1 **Development of hydrocolloid microgels as starch**
2 **granule mimetics: Hydrogel particles fabricated**
3 **from gelatin and pectin**

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12 **Journal:** Food Research International

13 **Submitted:** August 2015

14 **Revised:**

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Abstract

In this study, hydrocolloid microgels fabricated by electrostatic complexation of gelatin and pectin were developed as possible starch mimetics. The impact of covalent cross-linking on the physicochemical and structural properties of the microgels was investigated. Microgels were formed by acidifying a mixture of gelatin (0.5 wt%) and pectin (0.01 wt%) from pH 10 to 5 at 40 °C, followed by cross-linking with glutaraldehyde (0 to 2 mM). At low glutaraldehyde levels (< 0.5 mM), cross-linking occurred primarily within the microgels and did not affect particle dimensions, whereas at high levels (2 mM), cross-linking connected adjacent microgels leading to the formation of large flocs. Rheological and microscopic analysis showed that the degree of cross-linking impacted the thermal transitions of the microgels. A simulated oral processing study indicated that the melt-in-the-mouth behavior of the hydrocolloid microgels could be made to be similar to that of starch granules by controlling the degree of cross-linking. This study may be useful for designing starch mimetics with improved texture-modifying properties and reduced-calories.

Keywords: hydrogel particles; starch; gelatin; pectin; cross-linking; oral processing; microgels

1 Introduction

The food industry is interested in developing high-quality food and beverage products with reduced-calorie contents to combat health problems associated with overweight, obesity, and diabetes. Fat has the highest calorie density of the major macronutrients (fat, carbohydrates, and proteins) and therefore has been a primary target for calorie reduction in the food industry (McClements, 2015). Nevertheless, the removal of fat from many foods is challenging because it makes a major contribution to their desirable appearances, textures, mouthfeel, and flavor profiles. For this reason, there has been considerable interest in the development of fat mimetics that can replace some of the desirable attributes that are lost when the fat is removed. Starch granules have been widely used as fat mimetics in many emulsion-based products, such as dressings, sauces, yogurt, dips, and desserts (Beggs et al., 1997; Mounsey & O'Riordan, 2008; Sandoval-Castilla et al., 2004; Sikora et al., 2008). However, ingestion of high levels of digestible carbohydrates has recently been linked to the prevalence of diabetes, overweight, and obesity in many developed countries (Lustig et al., 2012; Walker & Parker, 2014). For this reason, there is now interest in also replacing digestible starches with low-calorie starch mimetics. These starch mimetics should be capable of providing similar physicochemical and sensory attributes to foods as conventional starch ingredients normally would (such as appearance, texture, and mouthfeel), but they should contribute fewer calories.

There are two important attributes of modified starch granules that have made them a popular fat mimetic. First, swollen starch granules are highly effective at increasing the viscosity of food systems (Rao, 2014). Second, starch granules are hydrolyzed by α -amylase in the saliva, which contributes to the desirable textural perception of foods (Butterworth et al., 2011). In

particular, the disintegration of starch granules within the mouth leads to a melting sensation that contributes to creaminess perception (Sarkar & Singh, 2012; Weenen, 2005). As mentioned earlier, overconsumption of starch has raised some health concerns. First, starch may not be as satiating as fat and therefore leads to overeating, which may actually contribute to an increase in obesity (Stubbs et al., 2001; Walker & Parker, 2014). Second, the rapid digestion of starch causes a sudden rise in blood glucose levels which may pose a risk for diabetes (Van Kleef et al., 2012). Consequently, it would be useful to develop a starch mimetic that could provide similar functionalities as starch granules (such as thickness and “melt in the mouth” behavior), but with more desirable nutritional attributes (such as lower calories and glycemic index) (Wu et al., 2014).

