1 **Design of Reduced-Fat Food Emulsions: Manipulating**
2 **Microstructure and Rheology through Controlled**
3 **Aggregation of Colloidal Particles and Biopolymers**

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Abstract

The objective of this study was to develop model reduced-calorie food emulsions with desirable textural and optical properties based on controlled aggregation of food-grade colloidal particles and biopolymers. The model food emulsion consisted of fat droplets (5 wt%), starch granules (4 wt%), and xanthan gum (0 to 0.02 wt%) under acidic conditions (pH 3). The fat droplets were stabilized by a protein-based emulsifier (whey protein isolate). Fat droplet aggregation was induced by adding anionic xanthan gum to promote bridging flocculation of the cationic protein-coated fat droplets. Thermal processing (95 °C) did not have a major impact on fat droplet aggregation, but it did promote starch granule swelling. The structural organization of the fat droplets could be regulated by altering xanthan levels. Relatively small droplet aggregates were formed at low xanthan concentrations that coated the starch granule surfaces. Conversely, large irregular shaped droplet aggregates were formed throughout the system at higher xanthan levels. The rheological and optical properties of the model emulsions could therefore be controlled by altering fat droplet organization. Addition of low levels of xanthan significantly increased the viscosity, yield stress, and complex modulus of the model food emulsions. However, high levels of xanthan led to the formation of large visible aggregates that would negatively impact sensory quality. This study has important implications for the development of cost-effective and clean-label reduced-fat products with desirable quality attributes, such as dressings and sauces.

Keywords: Reduced fat, emulsion, aggregation, rheology, xanthan, starch, whey protein, electrostatic

1 Introduction

There has been a strong focus in the food industry on the development of reduced-fat products due to health concerns associated with high-calorie diets (Lee, Lee, Lee, & Ko, 2013; Van Kleef, Van Trijp, Van den Borne, & Zondervan, 2012). However, reduced-fat food products often have limited consumer acceptance and commercial success due to undesirable changes in appearance, flavor, mouthfeel, and texture when fat is removed (Malone, Appelqvist, & Norton, 2003; McClements, 2015b; van Aken, Vingerhoeds, & de Wijk, 2011). For example, in oil-in-water emulsions, a decrease in fat content leads to a reduction in “lightness” due to weaker light scattering (McClements, 2002), a reduction in viscosity due to less energy dissipation (Derkach, 2009; Tadros, 2010), a change in flavor profile due to differences in ingredient partitioning (Taylor & Linfoth, 1996), and a reduction in creaming stability due to faster droplet movement (Chanamai & McClements, 2000). Various structural design approaches have therefore been developed to overcome some of the loss in desirable sensory attributes associated with reduced fat products (Fernández Farrés, Moakes, & Norton, 2014; Norton & Frith, 2001; Patel, Heussen, Dorst, Hazekamp, & Velikov, 2013; Simo, Mao, Tokle, Decker, & McClements, 2012; Wu, Degner, & McClements, 2014; Wu & McClements, 2015). Undesirable changes in optical properties (loss of “creamy” appearance) can be partly overcome by adding other types of colloidal particles that scatter light, such as protein, polysaccharide, or mineral particles (McClements, 2015b). The reduction in viscosity or modulus that occurs when fat droplets are removed from emulsion-based products is typically compensated for by adding thickening or gelling agents, such as proteins or polysaccharides. An alternative approach to maintaining high

viscosity or gel-like properties in reduced-fat emulsions is to induce aggregation of the fat droplets to form a three-dimensional network (Mao & McClements, 2012a).

Aggregation is often considered undesirable in dilute emulsions because it promotes creaming instability, which is considered a defect in many liquid-based products (Cao, Dickinson, & Wedlock, 1990; McClements, 2015a). However, in concentrated emulsions, aggregation results in the formation of a three-dimensional network of fat droplets that inhibits their movement and traps the continuous phase, thereby leading to an increased viscosity and even gel-like properties (Mao & McClements, 2012b; Santipanichwong & Supphantharika, 2009). Additionally, saliva-induced aggregation of fat droplets in the oral cavity may contribute to the desirable mouthfeel of some emulsion-based products, since this increases the perceived viscosity in the mouth (Silletti, Vingerhoeds, Norde, & Van Aken, 2007; van Aken, Vingerhoeds, & de Hoog, 2007). Consequently, manipulating the aggregation state of fat droplets in emulsions may provide a useful means of controlling their textural and mouthfeel properties and creating reduced fat products.

