Designing reduced-fat food emulsions: Locust bean gum–fat droplet interactions

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ABSTRACT

The influence of locust bean gum (LBG) on the physicochemical properties of model food sauces containing fat droplets was investigated. The particle size, microstructure, optical lightness, rheology, and storage stability of aqueous solutions and oil-in-water emulsions containing different LBG concentrations (0.05–1 wt%) were measured. Non-dissolved hydrogel microparticles were observed in both aqueous solutions and emulsions above a certain LBG level ($\geq$0.4%). The mean particle diameter ($d_{4,3}$) and apparent viscosity of the emulsions increased steeply when the LBG concentration exceeded about 0.2–0.4%, while the lightness and flocculation stability decreased. The emulsions were highly prone to creaming and phase separation at intermediate LBG concentrations (0.2–0.8%). The changes in the physicochemical properties of the emulsions with increasing LBG concentration were attributed to a number of factors: (i) viscosity enhancement; (ii) depletion flocculation; (iii) hydrogel formation. These results have important implications for the rational design and production of reduced-fat food emulsions, such as sauces, dressings, and deserts.

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1. Introduction

Awareness of the critical importance of diet on human health and wellness is growing among consumers, regulatory organizations, and the food industry (Chaput, Doucet, & Tremblay, 2012; Milliron, Woolf, & Appelhans, 2012). In particular, there has been increasing concern about the rise of chronic diseases related to overconsumption of calories, such as overweight, obesity, heart disease, hypertension, and diabetes. Academic, government, and industrial research laboratories have therefore been actively involved in formulating reduced-calorie foods, such as low-fat or fat-free versions of traditional food products (Hoefkens, Verbeke, & Van Camp, 2011; Nehir El & Simsek, 2012). However, reduction or elimination of fats often compromises the physicochemical attributes (optical properties, rheology, stability) and sensory quality (appearance, flavor profile, and textural attributes) of food products (Bayarri, Taylor, & Joanne, 2006; Gonzalez-Tomas, Bayarri, Taylor, & Costell, 2007; McClements, 2002a). This is because fats play multiple roles in determining the overall properties of food products, particularly when the fats are in an emulsified state. For example, fat droplets

influence the appearance (optical properties), flavor profile (molecular partitioning), textural attributes (rheology), and shelf-life (stability) of food emulsions (Benjamins, Vingerhoeds, Zoet, de Hoog, & van Aken, 2009; McClements & Demetriades, 1998; Mun et al., 2009).

Food hydrocolloids (such as starches, gums, and proteins) are widely utilized in reduced-fat foods to replace some or all of the desirable characteristics normally provided by the fat droplets (Bayarri, Chulia, & Costell, 2010; Torres, Janhoj, Mikkelsen, & Ipsen, 2011). The ability of hydrocolloids to modify emulsion properties depends on many factors, including their molecular characteristics (e.g., molar mass, branching, conformation, charge, hydrophobicity, concentration, and interactions) and their impact on bulk physicochemical properties (e.g., light scattering, thickening, gelling, stability) (Dickinson & Stainsby, 1988; McClements, 2005). Hydrocolloids may either be used in isolation or in combination to simulate specific fat droplet properties. When used in combination they may contribute independently, synergistically, or antagonistically to particular system properties depending on the nature of the molecular interactions involved (Bayarri et al., 2010; BeMillier, 2011; Dolz, Hernandez, Delegido, Alfaro, & Munoz, 2007). Food hydrocolloids increase the viscosity of aqueous solutions, and are therefore able to provide some of the textural attributes usually associated with high fat droplet contents (Bayarri et al., 2010; Korus, Juszczak, Witczak, & Achremowicz, 2004; Singh, Kaur, &

McCarthy, 2007). The ability of hydrocolloids to thicken aqueous solutions is also useful for inhibiting fat droplet creaming, which may occur when the fat droplet concentration is reduced below a level where the close-packing of droplets is no longer possible (Dickinson & Stainsby, 1988; McClements, 2005). Some hydrocolloids may also provide desirable optical properties to reduced-fat products due to their ability to scatter light waves and produce a cloudy appearance (Chantrapornchai, Clydesdale, & McClements, 1999). Finally, the addition of polysaccharide gums to foods may also provide desirable nutritional benefits, such as those associated with consumption of dietary fiber (Laneuville, Paquin, & Turgeon, 2005; Ward, 1997).

It should be pointed out that incorporation of hydrocolloids into emulsions may also have unintended adverse effects on reduced-fat systems. Hydrocolloid addition may accelerate the rate and extent of physical instability (creaming and phase separation) through depletion or bridging flocculation mechanisms if the type and amount of hydrocolloids added is not controlled (Coa, Dickinson, & Wedlock, 1990; Dickinson & Pawlowsky, 1997; Ye, Hemar, & Singh, 2004). Addition of some hydrocolloids may cause undesirable changes in the appearance, texture, or mouthfeel of commercial products, e.g., a slimy texture or appearance.