Our previous studies (Wu et al., 2014; Wu & McClements, 2015a) have demonstrated the potential of using gelatin-based hydrogel particles to replace swollen starch granules. These hydrogel particles were assembled from positively charged proteins and negatively charged polysaccharides using electrostatic complexation. Under optimized solution conditions (*e.g.*, pH, ionic strength, temperature, and shearing), the two biopolymers associate with each other and form a water-in-water emulsion with a polymer-rich dispersed phase and a solvent-rich continuous phase (Turgeon & Laneville, 2009; Turgeon et al., 2007; Wu et al., 2014). Gelation of the polymer-rich dispersed phase leads to the formation of hydrogel particles or microgels that have similar dimension as starch granules, and therefore provide a similar thickening effect (Wu et al., 2014). In addition, the ingredients used for the fabrication of these microgels have relatively low caloric density (protein = ~4 kcal/g and dietary fiber = ~0 kcal/g), and should therefore be suitable for creating reduced-calorie starch mimetics. Finally, microgels fabricated from gelatin and pectin have a melting temperature below body temperature (Wu &

McClements, 2015b), which may be useful for mimicking the “melt-away” mouth-feel of starch granules.

Nevertheless, there are still some technical challenges that need to be addressed when developing this type of starch mimetic. In particular, the relatively low melting temperature of pectin-gelatin microgels make them sensitive to thermal fluctuations that they might experience during food manufacturing, storage, and utilization. A major objective of the current study was therefore to examine the impact of covalent cross-linking of microgels on their thermal melting behavior. As a proof of principle, the hydrocolloid molecules within the microgels were cross-linked using a chemical agent: glutaraldehyde (Migneault et al., 2004). Glutaraldehyde can be used in certain food applications, provided there is no residue remaining in the final product, which would have to be established. In commercial practice, other types of cross-linking agents could be used, such as the food-grade enzymes transglutaminase and laccase (Zeeb et al., 2014). The effect of cross-linking on the microstructure and rheology of the microgels was investigated using simulated oral processing conditions, and their performance was compared to that of starch granules under similar conditions to establish their potential as starch mimetics.

2 Experimental Methods

2.1 Materials

Acid-processed gelatin derived from porcine skin (Type A, 30 mesh granule size, and 100 Bloom strength) was provided by Gelita USA (Sergeant Bluff, IA). Citrus pectin (Pectin LM35, degree of esterification: 29%) was donated by TIC gum (White Marsh, MD). Modified starch derived from waxy corn starch (hydroxypropyl distarch phosphate) was a gift from Tate & Lyle

(Hoffman Estates, IL). Porcine pancreas α -amylase (Type I-A, in PMSF treated saline suspension, Lot# SLBD9572V), mucin from porcine stomach (type II), ammonium nitrate, potassium phosphate, potassium chloride, potassium citrate, uric acid sodium salt, urea, lactic acid sodium salt, hydrochloric acid, sodium hydroxide, and sodium azide were purchased from Sigma-Aldrich (St. Louis, MO). Glutaraldehyde solution (25 %) was purchased from Thermo Fisher Scientific (Waltham, MA).

2.2 Preparation of starch paste

A weighed amount of modified starch (7 g) was dispersed into double distilled water (93 g) and stirred for 30 min to allow hydration. Subsequently, the suspension (100 g) was placed in a 150 g beaker and heated to 90 °C in a water bath (MGW Lauda KS6, LAUDA, Lauda-Königshofen, Germany) and held for 5 min. The sample was stirred manually to allow even distribution of heat during the thermal treatment. After heating, the starch paste was immediately cooled to room temperature (25 °C) using an ice-water bath. The moisture loss during the heat process was compensated for by adding back double distilled water.

2.3 Preparation of gelatin-pectin electrostatic complexes

2.3.1 Stock solutions preparation

Gelatin stock solution (2 wt%) was prepared by dispersing gelatin powder in double distilled water at 35 °C with continuous stirring for 1 h. Pectin stock solution (1 wt%) was prepared by dispersing pectin powder in double distilled water and stirring overnight at room temperature for complete hydration. The stock solutions were mixed with sodium azide (0.02 wt%) and stored at 4 °C to prevent microbial growth.