In previous studies, we developed a model system that consisted of a mixture of starch granules and protein-coated fat droplets so as to mimic the composition and microstructure of some important emulsion-based products used in the food industry, such as sauces, dressings, and dips (Wu, Degner, & McClements, 2013a; Wu, Degner, & McClements, 2013b). The appearance and textural properties of these model food systems could be manipulated by controlling the structural organization of the fat droplets. In general, the aggregation state of fat droplets can be controlled by altering the relative magnitude of the attractive and repulsive forces operating between them, such as electrostatic, steric, hydrophobic, depletion, and bridging interactions (McClements, 2015a). This can be achieved by altering the nature of the droplet

interface (such as surface hydrophobicity, thickness, or charge), solution conditions (such as pH and ionic strength), system composition (such as biopolymer type and concentration) and environmental conditions (such as temperature) (Damodaran, 2005; Dickinson, 2010). For protein-coated fat droplets, aggregation can be induced by: (i) adjusting the pH to around the isoelectric point (pI) of the adsorbed proteins; (ii) increasing the ionic strength; (iii) heating above the thermal denaturation temperature; (iv) adding adsorbing biopolymers; (v) or adding non-adsorbing biopolymers (Dickinson, 2010; McClements, 2004).

In the current study, we utilized a negatively charged polysaccharide (xanthan gum) for its ability to induce aggregation of positively charged protein-coated fat droplets (Laneuville, Paquin, & Turgeon, 2000). Xanthan gum is already widely used in reduced-fat food products for its ability to increase the viscosity of aqueous solutions (Lee et al., 2013; Samavati, Emam-Djomeh, Mohammadifar, Omid, & Mehdinia, 2012). In the current study, xanthan gum is mainly used for its ability to electrostatically cross-link oppositely charged fat droplets. This approach may be useful in the development of cost-effective and clean-label methods for formulating reduced-fat products with desirable quality attributes.

2 Experimental Methods

2.1 Materials

Whey protein isolate (WPI) was donated by Davisco Foods International (Le Sueur, MN, US). The WPI contains 97.9 wt% protein, 0.25 wt% fat, and 1.9 wt% ash according to the product specification. Canola oil and modified starch (hydroxypropyl distarch phosphate, derived from waxy corn starch) were provided by ConAgra Foods (Omaha, NE, US). Xanthan gum was

a gift from TIC Gums (Pre-Hydrated® Ticaxan® Xanthan, White Marsh, MD, US). According to the manufacturer, the average molecular weight of the xanthan gum was 500 kDa. Hydrochloric acid (HCl), sodium azide, and technical grade Nile Red dye (CAS#7358-67-3) were purchased from Sigma-Aldrich (St. Louis MO, US). Type A immersion oil was purchased from Nikon (Melville, NY, US). Double distilled water was used to prepare all solutions.

2.2 Preparation of model dispersions

2.2.1 Preparation of stock solutions

An aqueous emulsifier solution was prepared by dispersing WPI powder in double distilled water and then stirring for at least one hour at room temperature to ensure protein hydration and dissolution. A coarse oil-in-water emulsion, containing 25 wt% canola oil, was prepared by mixing emulsifier solution and oil (1:10 protein-to-oil ratio) together using a high-speed blender (Tissue Tearor Model 985370-395, BiosPec Products Inc., Bartlesville, OK, US) at 15,000 rpm for 2 min. The coarse emulsion produced was further homogenized by passing it through a high-shear fluid processor (Microfluidizer Model 110 L, Microfluidics, Newton, MA, USA) three times at a pressure of 10,000 psi.

An aqueous polysaccharide stock solution (0.5 wt%) was prepared by dispersing xanthan gum powder into double distilled water, followed by overnight stirring at room temperature. Sodium azide (0.01 wt%) was added to the xanthan gum stock solution and the emulsion to prevent microbial growth.

2.2.2 Preparation of dispersions

A series of 5 wt% oil-in-water emulsions with different xanthan gum concentrations (0, 0.005, 0.01, 0.02 wt%) were prepared by mixing different ratios of stock emulsion (25 wt% oil),

stock xanthan gum solution (0.5 wt%), and double distilled water. Pure starch dispersions (4 wt%) with various xanthan gum concentrations were prepared by mixing weight amounts of modified starch with xanthan stock solution and double distilled water. After mixing, the pH values of the pure emulsion and pure starch dispersions were adjusted to pH 3 using HCl solution.

Mixed emulsion-starch dispersions (model emulsion) containing 5 wt% emulsion, 4 wt% starch and various amount of xanthan gum (0, 0.005, 0.01, 0.02 wt%) were prepared by mixing different ratios of stock emulsion, xanthan stock solution, and modified starch powder. The pH of the mixed dispersions was adjusted to pH 3 or 7.

Selected samples (120 g) were placed in a 250 ml beaker and heated to 90 °C and then held for 5 min in a water bath (MGW Lauda KS6, LAUDA, Lauda-Königshofen, Germany). During heating, the samples were manually stirred occasionally to ensure uniform heat distribution. The loss of mass due to water evaporation was compensated for after the heat process by adding pH adjusted double distilled water. Unheated samples with the same composition were collected as well for comparison.

