1 **Protein encapsulation in alginate hydrogel beads:**
2 **Effect of pH on microgel stability, protein retention**
3 **and protein release**

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Abstract

Hydrogel beads (microgels) are promising delivery systems for encapsulation and release of proteins because they can be fabricated from food-grade biopolymers using mild processes. In this study, a model globular protein (whey protein) was encapsulated in calcium-alginate beads ($D_{43} = 290$ to $520\ \mu\text{m}$) fabricated using an extrusion device with a vibrating nozzle. Protein-loaded beads were fabricated at three different pH values (pH 3, 5 and 7) to study the influence of protein-alginate electrostatic interactions on protein encapsulation, retention, and release. Protein encapsulation and retention was highest at low pH, while protein release was highest at high pH. Confocal microscopy and spectrophotometry measurements indicated that increasing the pH could trigger protein release from alginate beads formed at pH 3. These results suggest that hydrogel beads are suitable for encapsulation and pH-triggered release of proteins, which may be advantageous for certain food applications.

Keywords: alginate; hydrogel beads; whey protein; pH conditions; release fraction

1. Introduction

Colloidal delivery systems are finding increasing application for the encapsulation, protection, and release of bioactive agents in the pharmaceutical, supplement, and food industries (Kesisoglou, Panmai, & Wu, 2007; McClements, 2015; Oehlke et al., 2014). Each type of bioactive agent has its own unique molecular and physicochemical properties, which means that delivery systems typically have to be specifically designed for each application. Proteins and peptides are commonly used as bioactive agents because they exhibit a range of biological activities, including nutritional, antimicrobial, antioxidant, flavor, anti-hypertension, anti-diabetic, and anticancer (Chen, Remondetto, & Subirade, 2006; Moller, Scholz-Ahrens, Roos, & Schrezenmeir, 2008; Samaranayaka & Li-Chan, 2011; Udenigwe & Aluko, 2012). Nevertheless, the incorporation of these bioactive proteins into food products is often challenging because of their sensitivity to chemical or biochemical degradation, their susceptibility to aggregation, and their potential for causing off-flavors (such as bitterness or astringency). For this reason, there has been considerable interest in encapsulating proteins within polymer particles to protect and release them in different environments (Castellanos, Flores, & Griebenow, 2006; Chang & Lin, 2000; Cheng, Liu, & He, 2010; Xie, Ng, Lee, & Wang, 2008). Studies have shown that proteins encapsulated within polymer matrices may be protected from unfolding and aggregation (Bhatia, Brinker, Gupta, & Singh, 2000). Moreover, these protein-loaded polymer particles can be designed to carry bioactive proteins to specific locations within the gastrointestinal tract (GIT) and release them at a controlled rate or in response to a particular trigger (Chen et al., 2006). Consequently, the functional attributes and biological activity of proteins can be enhanced by encapsulating them within microgels (Cheng et al., 2010; Gombotz & Wee, 2012).

Many different approaches have been developed to fabricate hydrogel beads suitable for protein encapsulation, including extrusion, emulsion, molding, and phase separation methods (Castellanos, Crespo, & Griebenow, 2003; Joye & McClements,

2014; Kim & Morr, 1996; Ribeiro, Hussain, & Florence, 2005). For certain applications, it is important that the fabrication method used does not promote unfolding or aggregation of the encapsulated proteins, as this may lead to a loss of their functional attributes or biological activity. Hydrogel beads fabricated from biopolymers (proteins and/or polysaccharides) are particularly suitable for the encapsulation of bioactive proteins because they often involve mild preparation conditions that do not alter protein properties, and can be prepared using food-grade ingredients (Chen et al., 2006; Joye & McClements, 2014; Shewan & Stokes, 2013). The extrusion-gelation method is one of the most commonly used and effective methods of producing hydrogel beads (Gombotz & Wee, 2012). In this method, a biopolymer solution containing the bioactive protein is injected into another “hardening” solution that promotes biopolymer gelation. This procedure results in the formation of hydrogel beads with bioactive proteins trapped inside a biopolymer matrix.