2.3.2 *Gelatin-pectin complex formation*

Aqueous solutions containing gelatin-pectin electrostatic complexes were prepared following similar procedures as those described previously (Wu & McClements, 2015a), but in this case different levels of cross-linking agent were included (0 - 2 mM glutaraldehyde). Gelatin stock solution was heated at 40 °C for 30 min to ensure it was in the random coil (liquid) state. The pH value of the stock solutions containing gelatin, pectin, and double distilled water were first adjusted to pH 10.0 using NaOH solutions (2 and 1 M). Subsequently, a gelatin-pectin mixture (pH 10, 1600 g) was prepared by mixing weighed amounts of the stock solutions and pH-adjusted double distilled water to obtain a final concentration of 0.5 wt% gelatin and 0.01 wt% pectin. The mixtures were then heated to 40 °C with continuous stirring at a shear rate of 300 rpm (Fisher Scientific Isotemp Basic Stirring Hotplates, Fisher Scientific, Pittsburgh, PA). When the temperature was stabilized, a total of 3100 µL of 1 M HCl solution was titrated into the mixture at a rate of 50 µl per 30 s using an automated electronic pipette (Rainin SE4, Mettler Toledo, Oakland, CA). As a result the biopolymer mixture reached a final pH of 5. The required amount of 0.1 M HCl was determined in a preliminary experiment. Once the pH value was adjusted, a fixed volume of 2.5 M glutaraldehyde was titrated into the mixture to reach final cross-linking agent concentrations ranging from 0.1 to 2 mM. A mixture without glutaraldehyde was prepared as a control. The total heating time after pH adjustment was 20 min for all the samples. The mixtures were then cooled in an ice-water bath to 25 °C and were kept stirring at 300 rpm at room temperature prior to further analysis. The pH and temperature of the samples were monitored using a pH meter (Metrohm® 827 pH Lab Meter, Metrohm, Riverview, FL). All the mixtures were prepared in a 2000 mL beaker and were stirred using magnetic stir bars (Octagonal, 0.5 inch diameter; 3 inch length, Thermo Fisher Scientific, Waltham, MA). After

formation the hydrogel beads were stored for 24 hours at ambient temperature prior to analysis. Previous studies have reported that the majority of cross-linking in polysaccharide-gelatin hydrogels occurs within the first 2 hours, and therefore we assumed that the microgels were full hardened prior to further analysis (Buhus et al., 2009).

2.3.3 *Collection of hydrogel particles*

The hydrogel particle dispersions were centrifuged at 6000 rpm at 25 °C for 40 min using a centrifuge (Sorvall RC6 Plus, Thermo Scientific, Agawam, MA) with a fixed-angle rotor (Fiberlite, PIT F10S-6 × 500Y, Santa Clara, CA). After centrifugation, the supernatant was discarded and the centrifuge bottles were inverted for 3 min to remove excess supernatant from the sediment. Subsequently, the sediment which consisted of the gelatin-pectin complex, was collected for rheological and microscope analysis.

2.4 Particle size determination

The mean particle diameter and particle size distribution of the electrostatic complexes were determined using static light scattering (Mastersizer 2000, Malvern Instruments, Ltd., Worcestershire, UK). The samples were pipetted into an optical measuring cell (Hydro 2000 SM, Malvern Instruments Ltd, Worcestershire, UK) containing pH-adjusted double distilled water (pH 5). The samples were continuously stirred at 1200 rpm during measurement. The particle size distribution and volume-weighted mean diameter ($D_{4,3}$) were calculated from the resulting light scattering pattern using the instrument software (Mastersizer 2000, version 5.60) based on Mie theory (ISO, 2009). A refractive index of 1.33 was set for the continuous phase (water) as provided by (ISO, 2009); the typical optical parameters (1.45 as the refractive index, and 0.001

as the absorption value) for proteins provided by the software database was used for the dispersed phase (gelatin-pectin complex).

2.5 Rheological analysis

2.5.1 Sol-gel transition of hydrogel particles

The effect of glutaraldehyde on the sol-gel transition of hydrogel particle suspensions was evaluated using a strain/stress-controlled rotational rheometer (Kinexus, Malvern Instruments Ltd, Worcestershire, UK). A serrated parallel plate-plate geometry was used to avoid “wall-slip”, which is a common problem when analyzing colloidal suspensions (Buscall, 2010). The upper plate was 40 mm in diameter and the lower plate was 65 mm in diameter. A small amplitude oscillation test coupled with a temperature ramp was performed on the samples to characterize the melting process of the hydrogel particle suspensions as well as their viscoelastic properties. The strain and oscillation frequency of the test were set at 0.5% and 1 Hz (6.283 rad/s), respectively, and the gap between two plates was set at 0.5 mm. The samples were heated from 15 °C to 45 °C at a ramping rate of 2 °C/min. The change in viscoelastic properties was reported as storage modulus (G'), loss modulus (G''), and complex modulus (G^*) *versus* temperature. The cross-over point of the storage modulus (G') and the loss modulus (G'') was analyzed by the instrument software and was taken to be the melting point (T_m).