2.3 Characterization of particle properties

The ζ -potential of the samples with or without heat treatment was characterized by a particle micro-electrophoresis instrument (Zetasizer NanoZS, Malvern Instruments, Ltd., Worcestershire, UK) as described in our previous study (Wu et al., 2013a). Namely, samples were diluted 200-times with pH-adjusted double distilled water (at a pH matching that of the continuous phase in the emulsion) and were introduced into a folded capillary cell (Malvern Instruments, Ltd., Worcestershire, UK). The instrument software (Version 6.30, Zetasizer, Malvern Instruments,

Ltd., Worcestershire, UK) recorded the electrophoretic mobility data and converted it into ζ -potential values. The ζ -potential calculation depends on the viscosity of the liquid surrounding the particles. In our experiments all the samples were diluted so much that the solution viscosity was close to that of water regardless of the initial biopolymer concentration.

The particle size of the samples with or without heat treatment was determined by static light scattering (Mastersizer 2000, Malvern Instruments, Ltd., Worcestershire, UK) following the same procedures as described in our previous studies (Wu et al., 2013b). Samples were diluted with pH adjusted double distilled water (at a pH matching the continuous phase) to avoid multiple scattering effects. The instrument software (Mastersizer 2000, Version 5.60) calculated the particle size distribution and mean diameters based on Mie theory (ISO, 2009). It was assumed that the particles had a refractive index of 1.43 and the surrounding liquid had a refractive index of 1.33. In reality, the starch granules and fat droplets will have different refractive indices, however, the particle size determination is much less sensitive to refractive index for large particles (such as starch granules) and therefore the results should still provide a good indication of the actual size. The volume-weighted mean diameter ($D_{4,3}$) is reported in this study.

The effect of xanthan gum on the microstructure of model dispersions was examined by Differential Interference Contrast (DIC) and confocal microscopy (Nikon microscope D-Eclipse C1 80i, Nikon Corporation, Melville, NY, USA). An oil immersion objective lens (60 $\times$, 1.40 NA) with a 1.0 $\times$ camera zoom was used to observe the specimens. Confocal microscopy is used to visualize the oil fraction in the samples. For the confocal images, a few drops of Nile red solution (1 mg/ml ethanol) were added to the dispersions before heat treatment to dye the oil phase. Nile red was then excited by an air-cooled argon ion laser (Model IMA 1010BOS, Melles

Griot, Carlsbad, CA, US) at 488 nm, the emission spectra of which were detected in the 605 channel with a long pass (LP) filter (HQ 605LP/75m). The confocal images were examined using the image processing software (EZ-C1 Version 3.8, Nikon, Melville, NY, US). All microscopy images were captured by a CCS camera (CCD-300-RC, DAGE-MTI, Michigan City, IN, USA) and were processed by an image processing software (Micro Video Instruments Inc., MA, USA).

2.4 Determination of rheological properties

The rheological properties of the samples were characterized by large and small deformation tests using a dynamic shear rheometer at 25 °C (Kinexus, Malvern Instruments, Ltd., Worcestershire, UK). All the tests were performed using serrated parallel plates with diameters of 65 mm for the lower plate and 40 mm for the upper plate at a gap of 1 mm in order to eliminate wall-slip issues of colloidal systems (Buscall, 2010). The shear stress *vs.* shear rate (0.01 - 30 s⁻¹) test was conducted on the samples and the results are presented as shear stress and apparent viscosity *versus* shear rate profiles. The yield stress of samples was determined by a stress sweep measurement. Namely, stress ramped from 0.1 to 10 Pa and the stress at which the viscosity reached maximum was taken to be the yield stress. The viscoelastic properties of the samples were characterized using a small amplitude oscillation test. The oscillation test was performed at a 1% strain, and frequency sweep from 10 Hz (62.8 rad/s) to 0.01 Hz (0.06 rad/s). The viscoelastic property of the samples was presented as complex modulus (G*) *versus* frequency profile.

2.5 Appearance

The heated model emulsions (20 ml) with various xanthan concentrations (pH 3) were placed in the cup of a couette geometry using the Kinexus rheometer (Malvern Instruments, Ltd., Worcestershire, UK). The bob was lowered into the cup and retracted at fixed speed and force as set by the default setting of the instrument, thus a thin layer of sample covered the surface of the bob and its appearance was captured by a digital camera (Panasonic DMC-ZS8, Panasonic, NJ, US). In addition, the appearance and texture (consistency) of the heated model emulsion were visualized by tilting a spoonful of sample and recording the resulting appearance using a digital camera.

2.6 Statistical analysis

Each individual sample was measured in triplicate for all the tests and all the experiments were repeated twice using freshly prepared samples. The mean and standard deviations were calculated based on this data.