This study is part of an ongoing research program within our laboratory to understand the major factors influencing the formulation of high quality reduced-fat food emulsions. The main aim of this study was to understand the influence of a hydrocolloid (locust bean gum, LBG) on the physical properties and stability of model reduced-fat sauces. For the sake of clarity, the study is divided into two parts. In this paper, we examine the influence of LBG addition on the properties of a simple model sauce that consisted of fat droplets dispersed within water. In a subsequent paper, we will examine the influence of LBG addition on the properties of a more complex model sauce consisting of a mixture of fat droplets and swollen starch granules.

LBG is a non-ionic highly branched water-soluble polysaccharide (Cui, 2005). It was selected for this study because it is widely used in the food industry to modify the texture and stability of food and beverage products. A previous study has examined the possibility of replacing starch with LBG and other hydrocolloids within model salad dressings (Dolz, Hernandez, & Delegido, 2008). These researchers reported that LBG addition could be used to replace part of the textural characteristics lost when starch was removed from the samples.

2. Materials and methods

2.1. Materials

Fat (Canola oil) and locust bean gum (LBG) were provided by ConAgra Foods (Omaha, Nebraska, USA). Tween® 80 was purchased from Sigma–Aldrich (St Louis, MO). The average molecular weight (MW) of the LBG used in this study was determined to be 1800 kDa by size-exclusion chromatography (Spectrometry Facility, University of Massachusetts, Amherst, MA). The average molecular weight was estimated using Pullulan starch as a standard (Maximum MW = 800 kDa) and double distilled water as a solvent. This molecular weight corresponds to a viscometric radius of LGB of about 90 nm (Pollard, Eder, Fischer, & Windhab, 2010).

2.2. Methods

2.2.1. Locust bean gum dispersions

Locust bean gum dispersions with concentrations ranging from 0.05 to 1.0% were prepared. A weighed amount of LBG powder was dispersed in double distilled water (room temperature) and

heated to 90 °C for 5 min holding time with continuous stirring (~400 rpm). The heated suspensions were cooled to room temperature in a water bath and were then analyzed for their physical properties. After this process, the LBG solutions had pH values around 6.3 ± 0.5.

2.2.2. Oil-in-water emulsions

Oil-in-water (O/W) emulsions containing 10 wt% canola oil and 1 wt% Tween 80 were prepared. Firstly, surfactant solutions were prepared by dispersing weighed amount of Tween 80 in double distilled water and then warming at 40 °C until the surfactant was fully dissolved. Coarse emulsions were then produced by blending the oil phase and the aqueous surfactant solution together using a handheld mixer at 15,000 rpm for 60 s. The coarse emulsions produced were then passed through a two-stage homogenizer at 5000 psi for 4 passes to reduce the droplet size and degree of polydispersity (LAB 1000, APV-Gaulin, Wilmington, MA).

2.2.3. Locust bean gum and emulsion mixed systems

A series of emulsions were prepared that had the same fat content (5%) but different LBG concentrations (0–1%). A weighed amount of LBG was dispersed in the 5% O/W emulsions and the mixtures were heated to 90 °C for 5 min holding time with continuous stirring (~400 rpm). The heated mixtures were then cooled to room temperature in a water bath prior to analysis. The mixed systems had pH values ranging from 6.0 to 6.3, with the pH increasing slightly with increasing LBG content.

2.3. System characterization

2.3.1. Particle size

The particle size distribution of all the systems was measured using a laser diffraction particle size analyzer (Mastersizer 2000, Malvern Instruments, Ltd., Worcestershire, U.K.). Samples were diluted by adding small aliquots into a measurement chamber containing water until the instrument gave an optimum obscuration rate between 10 and 20%. The particle size distribution was calculated from the light scattering pattern using Mie theory. A refractive index of 1.33 was used for the aqueous phase, 1.472 for the oil phase (emulsions) and hydrogel phase (LBG microparticles). The actual refractive index of LBG microparticles was unknown, and so the particle size data obtained by light scattering on these systems should be treated with some caution. Particle size measurements are reported as volume-weighted mean diameters ($d_{4,3}$) and surface-weighted mean diameters ($d_{3,2}$).

2.3.2. Microstructure analysis

The microstructure of all systems was examined using optical microscopy with a 60× objective lens and 10× eyepiece (Nikon D-Eclipse C1 80i, Nikon, Melville, NY, U.S.). A small aliquot of each sample was placed on a microscope slide and covered with a cover slip prior to analysis. The microstructure images were analyzed using image analysis software (Nikon, Melville, NY).

2.3.3. Optical properties (lightness)

The lightness (L^*) of the samples was measured using a colorimeter (ColorFlez EZ, HunterLab, Reston, VA) with a tristimulus absorption filter (HunterLab, 2008). The lightness value ranges from 0 (i.e., black) to 100 (i.e., white) (HunterLab, 2008; Leon, Mery, Pedreschi, & Leon, 2006).

