris*, a bacterium commonly found on the leaves of plants such as those belonging to the cabbage family. The backbone chain of xanthan molecules is similar in structure to that of cellulose molecules; however, its trisaccharide side chain wraps around the main chain, making the xanthan molecule a relatively stiff rod (Garcia-Ochoa, Santos, Casas, & Gomez, 2000).

The main purposes of the present study were to elucidate the effects of xanthan gum on the rheological properties of rice starch-based filled hydrogels, and to study the lipid digestion and bioaccessibility of β-carotene incorporated within filled starch hydrogels to determine how changing the physical properties of the food matrix can affect β-carotene bioaccessibility and lipid digestion.

2. Materials and methods

2.1. Materials

The rice starch isolated from native rice (*Ilmi byeo*, Korea) in a laboratory, using an alkaline steeping procedure (Kim, Song, & Shin, 2010) was used in current study. The isolated rice starch had an amylose content of 14%. Xanthan gum was kindly provided by MSC Co. (Yangsan, Korea). Pepsin (from porcine gastric mucosa), mucin (from porcine stomach), pancreatin (from porcine pancreas), β-carotene, and bile (porcine) extracts were purchased from Sigma-Aldrich (St. Louis, MO, USA). Whey protein isolate (WPI, product code: 9500) was provided by Protient, Inc. (St. Paul, MN, USA). Soybean oil (Ottogi Corp., Pyeongtaek, Korea) was obtained from a local store.

2.2. Methods

2.2.1. Rheological measurement

The dynamic viscoelastic properties of the filled hydrogels in the presence and absence of xanthan gum were examined using a rheometer (AR 1500 ex, TA Instruments Ltd., New Castle, DE, USA). Rice starch powder was dispersed into WPI-stabilized emulsion and then heated at 95 °C for 10 min in the presence and absence of xanthan gum. The resulting samples were placed between the parallel plates in a rheometer (4 °C). Samples were surrounded by a thin layer of silicone oil to prevent sample dehydration. After hardening the gel for approximately 1 h, a dynamic frequency sweep test was performed at 25 °C at a constant strain (1.0%), over a frequency range between 0.63 and 63 rad/s. Dynamic rheological measurements were performed in triplicate.

2.2.2. Preparation of β-carotene encapsulated in oil-in-water (O/W) emulsion

First, β-carotene (0.3%, w/w) was dissolved in soybean oil. For complete dissolution, soybean oil containing β-carotene was sonicated (Ultrasonic Cleaner-Powersonic 410, Hwashin, Seoul, Korea) for 15 min and heated at 60 °C for 30 min with agitation. For the aqueous phase, WPI (0.6 wt%, w/w) was dispersed in 10 mM phosphate buffer (pH 7) for 3 h with mild stirring. A stock emulsion (6 wt% oil) was prepared by homogenizing soybean oil containing β-carotene and WPI solution using a high-speed blender (ULTRA-TURRAX model T25 digital, IKA, Germany) for 2 min and then passing the solution through a microfluidizer (Picomax MN 250A, Micronox, Seongnam, Korea) three times at 10 kpsi. In this study, to prepare O/W emulsion containing 4% oil, 6% oil stock emulsion was diluted with phosphate buffer (10 mM, pH 7) and adjusted to the desired concentration. To avoid the degradation of β-carotene by UV light, all samples were prepared, stored, and tested in the bottles covered with aluminum foil.

2.2.3. Preparation of filled starch hydrogels containing xanthan gum

Rice starch-xanthan gum mixtures (7 wt% total solid, w/w) of different gum concentrations (0, 0.1 wt%, 0.5 wt%, 1.0 wt%, and 2.0 wt %) were dispersed into the diluted O/W emulsion. Mixtures were then stirred for blending and heated at 95 °C for 10 min with mild agitation for starch gelatinization. The hot samples were placed on flat petri dishes at room temperature for 12 h to form the gel structure, following which the samples were stored at 4 °C, until further use.

2.2.4. In vitro digestibility test

The *in vitro* digestibility of the samples was measured using an *in vitro* GIT model that has been reported in previous studies (Lopez-Pena et al., 2016; Salvia-Trujillo, Qian, Martin-Belloso, & McClements, 2013; Sarkar, Goh, Singh, & Singh, 2009).