When designing a suitable hydrogel bead for this type of application it is important that it has good encapsulation, retention, and release properties (Cheng et al., 2010; Shewan & Stokes, 2013; Zhang, Zhang, Chen, Tong, & McClements, 2015). There are a number of potential strategies to control the retention and release properties of hydrogel beads (Zhang et al., 2015). First, the pore size of the hydrogel matrix can be controlled by manipulating the amount of biopolymer and cross-linking agent used: larger pores typically lead to faster release. Second, the overall dimensions of the hydrogel beads can be controlled using different fabrication methods or preparation conditions: smaller beads typically lead to faster release. Third, specific interactions between the bioactive agents and biopolymer molecules in the hydrogel matrix can be utilized, *e.g.*, hydrophobic, hydrogen bonding, or electrostatic interactions. Fourth, the hydrogel beads can be designed to dissociate upon exposure to specific environmental conditions, thereby releasing their payload. Fifth, the hydrogel beads can be coated with layers of biopolymers or other materials to alter their permeability.

The mechanism that is important for a particular delivery system depends on its composition and structure.

In the current study, we investigated the utilization of alginate as a building block for creating protein-loaded hydrogel beads that could release encapsulated proteins in response to pH changes. Alginate is a naturally occurring anionic polymer isolated from brown algae that has the ability to form strong hydrogels (Lee & Mooney, 2012). Protein-loaded alginate beads can be fabricated by extruding a solution of sodium alginate containing the bioactive protein into a divalent crosslinking solution, such as one containing Ca^{2+} ions (Gombotz & Wee, 2012). Biopolymer gelation primarily occurs due to exchange of Na^+ ions from carboxylic acids on the sodium alginate molecules with Ca^{2+} ions from the crosslinking solution, which leads to the formation of cross-links with a characteristic “egg-box” structure (Chan, 2011; Lertsutthiwong, Noomun, Jongaroonngamsang, Rojsitthisak, & Nimmannit, 2008; Ribeiro, Neufeld, Arnaud, & Chaumeil, 1999). The internal environment of alginate hydrogels has been shown to have little impact on the functional properties of many globular proteins (Gombotz & Wee, 2012). In addition, proteins can be retained within alginate beads that have sufficiently small pore sizes and/or specific attractive interactions between the proteins and alginate molecules (Li, Hu, Du, Xiao, & McClements, 2011). However, the proteins can be released when the system conditions are changed to increase the pore size and/or reduce the attractive interactions.

In the present study, whey protein was incorporated into hydrogel beads fabricated from calcium alginate using an automated extrusion device with a small vibrating nozzle. We hypothesized that the retention and release of the protein molecules would depend on their electrical characteristics because this would influence their interactions with the alginate molecules in the hydrogel matrix. The solution pH was therefore altered relative to the isoelectric point (pI) of the whey proteins so as to obtain highly positive (pH 3), approximately neutral (pH 5) and highly negative (pH 7)

proteins. The influence of pH on the encapsulation, retention and release of the proteins was then measured. The information obtained from this study may be useful for the rational design of more effective delivery systems for bioactive proteins in the food and other industries.

2. Materials and methods

2.1 Materials

Whey protein isolate (WPI) was kindly provided by Davisco Foods International Inc. (Le Sueur MN). The company reported that the WPI contained 97.9 wt.% protein and 0.2 wt.% fat. Alginic acid (sodium salt), calcium chloride dihydrate ($\text{CaCl}_2 \cdot \text{H}_2\text{O}$) and fluorescein isothiocyanate (FITC) isomer I was purchased from the Sigma Chemical Company (St. Louis, MO). Double distilled water was used to make all solutions.

2.2. Methods

2.2.1. ζ -potential measurements

ζ -potential *versus* pH profiles of WPI and alginate solutions was measured using a commercial micro-electrophoresis instrument (Zetasizer Nano ZA series, Malvern Instruments Ltd. Worcestershire, UK). Samples were diluted using 10 mM phosphate buffer (at the same pH as the sample) prior to analysis to keep the instrument attenuation value between 5 and 10.