2.3.4. Flow behavior and apparent viscosity

The apparent shear viscosity of all systems was measured using a dynamic shear rheometer (Kinexus Rheometer, Malvern

Instruments Ltd., MA, U.S.A.) with a cup-and-bob measurement cell. All measurements were performed using shear rates from 0.1 to 100 s^{-1} at 37 °C. The control and analysis of the rheological measurements were performed using instrument software (Kinexus rSpace, version 1.30, Malvern Instruments Ltd., MA). The apparent shear viscosity results are reported at 10 s^{-1}, a rate that is similar to that reported for the mastication of semi-solid foods in the mouth (Shama & Sherman, 1973). In reality, the most appropriate shear rate(s) to use to accurately simulate oral conditions is likely to be highly system dependent. We found similar trends across the range of shear rates measured in this study (0.1–100 s^{-1}), and so our findings should be generally applicable. The experimental rheological data were fitted using a power law model: $\tau = K\gamma^n$, where τ is the shear stress, γ is the shear rate, K is the consistency index, and n is the power law index.

2.3.5. Storage stability

The physical stability of heated mixed systems (LBG + O/W emulsions) was determined visually during 48 h storage. Each fresh sample was poured into a glass test tube, sealed with a cap, and then stored at room temperature (25 °C). A system was deemed unstable to gravitational separation when its appearance becomes inhomogeneous, e.g., due to the formation of distinct layers at the top (droplet-rich) and/or bottom (droplet-depleted). In systems where a distinct cream layer was formed at the top the instability was determined by measuring the height of the cream layer (H_c) and the total emulsion (H_t):

$$\text{Extent of Creaming} = (H_c/H_t) \times 100 \qquad (1)$$

2.4. Statistical analysis

Each experiment was carried out in duplicate or triplicate, and the mean and standard deviations were calculated from these values using Microsoft Excel 2011.

3. Results and discussion

3.1. Particle size distribution and microstructure

3.1.1. Locust bean gum solutions

In principle, one would expect that the polysaccharide molecules would completely dissolve in the heated aqueous LBG solutions. In practice, we observed that relatively large particles persisted within the solutions containing relatively high LBG concentrations. For example, 1% LBG solutions had a relatively large mean particle diameter ($d_{4,3} \sim 239$ μm), which can be attributed to a population of large particles in the particle size distribution (Fig. 1a). On the other hand, we did not observe any measureable particles in the polysaccharide solutions with lower LBG concentrations (<0.8%). Optical microscopy images of the 1% LBG solutions also show evidence of relatively large particles (Fig. 2a). The physicochemical origin of this effect may be due to a steric exclusion effect that occurs when polysaccharide molecules overlap in aqueous solutions. Polymer overlap is unfavorable because it reduces the conformational entropy of the system, and therefore the polymers may adopt a conformation that reduces the extent of the overlap. This could be achieved by the polymer molecules associating with their neighbors to form ordered regions, such as liquid crystalline phases or microgels (Chronakis & Ramzi, 2002). It is known that many polysaccharides form helices in aqueous solutions that can promote polysaccharide–polysaccharide association (Ikeda & Shishido, 2005; Ikeda & Zhing, 2012). The LBG microparticles observed in this study may therefore be the result of polymer association at high LBG concentrations.

3.1.2. Emulsions

The particle size distribution of the emulsions was also measured so as to determine the initial size of the fat droplets used in the mixed systems. The emulsions had mean particle diameters ($d_{4,3}$) of ∼0.24 μm as measured by light scattering (Fig. 1a), and small oil droplets were observed in the optical microscopy images (Fig. 2b).
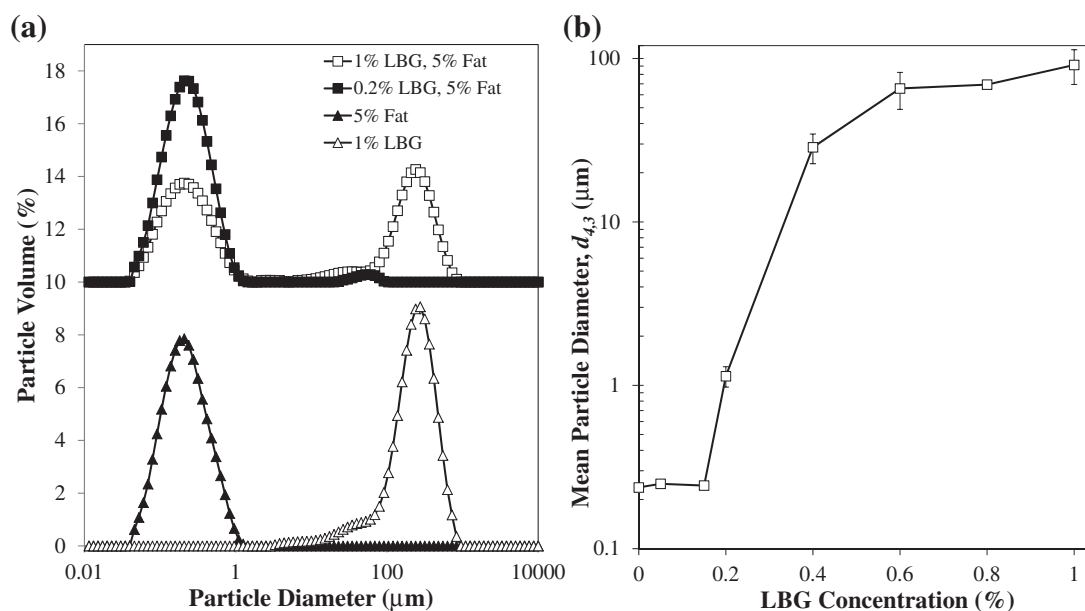

