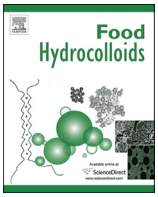
Food Hydrocolloids 63 (2017) 170–177

Contents lists available at ScienceDirect

Food Hydrocolloids

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



Droplet size distribution, rheological behavior and stability of corn oil emulsions stabilized by a novel hydrocolloid (Brea gum) compared with gum arabic

Virginia Castel*, Amelia C. Rubiolo, Carlos R. Carrara

Instituto de Tecnología de Alimentos, Facultad de Ingeniería Química, Universidad Nacional del Litoral, Santa Fe, Argentina

ARTICLE INFO

Article history:
Received 29 June 2016
Received in revised form
19 August 2016
Accepted 28 August 2016
Available online 30 August 2016

Keywords:
Brea gum
Emulsion
Rheology
Stability

ABSTRACT

The emulsifying properties of Brea gum (BG), the exudate from *Cercidium praecox*, were studied in comparison to gum Arabic (GA). Droplet size distributions, rheological properties and stability of corn oil emulsion stabilized with BG and GA solutions were analyzed. The results showed that an increase in BG concentration led to a decrease in Z-average diameter and to an increase in emulsion apparent viscosity and stability. All emulsion flow curves presented shear-thinning behavior at low shear rates and Newtonian plateau at high shear rates. The mechanical spectra showed droplets tending to arrange as a network in the emulsions which was related to the high stability. BG emulsion presented higher viscosity and stability than GA emulsion at the same concentration suggesting that BG could replace GA in some industrial applications.

1. Introduction

Emulsions are widely used in the formulation of food, pharmaceutical, and cosmetic products. In the food industry, many food products are in the form of oil-in-water (o/w) emulsions consisting of small lipids droplets dispersed in an aqueous phase, such as salad dressing, dips, sauces, milk, cream, batters, beverages and desserts. On the other hand, emulsification is one of the important steps in microencapsulation technology (Jafari, He, & Bhandari, 2006). In microencapsulation of food oils and flavours through spray drying, it has been proved that emulsion stability and droplet size are of high relevance in the retention of volatiles and shelf-life of encapsulated oils by reduction of surface oil content of encapsulated powder particles (Jafari, He, & Bhandari, 2007a; Liu et al., 2001; Soottitantawat et al., 2005). According with many studies, the lower the emulsion particle size, the higher is the encapsulation efficiency (Ishido, Hakamata, Minemoto, Adachi, & Matsuno, 2002; Soottitantawat et al., 2005; Soottitantawat, Yoshii, Furuta, Ohgawara, & Linko, 2003). So, in the recent years, production of emulsion with minimum droplet size and a narrow distribution has been of interest of many food researchers.

However, emulsions are not thermodynamically stable systems and can phase separate through different physicochemical processes, such as gravitational separation, flocculation, coalescence and Ostwald ripening (McClements, 2005). To improve emulsion stability, a widely used method is the addition of emulsifiers and stabilizers. Emulsifiers are surface-active molecules that readily adsorb at the oil-water interface, facilitate emulsion formation by lowering the interfacial tension and form a protecting film (Guzey, Kim, & McClements, 2004; Krstonošić, Dokić, Nikolić, & Milanović, 2015; McClements, 2005). They also prevent droplet aggregation by generating repulsive forces between droplets (Wang, Wang, Li, Adhikari, & Shi, 2011). Stabilizers are used to provide long-term emulsion stability, some of them by adsorbing into the interface while others only modifying the viscosity of the continuous phase due to their non-adsorbing character. Usually proteins, such as whey protein isolate or β-lactoglobulin, are the main emulsifiers whereas polysaccharides contribute to the emulsion stability through their thickening and steric stabilizing characteristics (Bouyer et al., 2011; Wang et al., 2011).