3 Results & Discussion

3.1 Effect of xanthan on microscopic characteristics

3.1.1 Fat droplets in simple emulsions

In this section, the influence of xanthan gum addition and thermal treatment on the properties of simple emulsions (protein-stabilized fat droplets in water) was investigated. Under acidic conditions (pH 3), simple emulsions containing various xanthan concentrations were highly positively charged and their electrical characteristics were unaffected by heat treatment

(**Figure 1**). This is because the electric properties of emulsions are mainly determined by the nature of the emulsifier adsorbed to the fat droplet surfaces. WPI is an amphoteric biopolymer with an isoelectric point (pI) around pH 4.6 due to the presence of ionizable carboxyl ($-\text{COOH} \leftrightarrow -\text{COO}^-$) and amino ($-\text{NH}_2 \leftrightarrow -\text{NH}_3^+$) groups (Wu et al., 2013b). Consequently, when the pH is lower than the pI of WPI, the emulsion droplets are positively charged, and when the pH is higher than the pI, they are negatively charged.

The addition of xanthan reduced the ζ -potential of the model emulsions: the ζ -potential of the heated model emulsion, for example, dropped from +62.3 to +39.0 mV when the xanthan content was increased from 0 to 0.02 wt%. Xanthan is a negatively charged polysaccharide with a high charge density (Lii, Liaw, Lai, & Tomasik, 2002). At pH 3, the anionic xanthan molecules would therefore be expected to interact with the cationic protein-coated droplets due to electrostatic attraction leading to partial charge neutralization (McClements, 2015a).

Polysaccharide addition also altered the particle size distribution (PSD) of the emulsions. In the absence of xanthan gum, the emulsions had a monomodal distribution (**Figure 2**) and a mean particle diameter ($D_{4,3}$) around 0.25 μm . As the xanthan gum concentration increased, the peak in the PSD shifted to higher values and became broader (**Figure 2**) and the mean particle diameter increased (**Table 1**), indicating that particle aggregation had occurred. At all xanthan concentrations, thermal treatment did not have a significant impact on the mean particle diameter and did not cause an appreciable change in the PSD (**Table 1, Figure 2**). Further insights into the effects of xanthan gum on the microstructure of the emulsions was obtained using confocal microscopy (**Figure 3**). In the absence of xanthan, the emulsions contained small fat droplets that were evenly distributed throughout the continuous phase, which was in agreement with the light scattering measurements (**Table 1, Figure 2**). The good aggregation stability of these emulsions

can be attributed to the high positive charge on the protein-coated fat droplets causing a strong electrostatic repulsion that prevented droplets from coming close together. It should be noted that a small population of aggregated droplets was observed in the simple emulsion (data not shown), which likely resulted from some irreversible aggregation of the fat droplets when the system was adjusted from the original conditions (pH 7.0) to the final conditions (pH 3.0), since the emulsion had to pass through the isoelectric point of the protein-coated droplets.

In the presence of xanthan, the fat droplets in the simple emulsions flocculated and formed large fibrous structures (**Figure 3**). In addition, there was a strong dependence of emulsion microstructure on xanthan content: the fat droplets became more highly aggregated as the xanthan level increased. Indeed, when the xanthan concentration reached 0.01 wt% the aggregates were actually visible to the naked eye. The most likely origin of droplet aggregation in these emulsions is bridging flocculation, *i.e.*, the ability of negatively charged xanthan molecules to link together two or more positively charged fat droplets (Cheftel & Dumay, 1993; Laneuville et al., 2000). Laneuville et al. (2000) reported that the nature of the electrostatic complexes formed between xanthan and WPI depended on the polysaccharide-to-protein ratio and the polysaccharide molecular weight.

3.1.2 Model emulsions: Fat droplet-starch granule mixtures

In this section, we examined the effect of xanthan gum addition, thermal treatment, and pH on the properties of fat droplet-starch granule mixtures (model emulsions). It was found that heat treatment had limited effect on the electrical properties of the particles in the model emulsions (**Figure 1**). At pH 3, the particles in the pure starch suspension had a relatively low net charge, and so the overall electrical properties of the acidic model emulsions would be expected to be

primarily determined by those of the fat droplets. Thus, the high positive charge measured for the model emulsions at pH 3 can be attributed to the cationic nature of the protein-coated fat droplets below their isoelectric point. As described in Section 3.1.1, negatively charged xanthan gum molecules can electrostatically interact with positively charged fat droplets at pH 3, which accounts for the observed decrease in the positive charge in the mixed systems as increasing amounts of xanthan were added (**Figure 1**). At pH 7, a high negative charge was measured in the mixed systems, which can be attributed to the fact that the protein-coated fat droplets were above their isoelectric point and therefore negatively charged, and because this type of modified starch granule is known to gain some negative charge at neutral pH (Wu et al., 2013b).