Before the filled starch hydrogel samples were reacted with the simulated saliva fluid (SSF), the samples were processed by compression and grinding using a mortar and pestle for 2 min to prepare masticated gel samples. The pretreated samples were added to SSF containing α-amylase (Sarkar, Goh, & Singh, 2009) in a 1:1 v/v ratio to mimic the oral phase. After adjusting the pH of the mixture to 6.8, it was reacted at 37 °C for 10 min with gentle agitation. For the gastric phase, the sample from the oral phase was added to simulated gastric fluid (SGF). After the pH was adjusted to 2.5, the mixtures were held at 37 °C for 2 h with gentle agitation. SSF contained 2 g of sodium chloride (NaCl) and 3.2 g of pepsin in 1 L of water, and pH was adjusted to 2.5 by using HCl. 30-mL samples from the gastric phase were transferred to 100-mL glass beakers and were placed in a shaking water bath (37 °C). The pH of the sample was set at 7.0. To mimic the small intestine phase, 1.5 mL of the salt solution (10 mM calcium chloride ($CaCl_2$) and 150 mM NaCl) and 3.5 mL of the bile extract solution were added to the reaction vessel. Then, 2.5 mL of pancreatin suspension (187.5 mg/2.5 mL phosphate buffer) was added to the mixture and it was digested at 37 °C for 2 h.

Samples were taken at regular intervals of time (in minutes) during lipid digestion, and the volume of 0.25 M NaOH solution required to neutralize any free fatty acids (FFAs) released due to lipid digestion was measured using a pH meter (Orion 420A +, Thermo Electron Corporation, Massachusetts, USA). The percentage of FFAs released was calculated from the volume of sodium hydroxide solution required to neutralize FFA, using the following equation:

$$\text{FFA}\ (\%) = 100 \times \frac{V_{NaOH} \times m_{NaOH} \times M_{Lipid}}{W_{Lipid} \times 2}$$

where V_{NaOH} is the volume of the titrant (NaOH) in liters, m_{NaOH} is the molarity of NaOH, M_{Lipid} is the molecular weight of soybean oil, and W_{Lipid} is the weight of oil in the digestion system in grams. Blanks (samples without oil) were also run, and the volume of titrant used for these blank samples was subtracted from the corresponding test samples that contained oil.

2.2.5. Determination of β-carotene bioaccessibility

The bioaccessibility of β-carotene was determined after *in vitro* digestion processing of the samples. Digesta were collected after the small intestinal stage, and centrifuged at 2517 × *g* for 30 min at 25 °C (Supra 22 K, Hanil Science Inc., Incheon, Korea). The supernatant was then filtered using a syringe filter (0.45 μm Hydrophilic, Sartorius, Göttingen, Germany) to remove any large residual particles. Aliquots of 5 mL of the raw digesta or the filtered supernatant were mixed with 5 mL of chloroform, stirring, and then centrifuged at 315 × *g* for 10 min. Finally, solubilized β-carotene was collected from the bottom layer, and the process was repeated using the top layer to obtain more β-carotene containing micelle phase. The bottom chloroform layer obtained after centrifugation was combined with the previous one and analyzed spectrophotometrically (UV-1650 PC, Shimadzu, Kyoto, Japan) at 450 nm. Chloroform was used as the reference. Thereafter bioaccessibility was calculated according to the equation mentioned in

previous studies (Qian et al., 2012; Salvia-Trujillo et al., 2013).

2.2.6. Confocal laser scanning microscopy

Structural changes of the hydrogel matrices within the different stages of the GIT model were monitored under a confocal laser scanning microscopy (ZEISS LSM 510 META; Carl Zeiss, Hamburg, Germany). Initial samples and the samples taken from the different stages of the GIT model were dyed with Nile red that was previously dissolved at 0.1% (w/v) in ethanol. Small aliquots of samples were placed on glass slide, and fluorescence images (λ_{ex} = 543 nm, λ_{em} = 560 to 615 nm) were acquired.

2.2.7. Statistical analysis

All data presented were the mean ± standard deviation. Statistical analysis was performed using SPSS for windows (ver. 21.0, IBM Corp., Armonk, N.Y., USA). A one-way ANOVA test followed by a Duncan's multiple range test was conducted to identify statistical significances ($P < 0.05$).

3. Results and discussion

3.1. Influence of xanthan gum on rheological properties of filled starch hydrogels

The effect of xanthan gum on the rheological properties of filled starch hydrogels was examined (Fig. 1). The storage modulus (G′) of all the samples was greater than the loss modulus (G″) in all frequency ranges. As a result, tan δ (G″/G′) values were always < 1, indicating that filled starch hydrogel and mixtures of hydrogel and xanthan were elastic rather than fluidic (Wang et al., 2009). The values of G′ and G″ increased with the increase in xanthan concentration. Thus, xanthan gum elevated the values of the complex modulus of filled starch hydrogels, indicating that xanthan gum increased gel stiffness. Earlier studies have also reported similar results (Abdulmola, Hember, Richardson, & Morris, 1996; Kim & Yoo, 2006). However, in terms of tan δ, filled starch hydrogel-xanthan mixtures showed different results depending on the xanthan gum concentration (Fig. 1B). The tan δ values of the filled starch hydrogels prepared with xanthan gum were higher than those of the filled starch hydrogels without xanthan gum, at low concentrations of xanthan gum (< 0.5 wt%), indicating an increase in the viscous characteristics of the mixture.