2.2.3. Turbidity measurements

Turbidity *versus* pH profiles of WPI or WPI-alginate solutions was determined using a UV–visible spectrophotometer at 600 nm (Ultrospec 3000 pro, Biochrom Ltd., Cambridge, UK). The samples were contained within 1 cm path length optical cells, and phosphate buffer (PBS) was used as a control. Turbidity measurements were carried out on at least two freshly prepared samples.

2.2.4 Encapsulation of WPI in alginate beads

An aqueous alginate (2% w/v) solution was prepared by dissolving the powdered ingredient in distilled water, stirring at 60 °C for an hour, and then reducing the temperature to 35 °C. The WPI (2% w/v) and alginate (2% w/v) solutions were then mixed together (1:1 v/v) for 1 h with continuous stirring to form a uniform solution. The mixed alginate and WPI solutions were then adjusted to different pH values (pH 3, 5, or 7) prior to bead formation. In addition, the calcium chloride solutions used to induce gelation were also adjusted to the corresponding pH values (pH 3, 5, or 7). WPI-loaded hydrogel beads were then prepared using a commercial encapsulation unit (Encapsulator B-390, BUCHI, Switzerland) by injecting the WPI/alginate solutions (pH 3, 5, or 7) into the corresponding 10% calcium chloride solutions (pH 3, 5, or 7) with continuous stirring. The hydrogel beads were allowed to crosslink with Ca^{2+} for 1 h at ambient temperature. The hardened beads were then collected by filtration and subsequently washed with distilled water and buffer solution to remove any excess Ca^{2+} from their surfaces.

2.2.5 Particle size measurements

The mean particle diameter and particle size distribution of biopolymer mixtures (WPI and alginate) and hydrogel beads formed at different pH values (3, 5 and 7) were determined using static light scattering (Mastersizer 2000, Malvern Instruments, Worcestershire, United Kingdom). This instrument infers the size of the particles from measurements of their angular scattering pattern. Samples were diluted in 10 mM PBS buffer (pH 3, 5 and 7) by adding small aliquots into a measurement chamber.

2.2.6 Encapsulation efficiency and protein release

The ability of hydrogel beads formed at different pH values (pH 3, 5, and 7) to retain the protein was determined by immersing them within the corresponding phosphate buffer solutions (pH 3, 5 and 7) at room temperature. The concentration of

protein in the surrounding aqueous phase was then measured at various time intervals by recording the absorbance at 280 nm using the UV-visible spectrophotometer. The encapsulation efficiency (%) was determined by dividing the amount of protein remaining in the beads by the initial protein in the beads. The amount of protein in the beads was taken to be the difference between the initial protein and that released into the phosphate buffer. In some cases, the amount of protein released was determined for protein-loaded hydrogel beads formed at pH 3, and then incubated in solutions at higher pH values for 10 min.

2.2.7 Microstructure analysis

The microstructure of hydrogel beads formed at different pH values (pH 3, 5 and 7) was recorded, as well as the impact of pH on protein release from alginate-based beads. The microstructure of the protein-loaded alginate beads was examined using confocal scanning laser microscopy with a 20 × objective lens (Nikon D-Eclipse C1 80i, Nikon, Melville, NY, U.S.). The proteins were dyed prior to hydrogel bead formed by adding 0.1 mL of FITC dye solution (1 mg FITC /mL dimethyl sulfoxide) to 2 mL of sample and then storing at 5 °C overnight. The excitation and emission wavelength used for FITC were 488 nm and 515 nm, respectively. A relatively high gain value was used to observe the distribution of protein inside and outside of the hydrogel beads formed at different pH values, but a lower gain value was used to study the release of the proteins from the hydrogel beads after pH adjustment. Consequently, these images cannot be directly compared. The microstructure images for confocal microscopy were analyzed using image analysis software (NIS-Elements, Nikon, Melville, NY).

2.3. Statistical analysis

All experiments were carried out in triplicate using freshly prepared samples. Means and standard deviations were calculated from a minimum of three measurements using Excel (Microsoft, Redmond, VA, USA).