The effect of xanthan gum content on the particle size distribution (**Figure 4**), mean particle size (**Table 1**) and microstructure (**Figure 5**) of the different systems was characterized by static light scattering and optical microscopy. Measurements were also made on simple emulsions and on pure starch suspensions to identify the peaks associated with the fat droplets and starch granules in the PSDs of the mixed systems. At pH 3, in the absence of xanthan or heating, the mixed system had a bimodal distribution with a small peak (around 0.21 μm) and a large peak (around 26 μm), which were attributed to fat droplets and native starch granules, respectively. Microscopy measurements indicated that the fat droplets remained non-aggregated and homogeneously distributed throughout the continuous phase surrounding the native starch granules (**Figure 5**). When xanthan gum was incorporated into the unheated samples at pH 3 the small peak that corresponded to the non-aggregated fat droplets disappeared and there was an increase in the fraction of larger particles (**Figure 4a**). This effect can be attributed to the flocculation of the protein-coated fat droplets by xanthan gum, as was also observed in the simple emulsions containing no starch granules (**Figure 2**). Microscopy measurements indicated

that the starch granules were trapped within the flocs formed by the fat droplets in the presence of xanthan (**Figure 5**). It should be noted that all the components (emulsion, xanthan, and starch) were mixed together at pH 7 and then the system was adjusted to pH 3. Consequently, the starch granules may have been trapped within the flocs during the acidification process.

The PSD of the mixed systems containing 0.02 wt% xanthan gum at pH 7, indicated that there was still a population of small particles present, which corresponded to non-aggregated fat droplets. The polysaccharide did not promote droplet aggregation at this pH because the xanthan gum and fat droplets had similar charges (both negative) and therefore were not strongly attracted to each other. In addition, the level of xanthan gum added (0.02 wt%) was presumably too low to induce depletion flocculation.

After heating, the mean diameter of the particles in the starch suspension increased from around 26 to 43 μm (**Table 1**), which can be attributed to swelling of the starch granules due to water absorption and gelatinization (Wu et al., 2013a). The starch retained a granule-like structure after heating because it had been modified by covalent cross-linking, which prevented its thermal disintegration (Wurzburg, 2006; Wurzburg & Szymansk.Cd, 1970). The light scattering results suggest that the addition of xanthan gum did not affect the swelling of the starch granules in this case, *i.e.*, there was little change in mean particle diameter in the absence or presence of xanthan (**Figure 4b**, **Table 1**).

After heating, the PSDs of the fat droplet-starch mixtures (pH 3) only had a single peak around a particle diameter of 30 to 40 μm in the absence of xanthan gum (**Figure 4b**). This was probably because the large size of the swollen starch granules dominated the overall light scattering signal, obscuring the contribution from the fat droplets (Wu et al., 2013a; Wu et al., 2013b). In the presence of higher levels of xanthan gum (0.01 or 0.02 wt%), the PSDs contained

an additional small peak around 340 μm (**Figure 4b**), which was probably to the formation of large aggregates.

Additional information on the microstructure of the various systems was obtained by optical and fluorescence microscopy (**Figure 5**). Thermal treatment of the samples caused an appreciable change in their microstructure. The optical microscopy images showed that the starch granules were highly swollen after heating, and that they occupied a large volume of the sample. The confocal microscopy images indicated that the fat droplets were confined to the interstitial regions between the starch granules after thermal treatment. In the absence of xanthan gum, the fat droplets appeared to be evenly dispersed between the starch granules, which is to be expected because they should not be flocculated under these conditions (Section 3.1.1). In the presence of low levels of xanthan (0.005%), the droplets appeared to form filamentous aggregates that coated the surfaces of the starch granules and linked them together. In the presence of intermediate levels of xanthan (0.01 %), the droplets appeared to be present as fibrous structures that surrounded the starch granules. The formation of these fibrous structures was probably due to the fact that xanthan gum is a fairly rigid polyelectrolyte with a high charge density. It may therefore act as an anionic linear backbone for the cationic fat droplets to attach to through electrostatic attraction. For selected samples, the focal point of the microscope was adjusted to provide more information about the organization of the fat droplets, which showed that the fat droplet aggregates covered the starch granules (**Figure 6**). Similar results were observed in a previous study, where we found that anionic fat droplets formed a coating around swollen starch granules in the presence of intermediate concentrations of calcium ions (Wu et al., 2013a). At high xanthan levels (0.02 wt%), the fat droplets formed large fiber-like aggregates that were embedded within the starch gel (**Figure 5**).

3.2 Effect of xanthan on macroscopic characteristics

3.2.1 Influence of xanthan on rheological properties

In this section, the rheological properties of heated mixed dispersions (5 wt% oil, 4 wt% starch) containing various levels of xanthan gum were characterized and compared with starch suspensions. The unheated samples were not analyzed because the dense native starch granules rapidly sedimented to the bottom of the containers. The shear viscosity *versus* shear rate profiles of the model emulsions were measured (**Figure 7**). Additionally, the model emulsions were subjected to a shear stress ramping test to measure their yield stress and the obtained values are reported in **Table 1**. All the samples showed shear thinning behavior with a yield stress, but their rheological profile varied appreciably as the xanthan content was changed (**Figure 7, Table 1**). In the absence of xanthan, the model emulsion (5 wt% oil and 4 wt% starch) had a fairly similar rheological profile as pure starch suspensions. At low shear rate ($< 3 \text{ s}^{-1}$), the shear viscosity of the model emulsion was slightly higher than that of the pure starch suspension. In addition, the model emulsion had a slightly higher yield stress than the pure starch suspension (**Table 1**). This effect can likely be ascribed to the higher total effective volume fraction of the particles (starch granules plus fat droplets) in the mixed system (Wu et al., 2013b). As the shear rate increased ($> 3 \text{ s}^{-1}$), the shear viscosity fell below that of the pure suspension, which may be a result of a lubrication effect associated with the fat droplets (Genovese, Lozano, & Rao, 2007; Menut, Seiffert, Sprakel, & Weitz, 2012). Interestingly, the slopes of the log-log plots of shear viscosity *versus* shear rate were fairly similar for all of the samples studied, suggesting that they all exhibited similar structural changes as the shear rate was increased.