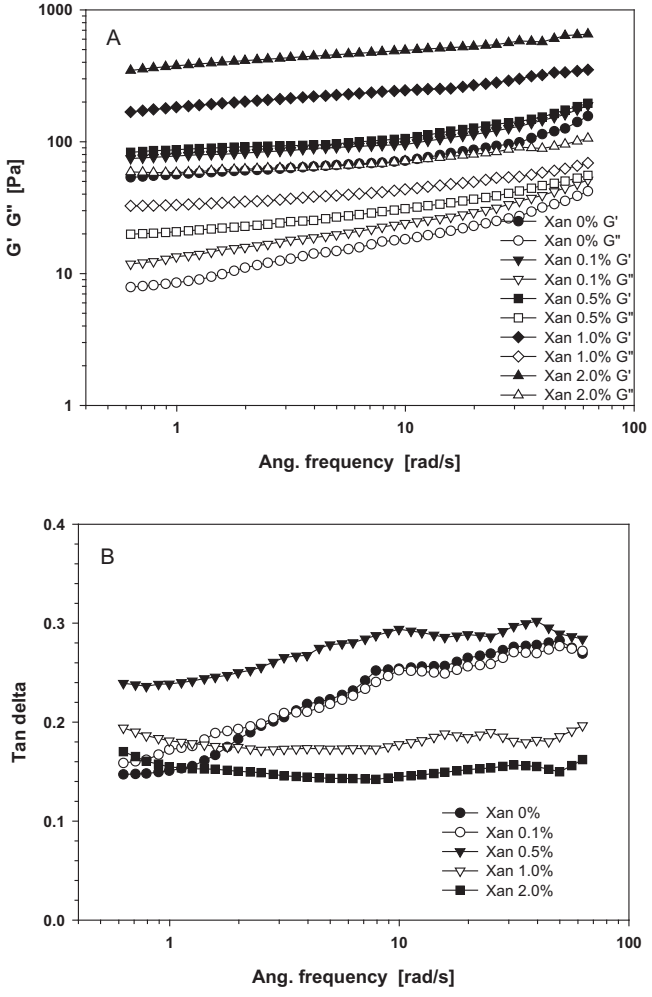

Fig. 1. Effect of xanthan gum (Xan) on the rheological properties of filled starch hydrogels during a frequency sweep at 25 °C. The number following Xan indicates the concentration of added xanthan gum in wt%.

However, as the xanthan concentrations were increased to 1.0 and 2.0 wt%, the tan δ values decreased again, and the values were lower than those of the filled starch hydrogels prepared without xanthan gum. This result indicated that the filled starch hydrogels containing 1.0 and 2.0 wt% of xanthan gum were more elastic gel-like compared to the filled starch hydrogels without xanthan gum, and thus, xanthan gum could reinforce the starch hydrogel network.

Generally, but not always, starch pastes and gels prepared with addition of hydrocolloids exhibit increases in viscosity and/or dynamic moduli. Previous studies have reported various mechanisms that are responsible for these positive effects of hydrocolloids on the rheological properties of starch pastes and gels. These mechanisms include an interaction between leached starch polymers and hydrocolloids, an increase in the local concentration of hydrocolloids in the continuous phase, and the effect of hydrocolloids on the physical properties of starch granules, as well as the amount of amylose leaching from the starch granules (BeMiller, 2011; Choi & Yoo, 2009; Mandala & Bayas, 2004). Some of these mechanisms may work together, and the proportion of their contributions varies depending on hydrocolloids, starches, and preparation methods (BeMiller, 2011).

Many of previous studies on interactions of xanthan gum in starch pastes seem to be consistent in at least one point, existence of incompatibility of xanthan molecules in starch pastes due to its rigid conformation and high molecular weight, forming microphase-separated domains where amylose, amylopectin, and xanthan molecules are mutually excluded (Abdulmola et al., 1996; BeMiller, 2011; Conde-Petit, Pfirter, & Escher, 1997; Russ, Zielbauer, Ghebremedhin, & Vilgis, 2016). Seemingly inconsistent rheological behavior of filled hydrogel with xanthan gum concentration in Fig. 1 could be explained in the same line as follows. At low xanthan concentrations (0.1 and 0.5%), incompatible xanthan molecules partly disrupted starch polymer network and increased viscous toughness, leading to an increase in tan δ (G″/G′) mainly contributed by the increase of loss modulus (G″). However, at high xanthan concentrations (1.0 and 2.0%), the same incompatibility effectively promoted depletion flocculation of swollen starch granules as proposed by (Abdulmola et al., 1996). The promoted association between swollen starch granules dominated rheological properties of filled hydrogel at high xanthan concentrations, resulting in the enhanced elasticity as evidenced by decreases in tan δ and its frequency dependency (Fig. 1B).