Fig. 1. a. Particle size distributions of single and mixed samples: 1% LBG solution; 5% O/W emulsion; 0.2% LBG + 5% O/W emulsion; 1% LBG + 5% O/W emulsion. The particle volume has been shifted up the y-axis by an increment of 10 for the two top curves so as to more clearly distinguish data sets. b. Influence of LBG concentration on mean particle diameter ($d_{4,3}$) of mixed systems containing LBG and 5% O/W emulsions.
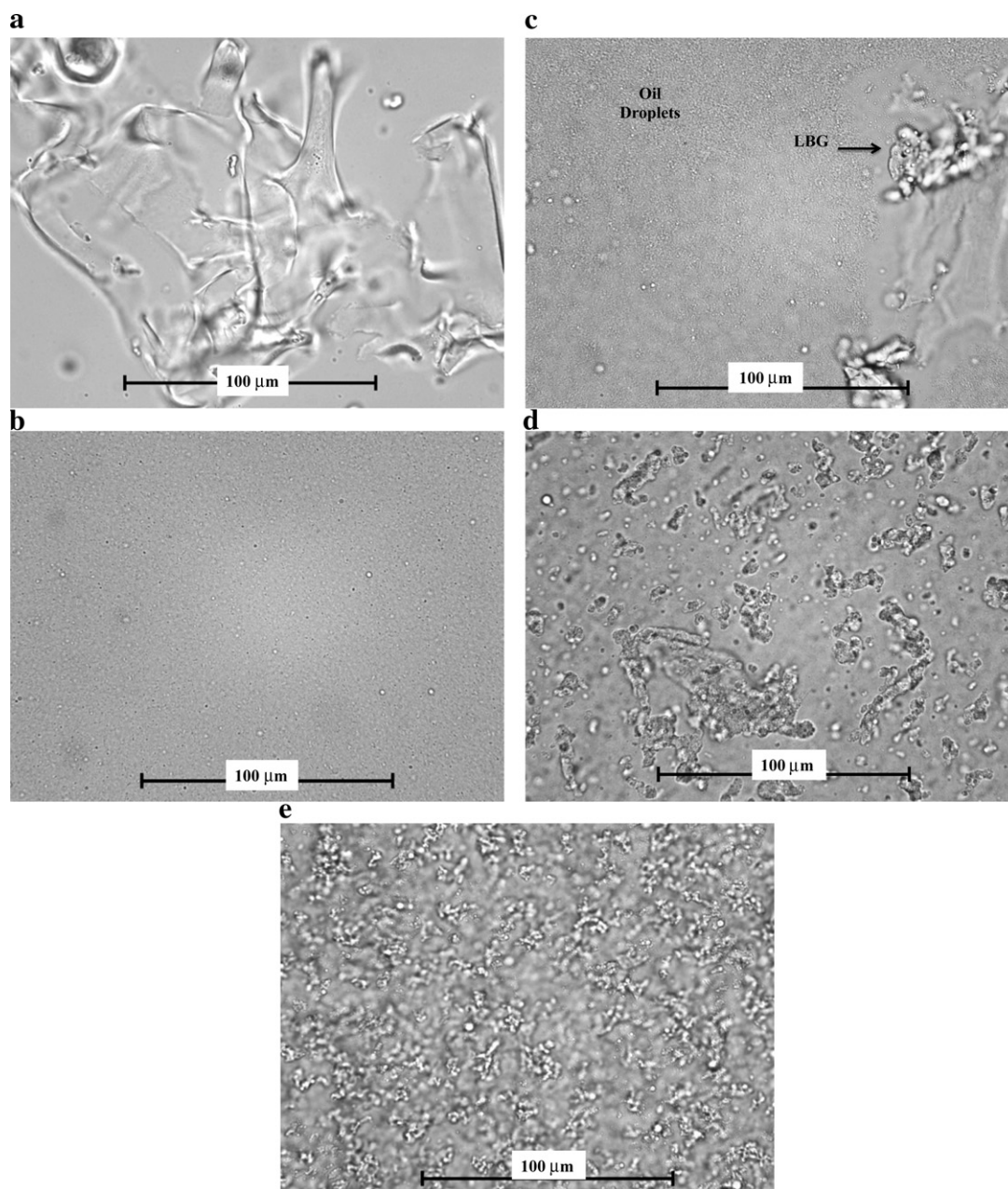
Fig. 2. Optical micrographs (60× magnification): (a) 1% locust bean gum solution; (b) 5% fat droplet emulsion; (c) 0.2% locust bean gum–5% fat droplet system; (d) 0.4% locust bean gum–5% fat droplet system; (e) 1.0% locust bean gum–5% fat droplet system. Scale at the bottom of each image represents 100 µm in length.