As the xanthan concentration increased, the shear viscosity and yield stress of the samples became greater than the control mixed system (0 wt% xanthan) and the pure starch paste (**Figure 7, Table 1**). Differences in the rheological characteristics of model emulsions containing different xanthan levels were attributed to differences in their microstructures (**Figure 5**). The anionic xanthan molecules linked the cationic protein-coated fat droplets together, thereby forming flocs that trapped some of the continuous phase within them. As a result, the effective volume fraction of the disperse phase in the suspensions increased, which led to an increase in the apparent shear viscosity and yield stress (Wu et al., 2013b). When the xanthan level was at 0.005 and 0.01 wt%, the fat droplet aggregates formed in the interstitial region between starch granules, thereby generating a network that trapped starch granules inside (**Figure 5**). Presumably, such structures were more difficult to deform under shear and contributed to the high viscosity and yield stress. In other words, these flocs consisted of starch granules and fat droplets that trapped more continuous phase and substantially increased the effective disperse phase volume fraction. At 0.02 wt% xanthan gum, large irregular fibrous aggregates were formed throughout the interstitial region separating the starch granules. Anisotropic particles often lead to higher viscosities in the low shear region than isotropic ones (van Hecke, 2010; Wolf, Frith, Singleton, Tassieri, & Norton, 2001).

Xanthan gum is widely used as a thickener to increase the viscosity of food products (Gamonpilas et al., 2011; Lee et al., 2013). One may therefore argue that the observed increase in viscosity was simply a result of the presence of the xanthan gum in the aqueous phase. We therefore compared the rheological profiles of the model emulsions containing 0.02 wt% xanthan (pH 3) with two controls: (i) starch suspensions containing 0.02 wt% xanthan (pH 3); and, (ii) model emulsions containing 0.02 wt% xanthan (pH 7). For the pure starch suspension, addition

of xanthan had little effect on the overall rheology (**Figure 6**). This was probably because the xanthan concentration used was too low to substantially contribute to the overall viscosity of the mixed system, and/or because the xanthan molecules could fit into the spaces between the starch large granules. The model emulsions containing xanthan had much lower shear viscosity and yield stress at pH 7 than at pH 3. The fat droplets in the model emulsions containing xanthan at pH 3 were highly aggregated, whereas the ones at pH 7 were not aggregated (**Figures 4 and 5**). This suggests that the increase in viscosity and yield stress in the mixed systems at pH 3 was mainly caused by fat droplet aggregation.

Viscoelastic properties of the model emulsions were characterized using a small amplitude oscillation test and plotted as complex modulus (G^*) *versus* frequency in **Figure 8**. The oscillation test on the model emulsions and starch suspensions revealed that all the samples exhibited predominantly elastic-like behavior with the storage modulus (G') higher than the loss modulus (G'') over the range of frequencies tested (data not shown). Complex modulus (G^*) values were used to indicate the stiffness of the viscoelastic properties of the model emulsions (Norton, Spyropoulos, & Cox, 2010). In the absence of xanthan, the model emulsion had slightly higher complex modulus than the starch suspensions, which was probably due to its higher total effective volume fraction. The presence of 0.02 wt% xanthan did not have an apparent effect on the G^* of the starch suspension. This may have been because the xanthan concentration was too low to have an appreciable effect, the xanthan did not interact with the starch granules, and/or the xanthan molecules could easily pack into the spaces between the starch granules. As the xanthan concentration increased, the model emulsions became stiffer (*i.e.*, increased G^*), which can be attributed to changes in the microstructure and state of fat droplet aggregation. In comparison, the model emulsion at pH 7 had the lowest G^* among all the samples, although it also contained

0.02 wt% xanthan. This further highlighted the importance of fat droplet aggregation on the rheological properties of the samples.