2.5.2 Characterization of rheological behavior under simulated oral processing conditions

For the starch samples, shear measurement with a fixed shear rate (20 s^{-1}) was conducted at 37 °C using the serrated parallel-plate geometry with a gap of 0.5 mm. Artificial saliva containing α -amylase was used to simulate the oral processing of starch suspensions (Chung et al., 2012). The chemical composition of the artificial saliva used is listed in **Table 1**. For this

experiment, 0.58 mL of the starch paste (containing 7 % starch) was added onto the lower plate and 0.1 mL of artificial saliva was loaded on top of the paste. The rheological properties of the mixture were then measured for a total of 10 min, and the shear viscosity *versus* time profile was reported. Additionally, artificial saliva in the absence of α -amylase was loaded on top of the starch paste, and the shear viscosity profile obtained thereafter was used as a control for comparison.

The rheological properties of the hydrogel particle paste (*i.e.*, sediment of the hydrogel particle suspension after centrifugation) were measured using a fixed shear rate (20 s^{-1}) coupled with a temperature ramp. The hydrogel particle paste (0.58 mL) was placed on the lower serrated plate and 0.1 mL of artificial saliva without α -amylase was added on top of the sample. Subsequently, the gap was set to be 0.5 mm and was stabilized at 25°C . The samples were then subject to heat ramp process from 25°C to 37°C at a rate of $5^\circ\text{C}/\text{min}$ to induce melting (disintegration) of the hydrogel particles; once the final temperature was reached, the samples were continuously heated at the final temperature so that the total heat process time was 10 min, and the shear viscosity *versus* time profile was reported. In this case, α -amylase was not added because the samples contained no digestible carbohydrates.

2.6 Microstructure analysis

The effects of glutaraldehyde on the microstructure of the hydrogel particles were observed using differential interference contrast (DIC) optical microscopy. A small drop of hydrogel particle suspension was placed on a microscope slide and then covered with a glass cover slip. The morphology of the specimens was observed with a $40\times$ objective lens and was captured using a CCD camera (CCD-300-RC, DAGE-MTI, Michigan City, IN, USA) with $1.0\times$ zoom.

The potential disintegration of the starch granules and microgels under simulated oral conditions was monitored using different approaches depending on the disintegration trigger: temperature (microgels) or enzymes (starch). The melting process of the hydrogel particle paste and the enzyme hydrolysis of the starch granules were observed with the aid of a temperature-controlled stage (LTS 120) and a temperature controller (PE 94) from Linkam Scientific Instruments Ltd (Tadworth, UK). For the hydrogel particle paste, a temperature ramp from 25 °C to 45 °C was performed at a rate of 2 °C/min. For the starch paste, a small drop of saliva solution containing α -amylase was added on top of the starch paste on a microscope slide, and then the slide was placed within the temperature-controlled stage at 37 °C. The disintegration process of both particles was video recorded by the CCD camera. All the images and videos were processed by a digital image processing software (Micro Video Instruments Inc, MA, USA). In future studies, it would be advantageous to utilize a similar simulated oral environment to compare the behavior of the microgels and starch granules under the same conditions, *e.g.*, use a temperature ramp and a saliva containing amylase for both systems.

2.7 Statistical analysis

All experiments were repeated twice using freshly prepared samples with all analysis performed in triplicate. The mean and standard deviations as well as Tukey's test (p value < 0.05) were conducted using MATLAB (Version R2014a, MathWorks, Natick, MA).