3 Results and discussion

3.1 Electrical characteristics of biopolymer molecules

An encapsulated protein can electrically interact with the charged biopolymer molecules used to fabricate the hydrogel beads and thereby influence its encapsulation, retention, and release properties. For this reason, the pH dependence of the ζ -potential of WPI and alginate solutions was determined using an electrophoresis method. The ζ -potential of the WPI solution went from negative at pH 7.0 (≈ -26 mV) to positive at pH 2.0 ($\approx +23$ mV), with a point of zero charge around pH 4.7 (**Fig. 1**), which can be attributed to changes in the protonation of acid ($-\text{COOH}$) and basic ($-\text{NH}_2$) groups on the protein molecules with pH. The ζ -potential of the alginate solution remained negative across the whole pH range studied (from pH 2 to 7), changing from strongly negative (≈ -68 mV) at pH 7 to slightly negative (≈ -10 mV) at pH 2. The magnitude of the negative charge on the alginate molecules was lower under acidic conditions than neutral conditions due to partial protonation of the carboxylic acid groups on the mannuronic and guluronic acid groups: $-\text{COO}^- \Rightarrow -\text{COOH}$ ($\text{pK} \approx 3.5$) (Lee & Mooney, 2012). The pH dependence of the ζ -potential of mixed WPI-alginate solutions was also measured by electrophoresis to obtain information about the interactions between the two biopolymers (**Fig. 1**). At relatively high pH values ($\text{pH} > 5.5$), the ζ -potential of the mixed system was between that of the two individual biopolymers, which suggests that both biopolymers contributed to the overall ζ -potential. At lower pH values, the ζ -potential of the mixed system tended towards that of the alginate molecules, which suggests that an electrostatic complex was formed between the two biopolymers whose charge characteristics were dominated by the presence of the anionic alginate molecules.

Based on the electrical characteristics of the two individual biopolymers (**Fig.1**), it would be expected that alginate and whey protein should be attracted to each other at low-to-intermediate pH ($\text{pH} < 4.7$) where they have opposite charges, but repel each other at high pH ($\text{pH} > 4.7$) where they have similar charges (negative charge).

However, the ζ -potential and turbidity measurements on the mixed systems suggest that the two biopolymers become associated at higher pH values (pH 5.5) even though they have similar charges (**Figs. 1 and 2**), which can be attributed to the binding of anionic groups on the alginate molecules to cationic patches on the surfaces of the protein molecules (Kayitmazer, Seeman, Minsky, Dubin, & Xu, 2013). Based on these measurements, we anticipate that whey proteins would be retained by the beads at relatively low pH values due to electrostatic attraction, but be released at high pH values due to electrostatic repulsion.

3.2 Turbidity characterization of mixed biopolymer systems

The formation of electrostatic complexes in biopolymer solutions can simply be monitored using turbidity measurements (Tsuboi et al., 1996). In this section, the turbidity *versus* pH profiles of the alginate solution, WPI solution, and WPI-alginate mixture were measured as a function of pH using a spectrophotometric method. The alginate solution had a very low turbidity at all pH values (data not shown), indicating that the alginate molecules did not aggregate. As expected, there was a large increase in the turbidity of the WPI solution around the protein's isoelectric point ($pI \approx 4.5$) due to protein aggregation associated with the low electrostatic repulsion between the whey protein molecules (Fig. 2). For the alginate-WPI mixtures, the solution turbidity was relatively low at high pH ($5 < pH < 7$) due to the high solubility of the individual protein and alginate molecules, and the fact that there would be a strong electrostatic repulsion between the two types of biopolymer in this pH range. When the pH was reduced further, there was a gradual increase in turbidity from pH 5 to 3, followed by a steep increase from pH 3 to a maximum value at pH 2. This result suggests that electrostatic complexes large enough to scatter light strongly were formed at pH values below about 4. As mentioned earlier, small electrostatic complexes may also be formed under conditions (pH 4.0 to 5.5) where both the WPI and alginate molecules have net negative charges due to electrostatic attraction between anionic groups on the polysaccharide and cationic patches on the protein surfaces (Chung, Degner, Decker, & McClements,

2013; da Silva & Jönsson, 2009; Kayitmazer et al., 2013). As reported in these studies, the presence of an anionic polysaccharide can suppress the aggregation of globular proteins that normally occurs around their isoelectric point. This effect can be attributed to the fact that the protein and polysaccharide molecules form an electrostatic complex, and there is a relatively strong electrostatic and steric repulsion between the complexes. In summary, these results have highlighted the fact that the alginate and whey protein molecules should bind strongly to each other at relatively low pH values due to electrostatic attraction.