Some gums like gum Arabic (GA) or Mezquite gum are capable to adsorb at the oil-water interface due to their amphiphilic character. The arabinogalactan proteoglycan (AGP) complex has been shown to be responsible for the interface activity and the emulsifying properties of GA (Acedo-Carrillo et al., 2006). The proteinaceous components of the AGP would embed in the oil phase, while

---

* Corresponding author. 1 de mayo 3250, 3000, Santa Fe, Argentina.
E-mail address: virginiacas@hotmail.com (V. Castel).

http://dx.doi.org/10.1016/j.foodhyd.2016.08.039
0268-005X/© 2016 Published by Elsevier Ltd.

the carbohydrates would extend out from the surface into the aqueous phase (Bouyer, Mekhloufi, Huang, Rosilio, & Agnely, 2013; Picton, Bataille, & Muller, 2000). Nowadays, GA is extensively used as an emulsifier/stabilizer in beverage emulsion for soft drinks and is the traditional gum of choice for flavor encapsulation via spray drying (Bertolini, Siani, & Grosso, 2001). Despite the excellent functional properties of GA, there is a need to find others natural emulsifiers that could replace GA due to its high price and variable supply.

Brea gum (BG) is a natural hydrocolloid exudated by the bark of *Cercidium praecox* tree (Ruiz & Pavon) Harms [= *Parkinsonia praecox* (Ruiz & Pavon) Hawkins] (Bertuzzi, Slavutsky, & Armada, 2012). In previous studies, it has been shown that BG consist in 83.7% of polysaccharide and 7.52% of protein which is higher than GA protein content (2.59%) and could differentiate interfacial properties of these gums (Castel et al., 2016). Moreover, the polysaccharide-protein complex present in BG molecular structure would give it high interfacial activity and good emulsifying properties (Castel et al., 2016).

On the other hand, there is a growing trend within the cosmetic and food industry to replace synthetic emulsifiers with natural and biodegradable products, 'label friendly' ones, such as phospholipids, proteins and polysaccharides (Guzey et al., 2004).

The aim of this study was to gain further understanding of the emulsifying properties of BG in comparison to GA. To this end, corn oil-in-water emulsions stabilized with BG and GA were analyzed in terms of the effect of gum concentration on emulsion droplet size, rheological behavior and stability.

2. Materials and methods

2.1. Materials

Nodules of crude BG were collected from trees in the area near to the city of Salta in Argentina, and donated by the promotion project of “Brea as non-wood forest product for the sustainable development of Wichí and Criollas communities of Chaco Salteño”. For BG purification, the nodules were dissolved in distilled water (15% w/w) and allowed to stand for 24 h to reach a complete hydration of the gum. The solution was centrifuged and filtered through Whatman No.1 filter paper to separate undissolved matters, and then was vacuum-filtered through a 0.5 μm fiberglass membrane to remove farther impurities. The purified solution was freeze-dried, and then grounded to obtain a powder. BG purified powder contained 1.14% w/w of fat and 4.23% w/w of ash as determined by standard methods (AOAC, 1995). Protein content determined by Kjeldahl method was 7.52% w/w, using 6.6 as N-protein conversion factor (Renard, Lavenant-Gourgeon, Ralet, & Sanchez, 2006).

GA was supplied by Colloïdes Naturels International (Rouen, France).

2.2. Emulsion preparation

BG purified powder was added to Milli-Q ultrapure water with 0.01% w/w sodium azide in a concentration of 5%, 10% and 20% w/w, the mixture was stirred at 25 °C until complete dissolution of the gum and kept overnight to warrant a full saturation of the polymer molecules. The same procedure was carried out to prepare a 20% w/w GA solution. Emulsions were prepared by a two step method:

(a) First, pre-emulsions were obtained by homogenizing 10% w/w corn oil with 90% w/w gum solution with an Omni mixer homogenizer (Ivan Sorvall, Inc., Norwalk, Conn.) for 5 min at 5000 rpm.

(b) Later, pre-emulsions were further emulsified by an ultrasonic treatment using a 20 Khz sonicator (Ultrasound generator, Sonics and Materials VCX-750, Newton, CT) at 75% AMP for 2 min with temperature controller setup to stop the ultrasonics when sample temperature reaches 30 °C.

Emulsions were named as follow: BG5 was the emulsion prepared with the solution of BG at 5% w/w, BG10 was prepared with 10% w/w BG solution, BG20 with 20% w/w BG solution and GA20 was prepared with GA solution at 20% w/w.

2.3. Emulsion droplet size analysis

The droplet size distribution of the emulsions was determined by dynamic light scattering using a Zetasizer Nano-ZS90 device (Malvern Instruments, Ltd., Worcestershire, UK) at 25 °C. Measurements were carried out after appropriate dilution of the emulsion samples with Milli-Q ultrapure water. The Z-average diameter of emulsion droplets and the polydispersity index (PdI) were automatically calculated by the instrument as mean of ten reading per sample.