3 Results & Discussion

3.1 Influence of glutaraldehyde on the microstructure of hydrogel particle

Glutaraldehyde has been widely used as a protein crosslinking agent; it can covalently link protein molecules through the available -OH or -NH₂ groups on the amino acid residues such as lysine and tryptophan (Migneault et al., 2004). Chemical cross-linking of protein molecules leads to structures that are more stable to environmental stresses (such as salt, pH, or organic solvents) than physically cross-linked ones (Migneault et al., 2004; Silva et al., 2004). Because gelatin molecules do not have appreciable quantities of amino acid residues with free -OH groups, the binding of glutaraldehyde mainly occurs at the α -amino group of the lysine molecules (Sharma & Bohidar, 2000). We added glutaraldehyde after the solution was adjusted to pH 5 to ensure that chemical cross-linking of the gelatin occurred after the hydrogel particles had been formed.

Our previous study demonstrated that hydrogel particles with different dimensions and morphologies could be produced depending on the formation temperature, which was attributed to thermally-induced alterations in the structure of the gelatin chains, *i.e.* coil-to-helical upon cooling (Wu & McClements, 2015b). For this reason, a formation temperature of 40 °C was used in the present study to eliminate microstructure variations caused by changes in gelatin configuration (Wu & McClements, 2015b). This temperature is above the melting point of gelatin, and so the majority of gelatin molecules should be in the random coil configuration.

In this section, the impact of cross-linking with glutaraldehyde on the microstructure of the gelatin-pectin complex was investigated using static light scattering (**Figure 1**) and optical microscopy (**Figure 2**). At the glutaraldehyde concentrations tested, all the electrostatic

complexes had monomodal distributions (**Figure 1a**). Relatively low glutaraldehyde concentrations (0.1 - 0.5 mM) did not appear to affect the dimensions of the hydrogel particles: their particle size distributions were similar to that of the control (without glutaraldehyde) and their volume-weighted mean diameters were not significantly different from the control ($p < 0.05$) (**Figure 1b**). However, the dimensions of the hydrogel particles increased significantly at relatively high glutaraldehyde concentrations (2 mM): the peak of the particle size distribution shifted rightward and the mean particle diameter ($D_{4,3} = 61.3 \pm 4.8 \mu\text{m}$) was nearly twice that of the control (**Figure 1**).

The optical microscopy measurements were in good agreement with the light scattering measurements (**Figure 2**). At low levels of the cross-linking agent (0 to 0.5 mM), spherical hydrogel particles were formed that had similar dimensions. These particles had relatively smooth surfaces, with the occasional presence of a circular cavity on their surfaces. As discussed in our previous study, the smooth surface can be attributed to the fact that the electrostatic complexation between gelatin and pectin was carried out at 40 °C (Wu & McClements, 2015b). At this temperature the gelatin molecules have a flexible random coil configuration, which allows the gelatin-pectin electrostatic complexes formed to minimize their surface free energies by adopting a spherical shape (Sharma & Bohidar, 2000; Wu & McClements, 2015b). The presence of a circular cavity on some of larger particles can be ascribed to the coalescence of two different hydrogel particles (Wu & McClements, 2015b). Interestingly, when the glutaraldehyde concentration reached 2 mM, some of the hydrogel particles appeared to be linked together. At low glutaraldehyde concentrations, the cross-linking of gelatin molecules probably occurred primarily within the hydrogel particles, since the local gelatin concentration was high within the particles. Conversely, at high glutaraldehyde concentrations, some cross-linking occurred

between different hydrogel particles, *i.e.*, the reaction rate was sufficiently fast for cross-links to form during a particle-particle collision. Interestingly, the aggregates formed at 2 mM glutaraldehyde contained smaller individual spherical particles than the individual particles formed at 0 to 0.5 mM glutaraldehyde. This may have occurred for a number of reasons. First, some coalescence may have occurred immediately after the solution was adjusted to low pH to induce electrostatic complexation and hydrogel particle formation. At the higher glutaraldehyde concentration, the cross-linking would have occurred more rapidly, which may have limited coalescence. Second, a higher glutaraldehyde concentration may have led to a more dense packing of the biopolymer molecules within the hydrogel particles, therefore leading to smaller particle dimensions. Third, the reaction of glutaraldehyde with certain groups on the biopolymer molecules (such as amino groups) may have changed their electrical characteristics, which would have altered the nature of any electrostatic interactions between the proteins and polysaccharides.

3.2 Influence of glutaraldehyde on the melting of hydrogel particles

In this section, we characterized the influence of chemical cross-linking by glutaraldehyde on the melting behavior of hydrogel particles. Small amplitude oscillation experiments were conducted which measured the viscoelastic properties of the hydrogel particle paste as a function of temperature (**Figure 3**).