3.2.2 Influence of xanthan on the appearance

The textural attributes of commercial food emulsions, such as sauces, dressings, dips and desserts, are often first perceived through visual observation of their flow characteristics. We therefore took digital photographs of the model emulsions (5 wt% oil, 4 wt% starch) when they were placed on a spoon that was then tilted (**Figure 9**). In the absence of xanthan gum, the model emulsions flowed freely when the spoon was tilted, forming a continuous stream of liquid. At 0.005 wt% xanthan, the model emulsions appeared much thicker and flowed in the form of lumps. At 0.01 or 0.02 wt% xanthan, the samples had a gel-like texture and they flowed much slower. These observations were in agreement with the instrumental rheological measurements: the shear viscosity, yield stress, and stiffness increased as the xanthan content increased (Section 3.2.1). Although xanthan gum has been widely used in low fat products for its thickening functionality, the amount of xanthan used is typically much higher than that used in the current study (Samavati et al., 2012). However, our research has shown that very low levels of xanthan can be used to greatly increase the viscosity of model emulsions based on their ability to induce bridging flocculation of protein-coated droplets through an electrostatic interaction.

Further insights into the visual appearance and perceived texture of model emulsions containing different levels of xanthan gum was obtained by pouring them into the measurement cell of a rheometer. The upper fixture of the rheometer (bob) was then dipped into the lower fixture (cup) at a fixed speed and force, and a digital photograph of the thin layer of sample attached to the bob was taken as it was retracted (**Figure 10**). In the absence of xanthan, the

model emulsion formed a thin film that appeared smooth and glossy. As the xanthan concentration increased, the appearance of the thin films became visibly rougher, which was likely due to the formation of large fat-droplet aggregates through electrostatic interaction. At the highest xanthan content (0.02 wt%), the aggregates were so large that they were clearly visible to the naked eye.

These observations have important consequences for optimizing biopolymer levels to use in commercial products. Although the rheological parameters of the model emulsions increase appreciably with increasing xanthan content, it may not be advisable to add too much xanthan, since the resulting appearance of the product may be unacceptable to consumers. In future studies, it would be useful to test the visual, textural, and flavor properties of these samples using sensory analysis to establish the optimum xanthan content. Alternatively, it may be possible to use a xanthan gum with a lower molecular weight than the one used in the current study or to use another negatively charged polysaccharide with different molecular characteristics to induce droplet aggregation. This is because the molecular weight, charge density, and flexibility of polysaccharide molecules has a strong impact on their electrostatic interactions with proteins (Laneuville, Sanchez, Turgeon, Hardy, & Paquin, 2005; Lii et al., 2002; Schmitt & Turgeon, 2011).

4 Conclusions

This study investigated the influence of xanthan gum on the microstructure and physicochemical properties of model emulsions containing starch granules and protein-coated fat droplets under acidic conditions. These model emulsions have many of the compositional and structural attributes of important commercial food products such as sauces, dressings, dips, and

desserts. At pH 3, xanthan addition promoted flocculation of the emulsion droplets, which was attributed to bridging of the cationic protein-coated droplets by the anionic polysaccharide molecules. The size, morphology, and location of the aggregates formed by the fat-droplets depended strongly on xanthan concentration. In the mixed dispersions containing fat droplets and starch granules, xanthan addition had a significant impact on the microstructure and bulk physicochemical properties of the model emulsions. At low xanthan levels, the fat droplets formed small aggregates and coated the surfaces of the swollen starch granules. At intermediate xanthan levels, in addition to small aggregates coating the starch surfaces, there was also a fraction of large fibrous aggregates present. At high xanthan levels, the fat droplets formed large aggregates with fibrous shapes that were distributed in the interstitial region between the starch granules. As the xanthan level increased, the viscosity, yield stress, and complex modulus of the model emulsions increased accordingly, which may be desirable for improving the textural attributes of reduced fat products. The origin of this effect was mainly attributed to the xanthan molecules promoting fat droplet aggregation, rather than due to their ability to thicken the aqueous phase. Thus the amount of xanthan required to form highly viscous or gel-like emulsions was much lower than the level typically required when xanthan is used solely as a thickening agent. However, addition of high levels of xanthan may have adverse effects on the overall appearance of food emulsions: emulsions were produced that had visibly rough surface textures. Therefore, future studies are needed using sensory analysis to test the potential of this type of controlled fat droplet aggregation to create reduced-fat products. Additionally, polysaccharides with different molecular weights, charge densities, or conformations could be investigated for their potential for modulating the rheology and appearance of emulsion-based products based on the bridging flocculation mechanism.

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Figure 1. Influence of xanthan concentration (0 - 0.02 wt% xanthan) and thermal treatment (90 °C, 5 min) on the ζ -potential of particles in simple emulsions (5 wt% oil), starch dispersion (4 wt% starch), and mixed dispersions (5 wt% oil + 4 wt% starch) at pH 3. Here: E = Emulsion; S = Starch granules; Mix = Oil droplets + Starch granules; U = Unheated; and H = Heated.

Figure 2. Influence of xanthan concentration (0 - 0.02 wt% xanthan) and thermal treatment (90 °C, 5 min) on the particle size distribution of simple emulsions (5 wt% oil) at pH 3. Here: E = Emulsion; X = Xanthan gum; U = Unheated; and H = Heated.