In a previous study, when methylcellulose was added to the filled starch hydrogel at 0.05, 0.1, and 0.2 wt%, methylcellulose only had a modest effect on the rheology of the starch hydrogel (Mun et al., 2016). However, the result obtained in the present study suggested that the addition of xanthan gum altered the viscoelastic properties of filled starch hydrogels. Thus, the xanthan concentration has a profound impact on the rheological properties of the xanthan-hydrogel mixture, despite a relatively small concentration in the mixture.

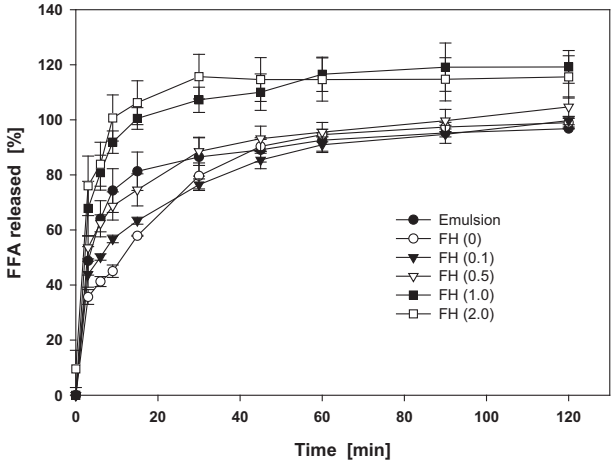

Fig. 2. Calculated FFA released from β-carotene-fortified emulsion and filled starch hydrogels (FH). The number following FH indicates the concentration of added xanthan gum in wt%.

3.2. Influence of xanthan gum on lipid digestibility

The digestibility of the lipid droplets within filled starch hydrogels, in the presence and absence of xanthan gum, was compared and the digestibility of the lipid droplets in emulsion was also examined (Fig. 2).

When the digestibility of the lipid droplets in emulsion was compared to that of filled starch hydrogel prepared without xanthan gum, the final extent of lipid digestion measured after 2 h-digestion was fairly similar, whereas the rate of digestion was relatively slower for the filled starch hydrogel. This result differed from the results obtained in previous studies (Mun et al., 2015; Mun et al., 2015). Previous studies have reported that the filled hydrogel had a higher initial rate and final extent of lipid digestion than those for the emulsion, suggesting that the starch hydrogel matrix kept the lipid droplets well dispersed in the GIT, which increased the specific surface area of lipid exposed to lipase. The difference in the results of the current and previous studies may be attributed to the difference in the composition of the simulated oral phase in the GIT: in previous studies, a mechanical simulation of mastication and α-amylase were not included in the oral phase (Mun et al., 2015; Mun et al., 2016). α-amylase is the most abundant enzyme in the saliva and is responsible for the initial digestion of starch-containing foods. However, it is often assumed that pancreatic amylase is responsible for starch digestion, which occurs mainly in the small intestine. Therefore, starch digestion with α-amylase in the oral phase has often been omitted in studies using the simulated GIT (Granger, Kivlighan, El-Sheikh, Gordis, & Stroud, 2007). The starch-based filled hydrogels fabricated in this study are soft solids; hence, it was hypothesized that the inclusion of a simulated mastication process and α-amylase in the oral phase would have an impact on the digestion of starch-based filled hydrogels.

Consequently, due to the inclusion of the mimicked mastication and α-amylase in the oral phase, the filled starch hydrogel and the emulsion behaved similarly within the simulated GIT. After exposure to the simulated gastric phase, the lipid droplets aggregated (Fig. 3). In the case of starch-based filled hydrogel that was not digested with α-amylase in the oral phase, the lipid droplets were dispersed evenly within the starch hydrogel as shown in previous studies (Mun et al., 2015; Mun et al., 2015). This result suggested that the reaction of starch hydrogel with α-amylase in the oral phase promoted aggregation of the protein-coated lipid droplets in the gastric phase. In other words, the changes in the properties of starch hydrogel caused by the action of α-amylase in the oral phase could affect the subsequent digestion of the lipid droplets in the gastric phase. Thus, the slower rate of digestion of the lipid droplets within starch-based filled hydrogels fabricated in this study might be attributed to the large aggregates of lipid droplets in the gastric phase. These large aggregated lipid droplets might be the reason for reducing the lipid surface areas available for lipase action, resulting in the slower rate of lipid digestion in the filled starch hydrogel.

The effect of xanthan gum on the behaviors of lipid digestion was also examined. Before the experiments were conducted, it was expected that filled hydrogels containing xanthan gum would have a lower FFA release rate than filled hydrogels without xanthan gum because the gel matrices of xanthan-containing hydrogels were further hardened and therefore, difficult to penetrate; however, the results obtained were contradictory. As the concentration of xanthan gum was increased from 0.1 to 2.0 wt%, lipid digestion occurred quickly and final digestion extent was also high. The starch-based filled hydrogels containing 1.0 and 2.0 wt% of xanthan gum had the fastest rate and highest overall extent of lipid digestion (Fig. 2). This result may be attributed to the effect of xanthan gum on strengthening the starch hydrogel network. In the absence of xanthan gum, the treatment of starch hydrogel with α-amylase altered the properties of the starch hydrogel by disrupting the hydrogel matrices and subsequently, the hydrogel matrices could not protect the protein-coated lipid droplets in the gastric phase, when samples moved from the oral to the gastric phase.