3.3 Particle size characterization of polymer mixtures at different pH

Three different pH values were selected for further study based on the expected differences in the electrostatic interactions between the whey protein and alginate molecules: electrostatic repulsion at pH 7; soluble complex formation at pH 5; and, insoluble complex formation at pH 3. The particle size distributions of biopolymer mixtures containing alginate and WPI at a ratio of 1:1 (w/w) were measured at different pH values (**Fig. 3**). At pH 7, the biopolymer mixtures appeared optically transparent, which suggested that they did not contain particles large enough to scatter light appreciably, consequently reliable light scattering measurements could not be made. At pH 5, the particle size distribution of the biopolymer mixtures was bimodal, suggesting there was a wide range of different-sized colloidal particles present. These particles were presumably held together by a weak electrostatic attraction between the whey protein and alginate molecules. Visually, these samples had a turbid appearance, which again indicated the formation of relatively large complexes in these samples. At pH 3, there was a monomodal distribution of relatively large particles present ($d \approx 500 \mu\text{m}$), which formed due to the strong electrostatic attraction between the cationic whey protein and anionic alginate molecules. The colloidal dispersions formed in the mixed systems at pH 3 had a higher lightness (white color) than those formed at pH 5, which suggests that the former particles scattered light more strongly. This may have occurred because the biopolymer molecules were packed more tightly together in the

complexes at the lower pH value (Kayitmazer et al., 2013), which led to a higher refractive index contrast.

3.4 Hydrogel beads formed at different pH

Relatively large alginate beads ($d > 1$ mm) are typically formed using the most commonly used extrusion method, which involves simply injecting an alginate solution into a calcium bath using a syringe or pipette (Elçin, 1995; Wee & Gombotz, 1994; Zeeb, Saberi, Weiss, & McClements, 2015). This bead size is too large for many commercial applications since it adversely impacts the physicochemical or sensory properties of the products they are incorporated into. In this study, we therefore fabricated relatively small alginate beads ($d < 500$ μm) using a specially designed extrusion device (Encapsulator) that uses a vibrating 120- μm nozzle and a syringe pump. These smaller beads may have several advantages over larger ones in terms of their impact on product appearance, rheology, mouthfeel and stability. On the other hand, smaller beads may have lower retention and higher release rates than large ones because of the reduced diffusion path of the encapsulated agents through the hydrogel matrix (Li et al., 2011). It is therefore important to design alginate beads so that they satisfy the different physicochemical requirements for each application.

The influence of fabrication pH on the properties of alginate beads was investigated by injecting WPI-alginate mixtures into Ca^{2+} solutions at pH 3, 5, and 7. As mentioned earlier, these three pH values were selected because they led to different electrostatic interactions between the protein and alginate molecules. The light scattering measurements indicated that the particle size distribution and mean diameter of the hydrogel beads was influenced by the fabrication pH: $d_{43} = 288, 471$, and 516 μm at pH 7, 5 and 3, respectively (**Fig. 4**). There are a number of factors that may contribute to the influence of pH on bead dimensions. First, the negative charge on the alginate molecules decreases with decreasing pH (**Fig. 1**), which may reduce the number of anionic groups on the alginate molecules that are available for cross-linking with calcium ions. Second, the charge on the protein molecules varied from negative

to positive with decreasing pH (**Fig. 1**), which will have altered any interactions between the protein and alginate molecules. Again, these interactions may also have interfered with the ability of the calcium ions to cross-link the alginate molecules. As a result of these effects, the kinetics of alginate gelation, as well as the nature of the gel network formed, may have been changed, which altered the dimensions of the beads formed.

The appearance of the suspensions of hydrogel beads changed from translucent to a whitish color when the fabrication pH was reduced from 7 to 3 (**Fig. 4**). In addition, there appeared to be some visible clumping of the particles at lower pH values. This result suggests that there may have been some aggregation of the protein-loaded alginate beads, which may have been due to a reduction in the electrostatic repulsion between the beads at lower pH values.