Figure 3. Influence of xanthan concentration (0 - 0.02 wt% xanthan) and thermal treatment (90 °C, 5 min) on the microstructure of simple emulsions (5 wt% oil) at pH 3. Here: X = Xanthan gum. Oil was dyed with Nile red which is showed in red color. The scale bars are 50 μ m in length. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Figure 4. Influence of xanthan concentration (0 - 0.02 wt%) on particle size distribution of mixed dispersions (5 wt% oil + 4 wt% starch) at pH 3 (a) before and (b) after thermal treatment (90 °C, 5 min). The particle size distribution of the mixed dispersion (5 wt% oil + 4 wt% starch) at pH 7 and starch dispersion (4 wt% starch) containing 0.02 wt% xanthan were plotted for comparison. Here: S = Starch granules; Mix = Oil droplets + Starch granules; X = Xanthan gum.

Figure 5. Influence of xanthan concentration (0 - 0.02 wt% xanthan) and thermal treatment (90 °C, 5 min) on the microstructure of mixed dispersions (5 wt% oil + 4 wt% starch) at pH 3. Here: X = Xanthan gum, S = Starch granules. For each condition, both confocal and DIC images were obtained at the same location of the microscope slide. For the confocal images, oil was dyed with Nile red which is showed in red color. Arrows show location of oil droplets and symbol “S” denotes the starch granules. The scale bars are 50 μm in length. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Figure 6. Confocal and DIC images of heated mixed dispersions (5 wt% oil + 4 wt% starch) containing 0.005 and 0.01 wt% xanthan at pH 3. Here: X = Xanthan gum, S = Starch granules. For each xanthan level, all the images were obtained at the same location of the microscope slide, and the top images and bottom images were obtained at different focal points. For the confocal images, oil was dyed with Nile red which is showed in red color. Arrows show location of oil droplets and symbol “S” denotes the starch granules. The scale bars are 50 µm in length. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Figure 7. Shear viscosity *versus* shear rate of heated mixed dispersions (5 wt% oil + 4 wt% starch) containing various xanthan levels (pH 3). The rheology profile of the mixed dispersion (5 wt% oil + 4 wt% starch) at pH 7 and starch dispersion (4 wt% starch) containing 0.02 wt% xanthan were plotted for comparison. Here: S = Starch granules; Mix = Oil droplets + Starch granules; X = Xanthan gum.

Figure 8. Complex modulus (G^*) *versus* oscillation frequency of heated mixed dispersions (5 wt% oil + 4 wt% starch) containing various xanthan levels at pH 3. The rheology profile of the mixed dispersion (5 wt% oil + 4 wt% starch) at pH 7 and starch dispersion (4 wt% starch) containing 0.02 wt% xanthan were plotted for comparison. Here: S = Starch granules; Mix = Oil droplets + Starch granules; X = Xanthan gum.

Figure 9. Representative images to demonstrate the consistency of mixed dispersions (5 wt% oil + 4 wt% starch) at pH 3 containing (a) 0 wt%, (b) 0.005 wt%, (c) 0.01 wt%, and (d) 0.02 wt% xanthan.

Figure 10. Appearance of mixed dispersions (5 wt% oil + 4 wt% starch) at pH 3 containing (a) 0 wt%, (b) 0.005 wt%, (c) 0.01 wt%, and (d) 0.02 wt% xanthan.

Table 1. Summary of yield stress and volume-based mean diameters of suspensions containing various xanthan levels. All measurements were at pH 3.0, unless stated otherwise. Here: S = Starch granules; Mix = Oil droplets + Starch granules; X = Xanthan gum.

Sample	Composition	Yield stress (Pa)	D _{4,3} (μm)	
			Unheated	Heated
0% Xanthan				
Starch	4% S	0.71 ± 0.30	26.0 ± 5.0	43.0 ± 1.0
Emulsion	5%E	-	0.2 ± 0.0	0.24 ± 0.0
Mix	0% X + 4% S + 5%E	3.9 ± 0.6	19.8 ± 6.2	42.6 ± 0.8
0.005% Xanthan				
Emulsion	5%E	-	17.8 ± 1.3	19.0 ± 1.2
Mix	0.005% X + 4% S + 5%E	11.3 ± 1.0	25.4 ± 4.5	42.8 ± 0.7
0.01% Xanthan				
Emulsion	5%E	-	70 ± 44	77 ± 44
Mix	0.01% X + 4% S + 5%E	12.75 ± 0.67	109.0 ± 1.6	55.5 ± 5.3
0.02% Xanthan				
Starch	0.02% X + 4% S	1.89 ± 0.05	22.4 ± 0.2	43.1 ± 1.0
Emulsion	5%E	-	334 ± 52	287 ± 119
Mix	0.02% X + 4% S + 5%E	16.5 ± 0.5	399 ± 18	108.2 ± 0.5
Mix (pH 7)	0.02% X + 4% S + 5%E	2.77 ± 0.13	13.1 ± 0.2	37.6 ± 0.0