Several reasons for the aggregation of the protein-coated lipid droplets in the gastric phase have been reported. First, the changes in pH and ionic strength that occur when lipid droplets reach the gastric phase can weaken the electrostatic repulsion between the droplets. Second, the proteins present on the surface of the lipid droplets can be hydrolyzed by pepsin, and this protein hydrolysis results in the instability of lipid droplets, causing them to aggregate (Sarkar, Goh, Singh, et al., 2009; Singh & Ye, 2013).

The filled hydrogels in the presence and absence of xanthan gum behaved differently within the simulated GIT, including in their reaction to α-amylase in the oral phase. When the filled hydrogels containing xanthan gum moved from the simulated oral phase to the gastric phase, the lipid droplets remained relatively small without aggregation. Also, they were evenly distributed within the hydrogel matrices, even though α-amylase was included in the oral phase (Fig. 3). This result suggested that the starch hydrogel matrices containing xanthan gum surrounding the lipid droplets could still protect the lipid droplets from aggregation in the gastric phase.

As mentioned in Section 3.1, when xanthan gum was added to the starch hydrogels, the gel strength increased. Therefore, the results obtained here suggest that the addition of xanthan gum could minimize the changes to starch hydrogel properties and the disruption of starch hydrogel structures by α-amylase and subsequently, relatively small and evenly distributed lipid droplets could be easily digested by lipase in the simulated small intestinal phase. The rate of lipid digestion by lipase can depend on different factors. The lipid droplet size is one of the important factors because it influences the lipid surface area available for lipase action (Salvia-Trujillo et al., 2013). The rheological properties of the medium and the composition of the droplet surfaces are also important factors (Verrijssen et al., 2015).

3.3. Influence of xanthan gum on β-carotene bioaccessibility

Finally, the bioaccessibility of β-carotene incorporated within filled starch hydrogels containing various concentrations of xanthan gum was examined and the results are shown in Fig. 4. The bioaccessibility is the fraction of compound which is released from the food matrix in the GIT tract and then becomes available for absorption and in general, mixed micelle is considered as a form available for absorption (McClements, Decker, & Park, 2009). It is well known that β-carotene must first be released from the food matrix and then its solubilization into the lipid phase, followed by its subsequent transfer to the mixed micelles, and finally, its uptake by the enterocytes (Yonekura & Nagao, 2007). It has already been demonstrated that the absorption of ingested hydrophobic β-carotene may be improved when β-carotene is co-ingested with lipids

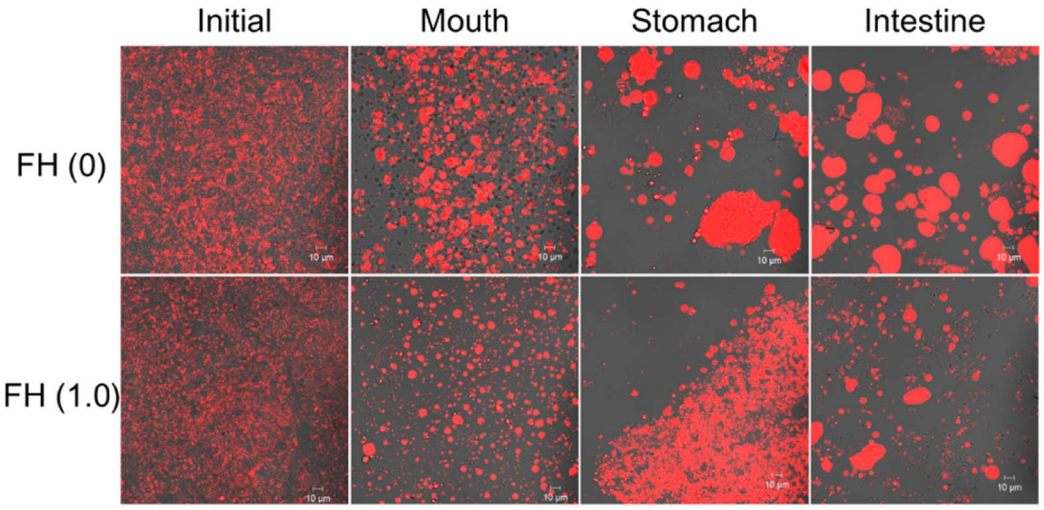

Fig. 3. Microstructures of filled starch hydrogels measured using confocal microscopy. FH (0), filled starch hydrogel prepared without xanthan gum; FH (1.0), filled starch hydrogel prepared with 1.0 wt% of xanthan gum.