3.1.3. Mixed systems

The measured particle size of the mixed systems depended on the concentration of LBG within the aqueous phase of the emulsions. The mixed systems containing 0.05 and 0.15% LBG had mono-modal particle size distributions ($d_{4,3}$ of 0.24 µm), which were similar to those observed for the control emulsions (without LBG). On the other hand, the mixed systems containing ≥0.2% LBG had bi-modal particle size distributions (Fig. 1a) and relatively large mean particle diameters (Fig. 1b). The large particles observed in the mixed systems at high LBG concentrations may have been due to aggregation of the fat droplets and/or due to the presence of non-dissolved LBG microparticles. Optical microscopy images suggested that the mixed systems containing high LBG levels (Fig. 2c–e) contained particles that had a similar appearance to the non-dissolved LBG microparticles observed at high polymer concentrations in the aqueous

solutions (Fig. 2a). However, the optical microscopy images also suggested that the fat droplets in the mixed systems were aggregated with each other and associated with LBG microparticles at high LBG concentrations (Fig. 2d–e). In general, there appeared to be a distinct change in the microstructure of the mixed systems between 0.2 and 0.4% LBG, with individual fat droplets being prominent at ≤0.2% LBG, but highly aggregated fat droplets and LBG microparticles being observed at ≥0.4% LBG.

3.2. Lightness (optical properties)

3.2.1. Locust bean gum solutions

The influence of LBG concentration on the lightness (L^*) of the LBG solutions and the mixed systems was determined. The lightness of the LBG solutions was similar to that of a polymer-free

aqueous solution at low LBG levels (0.05%), but increased steeply when the LBG concentration was increased from 0.15 to 0.2% (Fig. 3a). This increase in lightness suggested that the LBG solutions contained particles that scattered light (McClements, 2002b), which may have been the non-dissolved LBG microparticles observed by optical microscopy (Fig. 2a). Visually, these systems appeared relatively translucent and had brownish particles dispersed throughout them at higher LBG concentrations.

3.2.2. Mixed systems

The O/W emulsions (without LBG) had a higher lightness ($L^* \approx 95$) than the LBG solutions ($L^* \approx 37$). Visually, the emulsions were optically opaque with a milk-like appearance. For the mixed systems, the lightness decreased with increasing LBG concentration, with a steep decrease occurring between 0.2% and 0.4% LBG (Fig. 3b). These results therefore correspond to the light scattering experiments, which showed that a steep increase in mean particle size occurred over the same LBG concentration range (Fig. 1b). The lightness of an emulsion depends on the fraction of light backscattered from its surface, which depends on the size and aggregation state of the particles (McClements, 2002b). The aggregation of the fat droplets within the mixed systems due to the presence of LBG may therefore account for the observed decrease in lightness.

3.3. Flow behavior

The influence of LBG concentration on the flow behavior of aqueous solutions and emulsions was also examined. Overall, both types of system exhibited fairly similar rheological behavior and trends. Flow curves (shear stress vs. shear rate) for aqueous solutions containing different levels of LBG are shown in Fig. 4a. All the LBG solutions exhibited non-ideal (shear thinning) behavior, with the shear stress increasing non-linearly with increasing shear rate (or apparent viscosity decreasing with increasing shear rate (data not shown)). These results are in agreement with other authors who also reported shear thinning behavior in LBG-containing systems (Dolz et al., 2007; Mandala, Savvas, & Kostaropoulos, 2004). The rheological characteristics of the samples were characterized by fitting a power-law model to the experimental data and

calculating the consistency and power law index (Table 1). As expected, the consistency increased with increasing LBG concentration (indicating the samples became more viscous), whereas the power law index decreased (indicating the samples became more shear-thinning). The apparent viscosity of the aqueous solutions measured at a fixed shear rate (10 s^{-1}) increased with increasing LBG concentration, with a steep increase occurring above 0.4% LBG (Fig. 5). The polymer concentration where a steep increase in apparent viscosity occurs is related to the region where the polymer molecules start to overlap and interact with each other (Hunter, 1986; Pal, Yan, & Masliyah, 1992).

The flow behavior of polymer solutions (1% LBG), emulsions (5% fat), and mixed systems (1% LBG and 5% fat) were also compared directly (Fig. 4b). These measurements showed that the overall rheological properties of the mixed systems were dominated by the presence of the LBG, rather than the presence of the fat droplets. This is to be expected since the rheological properties of dilute colloidal dispersions can be described by the Einstein equation (Hunter, 1986; Pal et al., 1992):

$$\eta = \eta_0(1 + 2.5\phi) \tag{2}$$

Where η is the viscosity of the colloidal dispersion, η_0 is the viscosity of the continuous phase, and ϕ is the volume faction of the dispersed particles. This equation shows that the viscosity of a colloidal dispersion is proportional to the viscosity of the continuous phase. In our experiments, the viscosity of the continuous phase in the mixed systems (1% LBG solution) was much greater than the viscosity of the continuous phase in the emulsions (water). The addition of 5% fat droplets to the LBG solution would be equivalent to having a disperse phase volume fraction of about 0.05, which means that the viscosity of the mixed system should only be about 1.125 times higher than that of the LBG solution according to Equation (2).