At low glutaraldehyde concentrations (0 to 0.5 mM), the storage modulus (G') was initially higher than the loss modulus (G'') at low temperatures, indicating that the hydrogel particle exhibited mainly solid-like behavior. Both modulus decreased as the temperature was increased, but G' eventually became smaller than G'' above a critical temperature indicating that a solid-to-liquid transition had occurred (Winter & Mours, 1997). The temperature at which G' equals to

G' was taken to be the melting temperature of the hydrogel particle suspension. The melting temperature was observed to increase as the concentration of cross-linking agent used increased (Table 2).

At high glutaraldehyde concentrations (2 mM), G' remained higher than G'' when the temperature was increased from 15 to 45 °C, suggesting that the sample did not melt in this temperature range. Below the melting temperature, the rigidity of the hydrogel suspensions (G') increased with increasing cross-linking concentration.

The melting process was also monitored using the DIC microscope coupled with a temperature-controlled stage. The samples were heated from 25 to 45 °C, and the changes of the microstructure were video-recorded. Selected images of the samples taken (i) prior to heating (at 25 °C), (ii) at the melting temperature, and (iii) at the final temperature (45 °C) are shown in Figure 4. At 25 °C, the hydrogel pastes had fairly similar microstructures, regardless of the glutaraldehyde concentration. The hydrogel suspensions containing 0, 0.1 and 0.25 mM glutaraldehyde started to lose their spherical structure (*i.e.*, melt) around 36.1, 38.0 and 44.6 °C, respectively. These values correspond to the temperatures where they hydrogel suspensions were observed to melt in the rheology measurements. At 45 °C, the structure in the samples containing 0 and 0.1 mM glutaraldehyde had completely disappeared and the samples were observed to be fluid-like. In contrast, some spherical structures remained in the samples containing 0.25 mM glutaraldehyde at this temperature, which were only completely melted when it was held at 45 °C for extended periods (data not shown). On the other hand, the hydrogel particles containing 0.5 mM glutaraldehyde only underwent a slight change in their structure at their transition temperature (40 °C) and remained largely unchanged when they were further heated to 45 °C. Temperature appeared to have no effect on the microstructure of the hydrogel

particle paste containing 2 mM glutaraldehyde. Overall, these studies showed that cross-linking of the gelatin molecules using glutaraldehyde was an effective means of regulating the melting behavior of the gelatin-pectin hydrogel particles.

The melting behavior of hydrogel particles can have a pronounced influence on their functional properties. For example, heating could be used to trigger the release of encapsulated active agents from hydrogel-based delivery systems (Yeo et al., 2005). Alternatively, the disintegration of the hydrogel particles at elevated temperatures could be used to simulate the melt-in-the-mouth properties associated with starch granules (Sarkar & Singh, 2012; Weenen, 2005). Therefore, the melting behavior of the gelatin-pectin hydrogel particles may be important for mimicking the desirable sensory characteristics associated with ingestion of starch granules. It should be noted that the disintegration of starch granules is mainly promoted by enzyme (amylase) hydrolysis within the mouth, rather than by temperature changes. This effect is clearly seen in optical microscopy images of changes in starch granule properties when they are mixed with simulated saliva (**Figure 5**). The swollen starch granules disintegrate and disappear due to hydrolysis by the α -amylase in the simulated saliva solution. Interestingly, there was a similarity between the change in microstructure observed during hydrolysis of starch granules by amylase (**Figure 5**) and the melting of hydrogel particles (0 - 0.25 mM glutaraldehyde) during heating (**Figure 4**).

3.3 Characterization of rheological behavior under simulated oral processing conditions

In this section, the rheological properties of hydrogel particle pastes containing different cross-linking levels was characterized and compared to those of the starch paste. As discussed earlier, the complex modulus *versus* temperature profile was determined using an oscillation test

(**Figure 3**) to indicate changes in the gel strength of the hydrogel particle pastes. At relatively low temperatures, all the hydrogel particle pastes had a relatively high shear modulus. As the temperature was increased, the shear modulus decreased, with the extent of the decrease depending on glutaraldehyde level: the greater the cross-linking, the smaller the decrease. A steep decrease in shear modulus occurred over a narrow temperature range for suspensions containing relatively low glutaraldehyde concentrations (0 - 0.25 mM), indicating that melting occurred. On the other hand, this steep decrease was not observed in the suspensions containing higher glutaraldehyde levels (0.5 and 2 mM), which suggests that they did not fully melt over the temperature range studied. The rheology results were therefore in good agreement with the microscopy measurements reported in the previous section.