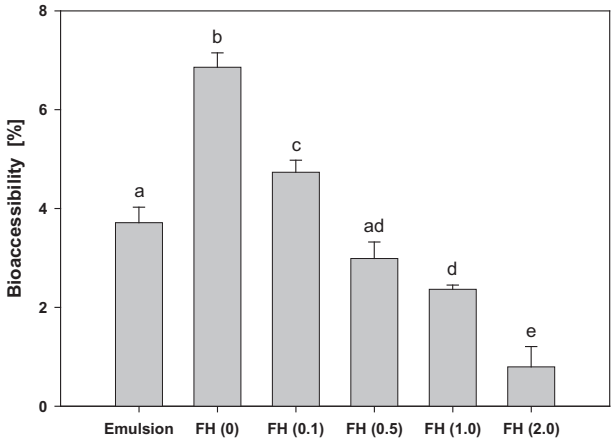

Fig. 4. Effect of xanthan gum on the bioaccessibility (%) of β-carotene incorporated in filled starch hydrogels (FH), after *in vitro* digestion. The number following FH indicates the concentration of added xanthan gum in wt%. Values with superscripted letters inside the figure are significantly different at $P < 0.05$.

because the mixed micelles are composed of bile salt and phospholipids from the bile and pancreatic juices and fat digestion products such as FFAs and monoacylglycerols (Iqbal & Hussain, 2009). Therefore, the amount of β-carotene in the micelle phase produced after completion of *in vitro* digestion process was measured for determining β-carotene bioaccessibility in present study.

The bioaccessibility value of β-carotene determined in this study ranged from 1% to 8%, and this value was lower compared to the bioaccessibility values determined from previous studies (> 10% for WPI-emulsion system) (Mun et al., 2015; Mun et al., 2016). This was possibly due to the fact that the supernatant assumed as the micelle fraction was filtered using a 0.45 μm filter in this study. Large particles (with d > 450 nm) would not be expected to get directly absorbed by epithelial cells (Xia, 2014).

The bioaccessibility of β-carotene was higher when β-carotene was incorporated within the starch hydrogel prepared without xanthan gum in comparison to its incorporation within the emulsion system, even though the emulsion had faster rate of lipid digestion. Previous studies have suggested that the higher bioaccessibility of β-carotene incorporated within the starch hydrogel than that within the emulsion system might be due to the ability of the hydrogel to prevent extensive lipid droplet aggregation, resulting in the easy adsorption of lipase onto the lipid droplet surfaces and the production of more FFAs and monoacylglycerols available for mixed micelle formation. However, in this study, the lipid droplets entrapped within the starch hydrogels were aggregated in the gastric phase due to the α-amylase treatment in the oral phase. This result suggested that other mechanisms might be able to affect the bioaccessibility. The starch hydrogels may have prevented the WPI from interacting with the mixed micelles; however, further study is required to understand this mechanism. Other study have reported that β-carotene can interact with β-lactoglobulin, which is a major constituent of WPI, inducing aggregation and precipitation of mixed micelles (Mensi et al., 2013).

In the presence of xanthan gum, the bioaccessibility of β-carotene reduced as the concentration of xanthan gum in the filled starch hydrogel increased (Fig. 4), although the rate and extent of lipid digestion gradually increased as the gum concentration increased. In the presence of 1.0 and 2.0 wt% of xanthan gum, the final extent of lipid digestion was relatively higher than those from the filled starch hydrogel without xanthan and the emulsion system. Generally, it has been explained that if the final extent of lipid digestion is higher *i.e.*, the amount of FFA released increases, the bioaccessibility of lipophilic bioactive material increases because there are more mixed micelles present to solubilize the bioactive material (Salvia-Trujillo et al., 2013). However, the results obtained in this study indicated that the bioaccessibility of β-carotene within the filled starch hydrogel fabricated with xanthan gum was lowered to various levels depending on the concentration of the xanthan gum. Although the exact mechanism underlying this phenomenon is not entirely clear, the possible reasons for this phenomenon can be suggested based on the mechanisms that have been reported in other studies (Espinal-Ruiz, Parada-Alfonso, Restreopo-Sánchez, Nárvaez-Cuenca, & McClements, 2014). First, the nature of the mixed micelles might have been altered when xanthan gum binds to bile salts, FFAs, or monoacylglycerols. Second, the formation of xanthan gum-gel aggregates might affect β-carotene micellization, and third, the direct binding of xanthan gum to released β-carotene molecules might result in the formation of a dense molecular complex (Mun et al., 2016; Yonekura & Nagao, 2007). Additional study is ongoing to elucidate the mechanisms that lower the bioaccessibility of β-carotene in filled starch hydrogels prepared with xanthan gum.