The influence of LBG concentration on the apparent viscosity of aqueous solutions and emulsions at a fixed shear rate (10 s^{-1}) is compared in Fig. 5, while its influence on the consistency and power law index of the same systems is shown in Table 1. The apparent viscosity increased with increasing LBG concentration
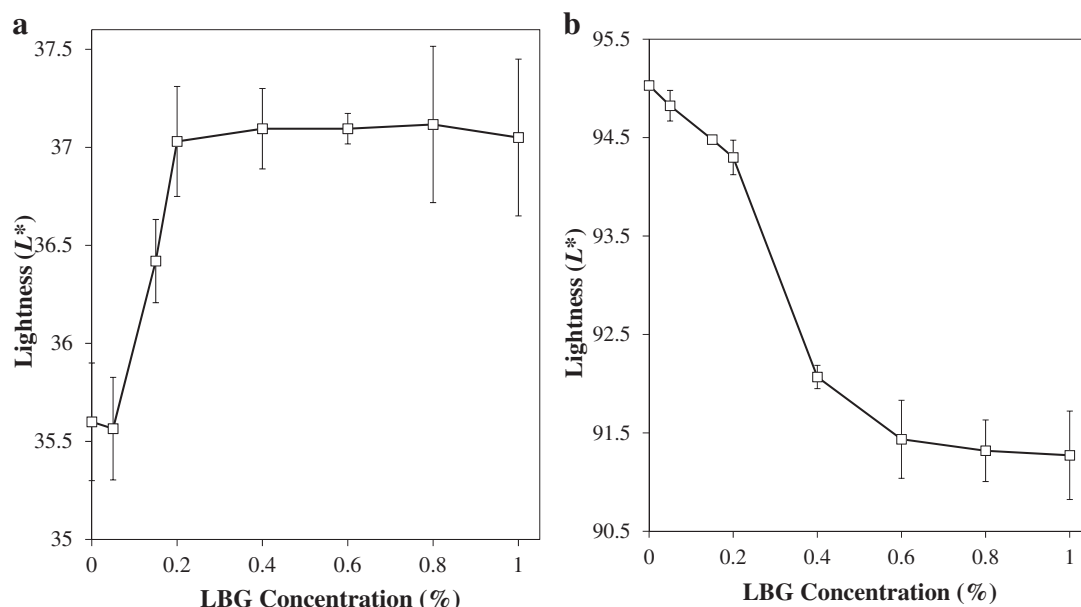

Fig. 3. Influence of LBG concentration on the optical properties (lightness, L^*) of a) locust bean gum solutions and b) locust bean gum–O/W emulsion mixed systems.
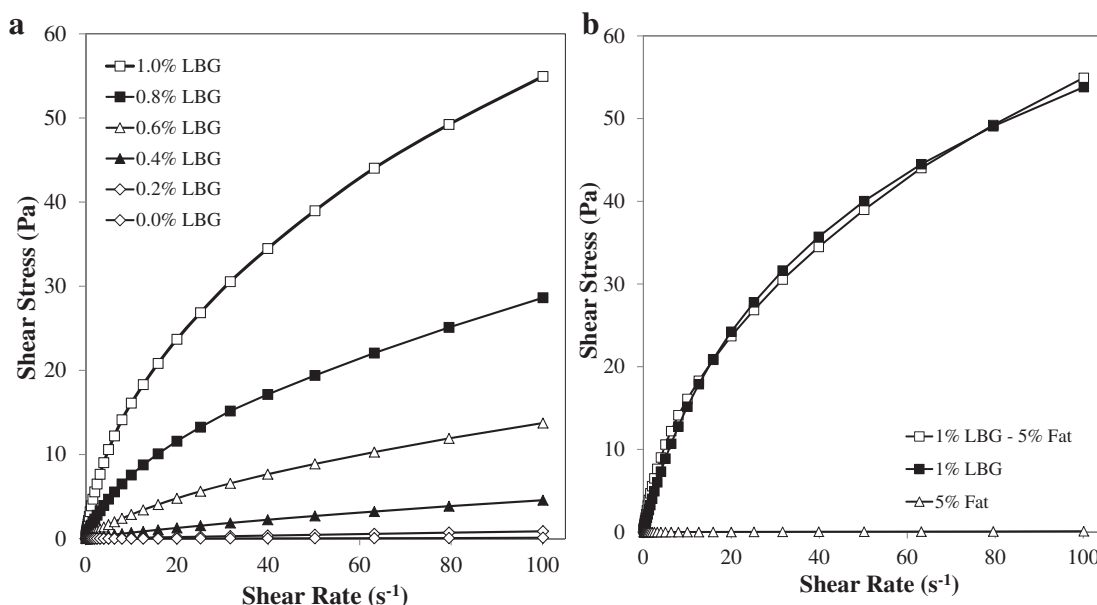
Fig. 4. Typical flow behavior (shear stress *vs.* shear rate) measured at 37 °C of (a) LBG solutions containing different LBG concentrations; (b) LBG solutions (1%), O/W emulsions (5% fat), and mixed systems (1% LBG and 5% fat).

fairly similarly in both systems (Fig. 5). In addition, the consistency increased and the power law index decreased with increasing LBG concentration in a similar manner in both systems (Table 1). As mentioned earlier, the fairly similar impact of LBG concentration on the rheological properties of the aqueous solutions and the emulsions can be attributed to the fact that the overall rheology was dominated by the properties of the LBG rather than the fat droplets. Having said this, there were some deviations between the experimentally measured apparent viscosity values and those predicted using Equation (2) at higher LBG concentrations (Fig. 5), which may have been due to fat droplet aggregation or changes in LBG microparticle structure discussed earlier. Our results suggest that flocs formed by the depletion mechanism were disrupted by shear, since the flow behavior of the mixed systems followed similar trends as the LBG solutions containing no droplets.