The structural disintegration of food in the mouth plays an important role in determining the textural perception of many food products. For example, swollen starch granules initially provide a thickening effect when first placed in mouth due to their high effective volume fractions (Rao, 2014), but then they provide a melt-in-the-mouth sensation when they are hydrolyzed by α -amylase in the saliva (Sarkar & Singh, 2012; Weenen, 2005). Our previous study demonstrated that hydrogel particle pastes had high viscosity and similar shear thinning behavior as starch pastes (Wu et al., 2014). In the current study, our experiments were designed to simulate oral processing conditions so as to investigate how well gelatin-pectin hydrogel particles mimic the behavior of starch granules in the mouth.

A starch paste was mixed with artificial saliva containing α -amylase and the viscosity was measured at typical body temperature (37 °C) at a fixed shear rate (20 s⁻¹). For comparison, a hydrogel particle paste was heated from 25 to 37 °C (to simulate placing a food in the mouth) and then its shear viscosity was measured at the same shear rate as the starch paste. The shear

rate of 20 s^{-1} was used to mimic oral processing conditions (Rao, 2007; Shama & Sherman, 1973). For the starch paste, the shear viscosity decreased rapidly during the first minute or two, and then decreased more slowly afterwards (**Figure 6**). This effect can be attributed to the reduction in the effective volume of the starch granules as they are broken down by the amylase. The importance of enzymatic digestion was highlighted in experiments that were carried out in the absence of amylase: in this case the viscosity of the starch paste remained relatively constant over time. The oral behavior of the hydrogel particle paste depended on the degree of cross-linking. At relatively low glutaraldehyde levels (0 and 0.1 mM), the viscosity dropped steeply when the temperature reached values close to body temperature (36.1 and 37.8 °C). This decrease in viscosity can be attributed to the melting of the hydrogel particles (**Figure 4**). Low levels of cross-linking may therefore be useful for producing hydrogel particles that have melting characteristics in the mouth similar to those of starch granules. Having said this, the final viscosity of the hydrogel particle paste was much higher than that of the starch paste (**Figure 6**), which can be attributed to the fact that starch granules are hydrolyzed into glucose molecules whereas hydrogel particles dissociate into large polymers (gelatin and pectin molecules). This may have important implications on the quality of a product, since the coating on the oral surface has been reported to have significant impact on texture perception (de Roos, 2005). It is interesting to note that there was a sudden increase in the viscosity of the hydrogel particle suspensions before they decreased (**Figure 6**). This was probably caused by the sudden alteration in the structure of the hydrogel particles when they melted, which would have affected their ability to increase the viscosity.

At 0.25 mM glutaraldehyde, the viscosity initially dropped but then it appeared to increase when the samples were held at 37 °C after the final temperature was reached (**Figure 6**). Visual

observations indicated that this sample became “sticky”, which was probably because its structure was only partially melted as its melting temperature was 44.6 °C. Consequently, the hydrogel particles may have adopted non-spherical shapes and undergone aggregation, which would have increased the shear viscosity. At higher glutaraldehyde levels (> 0.5 mM), a sharp transition in the shear viscosity upon heating was not observed (**Figure 6**), which can be attributed to the fact that the particles maintained their initial morphology. These results suggest that, in order to mimic the texture behavior of starch paste, the cross-linking degree should be kept to a minimum.