The protein encapsulation efficiency of the alginate beads depended on the pH used to fabricate them: pH 7 (11.6%) < pH 5 (19.1%) < pH 3 (58.6%) (**Fig. 5**). In addition, the confocal fluorescence microscopy images of the hydrogel beads indicated that the protein concentration (fluorescence intensity) within them was higher at the lowest fabrication pH (**Fig. 5**). This trend is related to the strength of the electrostatic interactions between the protein and alginate molecules. At pH 5 and 7, there will be a strong electrostatic repulsion between the anionic protein and anionic alginate molecules (**Fig. 1**), and therefore the protein molecules may not have been effectively trapped within the alginate beads during their formation. As mentioned earlier, at pH 5 alginate and protein molecules may bind together due to the attraction of anionic groups on the alginate molecules to cationic patches on the protein surfaces (even though both have a net negative charge). However, the attractive alginate-protein interactions may have been much weaker than the alginate-calcium interactions, and so the protein was released when the alginate solution was titrated into the calcium solution. At pH 3, there will have been a strong electrostatic attraction between the

cationic protein and anionic alginate molecules (**Fig. 1**), and therefore the two biopolymers may be held together more strongly during the cross-linking process. Consequently, the protein molecules are retained within the alginate beads during their fabrication at low pH values. It should also be noted that the pores within the alginate beads must have been sufficiently large to allow the protein molecules to diffuse through them (Gombotz & Wee, 2012). The size of the beads observed by confocal microscopy was inconsistent with that determined by light scattering measurements (Fig. 4). This was probably because the confocal images show contain only a single bead that is not representative of the full particle size distribution. Nevertheless, the confocal images do still provide some useful insights into the overall morphology of the beads at different pH values.

3.5 Protein release during storage

In this section, the rate of protein release from the alginate beads formed at different fabrication pH values was compared. At pH 5 and 7, there was a relatively rapid release of protein during the first 30 min of storage, followed by a more gradual release at longer storage times (**Fig. 6**). On the other hand, at pH 3, the release of protein occurred relatively slowly throughout the 6-hour storage period. The final amount of protein released at the end of the incubation period depended strongly on pH: pH 3 (24%) < pH 5 (78.9%) < pH 7 (95.3%). As discussed in the previous section, this effect can be attributed to differences in the electrostatic interactions of the alginate and protein molecules at different pH values. At pH 3, the protein and alginate have opposite charges and are strongly attracted to each other, and so the protein is better retained within the hydrogel beads. Conversely, at pH 5 and 7 the protein and alginate have similar charges and therefore tend to electrostatically repel each other, so the protein tends to leach out of the hydrogel beads. A similar effect has also been observed with electrically charged drugs trapped within alginate beads. For example, it has been reported that a cationic drug (chlorpheniramine maleate) showed a much slower release rate from alginate gels than an anionic drug (sodium salicylate) (Mumper,

Huffman, Puolakkainen, Bouchard, & Gombotz, 1994; Segi, Yotsuyanagi, & Ikeda, 1989).

The pH dependence of the protein release rate may also depend on changes in the structure of the alginate beads under different solution conditions. It was reported that calcium alginate beads tend to shrink at low pH due to the loss of negative charge on the alginate molecules when the carboxyl groups become protonated ($-\text{COOH}$, $\text{p}K_{\text{a}} \approx 3.5$) (Li et al., 2011). Conversely, they tend to swell when placed in higher pH solutions due to the fact that the alginate molecules become highly charged and repel each other (Zhang et al., 2015). Thus, the faster release of the protein molecules at pH 5 and 7 may have been partly due to the fact that the hydrogel network had larger pores at these higher pH values. Nevertheless, further work is required to measure the pore size of the alginate beads to confirm the importance of this mechanism, *e.g.*, using electron microscopy or size exclusion methods (Gombotz & Wee, 2012).