The steep increase in the apparent viscosity of the aqueous solutions and emulsions above a critical LBG level can be attributed to close packing of the dispersed particles within the system (Hunter, 1986; Pal et al., 1992). These dispersed particles may be fully dissolved and dispersed LBG molecules, LBG microparticles and/or fat droplets depending on the system. The apparent viscosity of concentrated colloidal dispersions can be described by semi-empirical models such as the one shown below (McClements, 2005):

$$\eta = \eta_0 \left(1 - \frac{\phi_{\text{eff}}}{\phi_c} \right)^{-2} \tag{3}$$

Here, ϕ_{eff} is the effective volume fraction of the dispersed particles and ϕ_c is a critical packing parameter (≈ 0.6–0.7). For the mixed colloidal dispersions used in this work: $\phi_{\text{eff}} = \phi_{\text{LBG},d} + \phi_{\text{LBG},m} + \phi_f$, where $\phi_{\text{LBG},d}$, $\phi_{\text{LBG},m}$ and ϕ_f are the effective volume fractions of the dissolved LBG, LBG microspheres, and fat droplets, respectively. This equation predicts that as the effective volume fraction of the dispersed particles increases and approaches the critical packing parameter there is a steep increase in apparent viscosity (McClements, 2005). In the present study, the steep increase in apparent viscosity that occurs around 0.4% LBG suggests that the highly hydrated LBG molecules and/or microparticles start to overlap with each other around this concentration. Interestingly this concentration is close to the value where the LBG microparticles were first observed, and where depletion flocculation is observed (see Section 3.4).

The formation of the highly viscous systems at higher LBG concentrations may have benefits for improving the texture and mouthfeel of reduced fat products. In addition, the fact that some of the fat droplets were trapped within LBG microparticles at high LBG levels may have a desirable impact on the flavor profile of reduced fat products. Previous studies have shown that the flavor release profile of non-polar flavor molecules is delayed when they are

Table 1
Parameters derived from power law model fit for the different systems with varying locust bean gum concentrations.

| LBG content (%)	LBG only			LBG + 5% fat droplets		
	Consistency (k_1) (Pa.s)	Power law index (n)	Correlation coefficient (r^2)	Consistency (k_1) (Pa.s)	Power law index (n)	Correlation coefficient (r^2)
0	0.0005	0.93	0.967	0.0005	1.15	0.989
0.05	0.0012	1.04	0.998	0.0014	1.04	0.995
0.15	0.0047	0.98	1.000	0.0055	0.98	1.000
0.2	0.0075	0.97	1.000	0.011	0.96	1.000
0.4	0.065	0.90	1.000	0.11	0.82	1.000
0.6	0.36	0.79	0.998	0.58	0.69	0.999
0.8	1.01	0.71	0.997	1.96	0.59	0.999
1.0	3.98	0.58	0.995	4.59	0.54	1.000
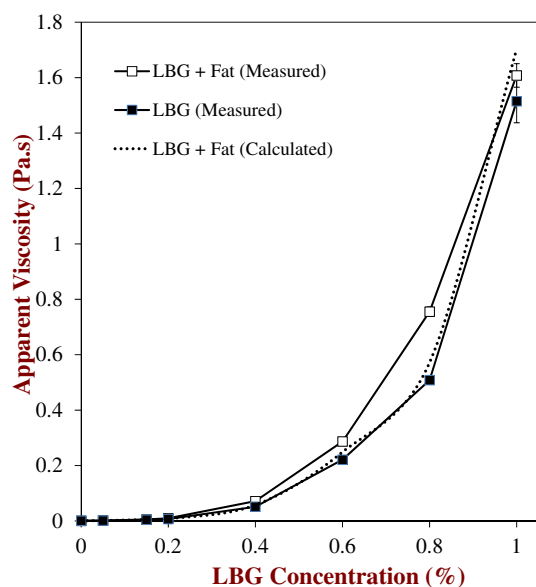
Fig. 5. Influence of LBG concentration on the apparent viscosities of aqueous solutions and emulsions containing 5% fat (10 s^{-1}, 37 °C). The calculated values for the mixed system were determined using Einstein's equation (see text for details).

Fig. 6. Influence of LBG concentration on the stability of 5% oil-in-water emulsions to creaming and/or phase separation. Samples were stored at room temperature (25 °C) for 48 h prior to measurements. Inset photograph shows instability of samples (0.2, 0.4, 0.6, 0.8 and 1% LBG, from left to right) after storage. Separation and/or inhomogeneity in samples containing 0.2, 0.8 and 1% LBG were not clearly noticeable from the photograph.

encapsulated within hydrogel particles because of the increase in their diffusion path length (Malone & Appelqvist, 2003; Malone, Appelqvist, & Norton, 2003). This phenomenon has previously been utilized to produce reduced-fat emulsions that have similar flavor release profiles as high fat emulsions (Malone et al., 2003). On the other hand, the relatively large size of the LBG microparticles may alter the mouthfeel of the product by being detected as individual entities.