4. Conclusions

The effects of xanthan gum on the lipid digestion and the bioaccessibility of β-carotene incorporated within a filled starch hydrogel were determined. The rate of lipid digestion was lower when the protein-coated lipid droplets containing β-carotene were entrapped within the starch hydrogels, which can be attributed to the action of α-amylase in the oral phase. Amylase changed the rheological properties of the filled starch hydrogel, resulting in the loss of its ability to protect the extensive aggregation of lipid droplets that formed in the gastric phase.

However, when xanthan gum was added to the filled starch hydrogels, lipid digestion rate and extent increased as the xanthan gum concentration increased. This might be due to the ability of xanthan gum to reinforce the structure of the gel network *i.e.*, xanthan gum possibly reconstructs the partially disrupted starch gel network and consequently, a gel network consisting of xanthan gum and starch gel could protect the protein-coated lipid droplets from being attacked by pepsin, and changes in environmental stresses, which are experienced in the gastric phase. β-Carotene bioaccessibility of filled hydrogels containing xanthan gum decreased, even though the rate and final extent of lipid digestion of the filled starch hydrogel prepared with xanthan gum were higher than those of the emulsion system and filled starch hydrogels without xanthan gum, indicating that more FFAs and monoacylglycerols were formed in the small intestinal phase. This may be attributed to the reaction of xanthan gum with specific components in the GIT or formation of xanthan gel aggregates. Taken together, the results of this study can provide useful information for designing functional foods in the food and nutraceutical industries.

Acknowledgments

This work was supported by Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries (IPET) through High value-added Food Technology Development Program (Project No. 315065-3), funded by Ministry of Agriculture, Food and Rural Affairs (MAFRA), Republic of Korea.

References

Abdulmola, N. A., Hember, M. W. N., Richardson, R. K., & Morris, E. R. (1996). Effect of xanthan on the small-deformation rheology of crosslinked and uncrosslinked waxy maize starch. *Carbohydrate Polymers, 31*, 65–78.

BeMiller, J. N. (2011). Pasting, paste, and gel properties of starch–hydrocolloid combinations. *Carbohydrate Polymers, 86*, 386–423.

Choi, H. M., & Yoo, B. (2009). Steady and dynamic shear rheology of sweet potato starch-xanthan gum mixtures. *Food Chemistry, 116*, 638–643.

Conde-Petit, B., Pfirter, A., & Escher, F. (1997). Influence of xanthan on the rheological properties of aqueous starch–emulsifier systems. *Food Hydrocolloids, 11*, 393–399.

Espinal-Ruiz, M., Parada-Alfonso, F., Restreopo-Sánchez, L.-P., Nárvaez-Cuenca, C.-E., & McClements, D. J. (2014). Interaction of a dietary fiber (pectin) with gastrointestinal components (bile salts, calcium, and lipase): A calorimetry, electrophoresis, and turbidity study. *Journal of Agricultural and Food Chemistry, 62*, 12620–12630.

Garcia-Ochoa, F., Santos, V. E., Casas, J. A., & Gomez, E. (2000). Xanthan gum: Production, recovery, and properties. *Biotechnology Advances, 18*, 549–579.

Granger, D. A., Kivlighan, K. T., El-Sheikh, M., Gordis, E. B., & Stroud, L. R. (2007). Salivary α-amylase in biobehavioral research. *Annals of the New York Academy of Sciences, 1098*, 122–144.

Iqbal, J., & Hussain, M. M. (2009). Intestinal lipid absorption. *American Journal of Physiology Endocrinology and Metabolism, 296*, E1183–E1194.

Kim, J. M., Song, J. Y., & Shin, M. (2010). Physicochemical properties of high amylose rice starches purified from Korean cultivars. *Starch, 62*, 262–268.

Kim, C., & Yoo, B. (2006). Rheological properties of rice starch-xanthan gum mixtures. *Journal of Food Engineering, 75*, 120–128.

Lopez-Pena, C. L., Zheng, B., Sela, D. A., Decker, E. A., Xiao, H., & McClements, D. J. (2016). Impact of ε-polylysine and pectin on the potential gastrointestinal fate of emulsified lipids: In vitro mouth, stomach and small intestine model. *Food Chemistry, 192*, 857–864.

Maiani, G., Caston, M. J. P., Catasta, G., Toti, E., Cambrodon, I. G., Bysted, A., et al. (2009). Carotenoids: Actual knowledge on food sources, intakes, stability and bioavailability and their protective role in humans. *Molecular Nutrition & Food Research, 53*, S194–S218.

Mandala, I. G., & Bayas, E. (2004). Xanthan effect on swelling, solubility and viscosity of wheat starch dispersions. *Food Hydrocolloids, 18*, 191–201.

Matalanis, A., Jones, O. G., & McClements, D. J. (2011). Structured biopolymer-based delivery systems for encapsulation, protection, and release of lipophilic compounds. *Food Hydrocolloids, 25*(8), 1865–1880.