It should be noted that the temperature ramping rate in this experiment was 5 °C/min, which may not reflect the temperature increase rate of foods after ingestion; the temperature increase rate of food is highly related to the food matrix and initial food temperature. Thus, depending on the real food application, it may be necessary to characterize the melting behavior of the hydrogel pastes using different heating rates. It should be noted that the melting temperature can be controlled not only by the degree of cross-linking, but also by the gelatin-to-pectin ratio. Consequently, it would be useful to investigate this parameter in future studies of the behavior of the hydrogel particles under simulated oral conditions. Sensory analysis of the samples would also be beneficial to evaluate the potential of cross-linked hydrogel particles to replace starch granules in various food applications. In addition, glutaraldehyde was used in this study as a cross-linking agent for the purpose of proof-of-concept. Many other approaches are available to induce covalent cross-linking of proteins, such as genipin, phenolic acids, and transglutaminase (Biscarat et al., 2015; Xiao et al., 2014; Zeeb et al., 2014), which will be more suitable for food applications.

4 Conclusions

In the present study, hydrogel particles were formed through gelatin-pectin electrostatic complexation, followed by chemical cross-linking of the gelatin molecules by glutaraldehyde. This study showed that the degree of chemical cross-linking influenced the physicochemical properties and functionalities of the hydrogel particles. At low glutaraldehyde concentrations, cross-linking occurred internally, which did not affect particle size or shape. Whereas, at high glutaraldehyde concentration, individual microspheres were linked together in addition to the internal cross-linking. The viscoelastic properties and melting behavior of hydrogel particle pastes can be controlled by regulating the degree of cross-linking: the samples showed increased melting point and more solid-like behavior as the glutaraldehyde level increased. Simulated oral conditions suggested that hydrogel particles could be designed to mimic the thickening and melt-in-the-mouth characteristics of starch granules. Further studies, such as sensory analysis, are needed to more comprehensively evaluate the potential applications of this type of hydrogel particle in commercial food products.

5 Acknowledgements

This project is partially supported by United States Department of Agriculture, NRI Grants (2011-03539 and 2013-03795), as well as a grant from ConAgra Foods (Omaha, Nebraska). Bicheng Wu also wanted to acknowledge the financial supports provided by the Thomas H. Smouse Memorial Fellowship from American Oil Chemists' Society, and the Eugene M. Isenberg Scholar Awards from UMass Amherst.

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TABLES

Table 1. Composition of artificial saliva solution used to simulate oral conditions (Chung et al., 2012).

Chemical type	Concentration (g/L) or activity (units/mL)
Sodium chloride	1.594
Ammonium nitrate	0.328
Potassium phosphate	0.636
Potassium chloride	0.202
Potassium citrate	0.308
Uric acid sodium salt	0.021
Urea	0.198
Sodium DL-lactate/lactic acid sodium salt	0.146
Mucin from porcine stomach	30
Type II Alpha amylase activity	93 units/mL

518 **Table 2.** Melting point of hydrogel particles containing various glutaraldehyde levels. The
 519 melting point was determined as the cross-over temperature for G' and G'' during heating.
 520

Glutaraldehyde (mM)	Melting point (°C)
0	36.1 ± 0.4
0.1	37.8 ± 0.2
0.25	44.6 ± 0.0
0.5	> 45
2	> 45

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522 FIGURES

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526 **Figure 1.** Influence of glutaraldehyde concentration (*i.e.*, degree of cross-linking) on (a) the
527 particle size distribution and (b) the volume-weighted mean particle diameters of gelatin-pectin
528 hydrogel particles. The designations *a* and *b* above the bars represent Tukey groups that differ
529 significantly with p value < 0.05 .

Figure 2. Representative DIC microscopic images of gelatin-pectin hydrogel particles containing (a) 0 mM, (b) 0.1 mM, (c) 0.25 mM, (d) 0.5 mM, and (e) 2 mM glutaraldehyde. All the scale bars are 50 µm in length.

Figure 3. Storage modulus (G') and loss modulus (G'') of gelatin-pectin hydrogel particles containing various glutaraldehyde levels (0 - 2 mM) obtained by small amplitude oscillation test coupled with temperature ramping (15 to 45 °C)

Figure 4. Changes in microstructure of the hydrogel particle paste containing various glutaraldehyde concentrations during the heating process (25 to 45 °C at 2 °C/min).

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Figure 5. Hydrolysis process of starch granules by α -amylase in the artificial saliva.

Figure 6. Complex modulus (G^*) of gelatin-pectin hydrogel particles containing various glutaraldehyde levels (0 - 2 mM) obtained by small amplitude oscillation test coupled with temperature ramping (15 to 45 °C)