3.4. Storage stability

Finally, we examined the influence of LBG concentration on the storage stability of mixed systems containing LBG and fat droplets. The stability of the mixed systems to gravitational separation was determined by visual observation after 48 h storage at room temperature (~25 °C). The stability of the mixed systems was strongly influenced by the concentration of LBG present (Fig. 6). At relatively low LBG concentrations (≤0.15%), the emulsions were stable to visible phase separation, but at higher LBG concentrations a distinct cream layer was observed at the top of the samples. In some samples, the serum layer below the cream layer was relatively clear, but in other samples the serum layer was highly turbid or opaque. The formation of the cream layer was attributed to droplet flocculation, which would lead to an increase in particle size and therefore accelerated creaming (Dickinson, 2003; Gulseren & Corredig, 2011; Jang, Nikolov, & Wasan, 2004). The driving force for flocculation was attributed to an increase in the osmotic attraction between the droplets resulting from the presence of non-adsorbed polymer in the aqueous phase, i.e., depletion flocculation (Coa et al., 1990; Jenkins & Snowden, 1996). At sufficiently low LBG concentrations (<0.2%), the emulsions were stable to flocculation because the repulsive forces acting between the droplets (mainly steric repulsion associated with the hydrophilic head-groups of the adsorbed surfactant molecules) was strong enough to overcome the attractive interactions (mainly van der Waals and weak depletion attraction) (Demetriades & McClements, 1998; Dickinson, 2003). At higher LBG concentrations, the depletion attraction was strong enough for the overall attractive interactions to dominate the repulsive interactions, leading to droplet flocculation and
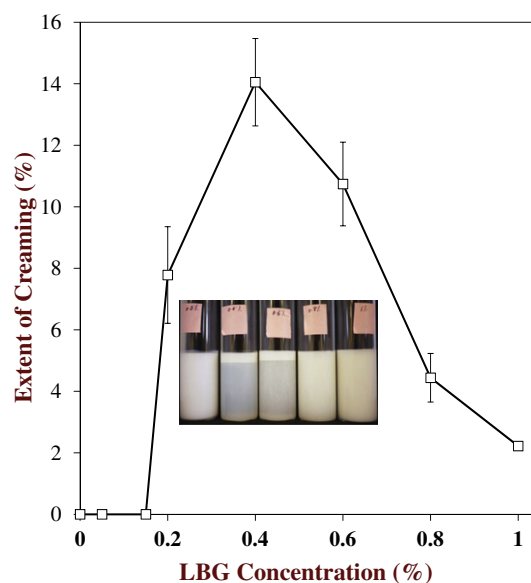
creaming. However, we observed a decrease in creaming as the LBG concentration was increased further (≥0.6%), which was attributed to the inhibition of droplet movement due to the formation of a three-dimensional network of aggregated fat droplets and/or an increase in the continuous phase viscosity (Dickinson et al., 1988; Nikiforidis, Biliaderis, & Kiosseoglou, 2012; Perrechil & Cunha, 2010). Although no clear serum layer was observed in the emulsions containing the higher LBG concentrations (0.6–1%), we did observe large white flocs dispersed throughout these systems and a whitish cream layer on top (Fig. 6 **inset**).

4. Conclusions

Hydrocolloids are commonly used in food emulsions as fat mimetics due to their viscosity-enhancing capacity. The current study demonstrated that the appearance, rheological properties, and stability of oil-in-water emulsions were strongly influenced by locust bean gum (LBG) concentration. The mean particle size and apparent viscosity of the emulsions increased with increasing LBG concentration, whereas the lightness and flocculation stability decreased. The emulsions appeared relatively stable to creaming and phase separation at low and high LBG concentrations, but exhibited pronounced phase separation at intermediate LBG levels. These changes in the bulk physiochemical properties of the emulsions were related to changes in the microstructure of the mixed systems, i.e., the structural organization and interactions of the LBG molecules and fat droplets within the system. We found that LBG formed non-dissolved microparticles at relatively high concentrations (≥0.4%), and that these microparticles trapped fat droplets inside them. We also found that high levels of LBG (≥0.2%) promoted fat droplet flocculation through a depletion mechanism, which resulted in rapid creaming at intermediate LBG concentrations (0.2–0.8%). At higher LBG concentrations, creaming was inhibited because the high viscosity of the aqueous phase and the formation of a three-dimensional network of aggregated fat droplets inhibited the movement of the fat droplets. These phenomena may play an important role in determining the flavor profile,

stability, and texture of reduced-fat products. It is known that trapping fat droplets within hydrogel particles helps balance the flavor profile of reduced-fat products by delaying flavor release so that they behave more similarly to high fat products. The tendency for creaming and phase separation to occur at intermediate LBG concentrations has important implications for optimizing the formulation of stable reduced-fat products. In general, the influence of a hydrocolloid on the stability and physicochemical characteristics of emulsions depends on its molecular characteristics (such as molar mass, conformation, charge, and hydrophobicity). Consequently, hydrocolloids other than the LBG used in this study may exhibit different kinds of behavior.

Acknowledgments

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