Matalanis, A., & McClement, D. J. (2012). Impact of encapsulation within hydrogel microspheres on lipid digestion: An in vitro study. *Food Biophysics, 7*, 145–154.

McClements, D. J., Decker, E. A., & Park, Y. (2009). Controlling lipid bioavailability through physicochemical and structural approaches. *Critical Reviews in Food Science and Nutrition, 49*, 48–67.

Mensi, A., Choiset, Y., Haertle, T., Reboul, E., Borel, P., Guyon, C., ... Chobert, J. M. (2013). Interlocking of β–carotene in beta-lactoglobulin aggregate produced under high pressure. *Food Chemistry, 139*, 253–260.

Mun, S. H., Kim, Y. R., & McClements, D. J. (2015). Control of β–carotene bioaccessibility using starch-based filled hydrogels. *Food Chemistry, 173*, 454–461.

Mun, S. H., Kim, Y. R., Shin, M. S., & McClements, D. J. (2015). Control of lipid digestion and nutraceutical bioaccessibility using starch-based filled hydrogels: Influence of starch and surface type. *Food Hydrocolloids, 44*, 380–389.

Mun, S. H., Park, S. J., Kim, Y. R., & McClements, D. J. (2016). Influence of methylcellulose on attributes of β–carotene fortified starch-based filled hydrogels: Optical, rheological, structural, digestibility, and bioaccessibility properties. *Food Research International, 87*, 18–24.

Pongsawatmanit, R., & Srijunthongsiri, S. (2008). Influence of xanthan gum on rheological properties and freeze-thaw stability of tapioca starch. *Journal of Food Engineering, 88*, 137–143.

Qian, C., Decker, E. A., Xiao, H., & McClements, D. J. (2012). Nanoemulsion delivery systems: Influence of carrier oil on β–carotene bioaccessibility. *Food Chemistry, 135*(3), 1440–1447.

Rao, A. V., & Rao, L. G. (2007). Carotenoids and human health. *Pharmacological Research, 55*(3), 207–216.

Russ, N., Zielbauer, B. I., Ghebremedhin, M., & Vilgis, T. A. (2016). Pre-gelatinized tapioca starch and its mixtures with xanthan gum and ι–carageenan. *Food Hydrocolloids, 56*, 180–188.

Salvia-Trujillo, L., & McClements, D. J. (2016). Enhancement of lycopene bioaccessibility from tomato juice using excipient emulsions: Influence of lipid droplet size. *Food Chemistry, 210*, 295–304.

Salvia-Trujillo, L., Qian, C., Martin-Belloso, O., & McClements, D. J. (2013). Influence of particle size on lipid digestion and β-carotene bioaccessibility in emulsions and nanoemulsions. *Food Chemistry, 141*, 1472–1480.

Sarkar, A., Goh, K. K. T., & Singh, H. (2009). Colloidal stability and interactions of milk-protein-stabilized emulsions in an artificial saliva. *Food Hydrocolloids, 23*(5), 1270–1278.

Sarkar, A., Goh, K. K. T., Singh, R. P., & Singh, H. (2009). Behaviour of an oil-in-water emulsion stabilized by β-lactoglobulin in an in vitro gastric model. *Food Hydrocolloids, 3*(6), 1563–1569.

Singh, H., & Ye, A. (2013). Structural and biochemical factors affecting the digestion of protein-stabilized emulsions. *Current Opinion in Colloid & Interface Science, 18*, 360–370.

Verrijssen, T. A. J., Smeets, K. H. G., Christiaens, S., Palmers, S., Van Loey, A. M., & Hendrickx, M. E. (2015). Relation between in vitro lipid digestion and β-carotene bioaccessibility in β-carotene-enriched emulsions with different concentrations of L-α-phosphatidylcholine. *Food Research International, 67*, 60–66.

Viturawong, Y., Achayuthakan, P., & Suphantharika, M. (2008). Gelatinization and rheological properties of rice starch/xanthan mixtures: Effects of molecular weight of xanthan and different salts. *Food Chemistry, 111*, 106–114.

Wang, B., Wang, L.-J., Li, D., Őzkan, N., Li, S.-J., & Mao, Z.-H. (2009). Rheological properties of waxy maize starch and xanthan gum mixtures in the presence of sucrose. *Carbohydrate Polymers, 77*, 472–481.

Xia, Z. (2014). *Influence of physical states (crystalized versus solubilized) of bioactive components and oil composition on bioaccessibility and bioavailability. Masters Theses May 2014 - Current. Paper 53*.

Yonekura, L., & Nagao, A. (2007). Intestinal absorption of dietary carotenoids. *Molecular Nutrition & Food Research, 51*, 107–115.

Zhang, R., & McClements, D. J. (2016). Enhancing nutraceutical bioavailability by controlling the composition and structure of gastrointestinal contents: Emulsion-based delivery and excipient systems. *Food Structure, 10*, 21–36.