3.6 pH-triggered protein release from hydrogel beads

The change in the electrical properties of the alginate and protein molecules with pH means that protein-loaded alginate beads may be designed to release the proteins in response to a pH trigger (Zhang et al., 2015). In this section, protein-loaded alginate beads were initially fabricated at pH 3 since they had a high loading capacity and good retention properties (Sections 3.4 and 4.5). The protein release from these hydrogel beads was then measured after they were stored at different pH values for 10 min. The storage pH of the beads had a pronounced impact on the amount of protein released, with the amount released increasing with increasing pH (**Fig. 7b**). For example, around 11% protein was released from the hydrogel beads after incubation at pH 3.5, whereas around 90% protein was released when they were incubated at pH 7. These results were confirmed by confocal fluorescence microscopy images of the hydrogel beads, which showed that the fluorescence intensity (protein) of the hydrogel beads decreased with increasing pH (**Fig. 7a**), which suggests that some of the whey protein

molecules had diffused out of the hydrogel beads at higher pH values. These results suggest that protein-loaded alginate beads could be fabricated at relatively low pH values (pH 3) to effectively retain the protein, and then the protein could be released by increasing the pH of the hydrogel beads environment. This type of pH-triggered hydrogel bead may be a useful tool for protection of proteins in the acidic environment of the stomach, and then releasing them in the neutral environment of the small intestine. If required, the initial hydrogel beads could be coated with extra layers of biopolymers (*e.g.*, chitosan or polylysine) using an electrostatic deposition approach to modulate their functional properties, such as stability, integrity, and permeability (Gombotz & Wee, 2012).

4. Conclusions

The effect of pH on the encapsulation, retention and release of whey proteins from alginate-based hydrogel beads was studied. Different pH values (pH 3, 5 and 7) were chosen to fabricate the hydrogel beads based on differences in the nature of the electrostatic interactions (attraction or repulsion) between the alginate and protein molecules. Protein-loaded hydrogel beads were successfully prepared using an encapsulation unit with a small vibrating nozzle to inject the whey protein/alginate mixture into a Ca^{2+} solution. The protein encapsulation efficiency and retention of the beads increased with decreasing fabrication pH ($\text{pH } 7 < \text{pH } 5 < \text{pH } 3$), which was attributed to the fact that there was a strong electrostatic attraction between the cationic protein and anionic alginate molecules at pH 3. Consequently, the protein molecules were held in the alginate beads more strongly. The hydrogel beads prepared in this study could be designed to release the protein molecules in response to a pH trigger. The amount of protein released increased as the pH of the surrounding solution was increased from pH 3 to pH 7, which was again attributed mainly to a weakening of the electrostatic attraction between the encapsulated protein molecules and the alginate hydrogel with increasing pH. These results have important implications for designing

hydrogel beads to encapsulate, retain, and release of proteins in food products and within the gastrointestinal tract.

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“Protein encapsulation in alginate hydrogel beads: Effect of pH on microgel stability, protein retention and protein release”

Zhang et al

Food Hydrocolloids

Graphical Abstract

Confocal images and protein encapsulation efficiency of hydrogel beads formed at different pH.

Figure 1. Dependence of the ζ -potential on pH for 0.1 wt% WPI solution, 0.1 wt% sodium alginate solution, and 0.1% WPI + 0.1 wt% sodium alginate solution (10 mM phosphate buffer).

Figure 2. Turbidity (at 600 nm) as a function of pH for aqueous solutions containing either 0.1% WPI and 0.1% sodium alginate or 0.1% WPI only.

Figure 3. Particle size distribution and appearance of biopolymer mixtures containing alginate and WPI (1:1 w/w) at pH 3 and 5. The solutions were transparent at pH 7, and therefore reliable particle size measurements could not be made.

Figure 4. Particle size distribution and appearance images of hydrogel beads formed at different pH (pH 3, 5 and 7).

Figure 5. Confocal images and protein encapsulation efficiency of hydrogel beads formed at different pH (pH 3, 5 and 7). The WPI (green) was stained with FITC.

Figure 6. Leakage of WPI from hydrogel beads formed at different pH as a function of incubation time. The beads were immersed into the according PBS buffer (pH 3, 5 and 7) at room temperature.

Figure 7. Protein release characterization from the hydrogel beads by changing pH from the initial condition (pH 3): (a) confocal images of hydrogel beads at initial and final pH. (b) WPI release fraction (%) after changing pH condition.

FIGURES

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