2.4. Microscopic analysis

Emulsions microstructure was visualized using an optical microscope (Leica Microsystems, Wetzlar, Germany) equipped with an attached camera. To this end, a drop of emulsion was placed on a thick glass slide and covered with a cover-slip ensuring no air or bubbles. Pictures were taken at 40× objective magnification.

2.5. Rheological determinations

Rheological measurements were carried out on a HAAKE RS80-Rheo stress rheometer (Haake Mess - Technik Gmbh, Alemania). A cone-plate geometry of 40 mm diameter, 4° cone angle, and 1 mm gap was employed in all measurements. The temperature was maintained at 25 ± 0.1 °C. The exposed surfaces of samples were covered with a thin layer of silicone oil to avoid dehydration. Apparent viscosity of emulsions was measured upon shear rate ramp-up from 0.1 to 100 s^{-1}. In oscillatory experiments, the storage modulus (G') and loss modulus (G'') were recorded versus frequency (0.5–100 Hz), in the linear visco-elastic region (LVR) determined previously. An appropriate shear rate stress of 0.05 Pa was selected by recording G' and G'' versus shear stress (0.01–1 Pa) at constant frequency of 1 Hz. This shear stress was applied in frequency sweep tests.

2.6. Emulsion stability analysis

The stability of the emulsions was determined through the use of a vertical scan analyzer Turbiscan MA 2000 (Formulaction, Toulouse, France). The samples were put in a flat-bottomed cylindrical glass cell and scanned from the bottom to the top. The light flux transmitted through the emulsion and the light backscattered as a function of the sample height (total height = 70 mm) were recorded simultaneously at 25 °C. The equipment was programmed to take measurements of Transmittance (T) and backscattering (BS) every 15 min during the first hour of the analysis (data not shown, to better visualize the changes undergone in the systems, one curve per day is presented in the graphic). After that, measurements were taken once each day.

The creaming destabilization kinetics was evaluated by measuring the Creaming index (CI) as the percentage of serum layer height (H_S) from the total emulsion height (H_T) (Gu, Decker, & McClements, 2005):

$$\text{Creaming index } (\%) = \frac{H_S}{H_T} \cdot 100 \tag{1}$$

To measure H_S and H_T, it was convenient to work in the reference mode (DeltaBS), which substracts the first curve ($t = 0$) from the subsequent ones in order to see the variations of profiles related to the initial state.

Also, a delay time (h), time in which emulsion destabilization reaches 5%, was measured as the time when the clarification peak (bottom zone) increase 5% of its height.

2.7. Statistical analysis

All experiments were performed at least three times and results are presented as the mean value with the standard deviation. Data was analyzed to detect significant differences using one-way analysis of variance (ANOVA). P values ≤ 0.05 were deemed statistically significant and LSD test was used for means comparisons. Statistical analyses were conducted using Statgraphics Centurion XVsoftware.

3. Results and discussion

3.1. Emulsion droplet size distributions

The droplet size distribution from emulsions stabilized with solutions of BG at 5% w/w (BG5), 10% w/w (BG10) and 20% w/w (BG20) and a solution of GA at 20% w/w (GA20) are shown in Fig. 1. BG5 presented a bimodal distribution with a mayor population of droplets with a mean diameter around 741.5 nm and a minor population with 4894 nm of mean diameter (Table 1). BG5 micrograph clearly showed the presence of larger particles than the other emulsions and also some particles forming little flocs were observed (Fig. 2A). The great polydispersity of BG5 was reflected by the PdI value that was significantly higher than the others (Table 1). The Z-average of this system (783.8 nm) was also the highest within the samples, however, this parameter represents an average of the entire distribution and does not describe each population, so it is inappropriate to characterize this system.

When BG concentration was increased to 10% w/w (BG10) and 20% w/w (BG20), monomodal distributions were obtained (Fig. 1) and Z-average and PdI values decreased (Table 1), being BG20 the system with the lower values. In these monodispersed systems, Z-average can adequately represent the average droplet diameter, but a difference between this value and the peak mean was observed. According to the manual of the size analyzer equipment, the calculated Z-average is lower than the peak mean when the cumulants fit only for the initial part of the correlation function, so in some way, the initial decay of the distribution is overestimated. These results are consistent with BG10 and BG20 micrographs (Fig. 2 C and E, respectively) where smaller particles than BG5 and no flocculation were observed.

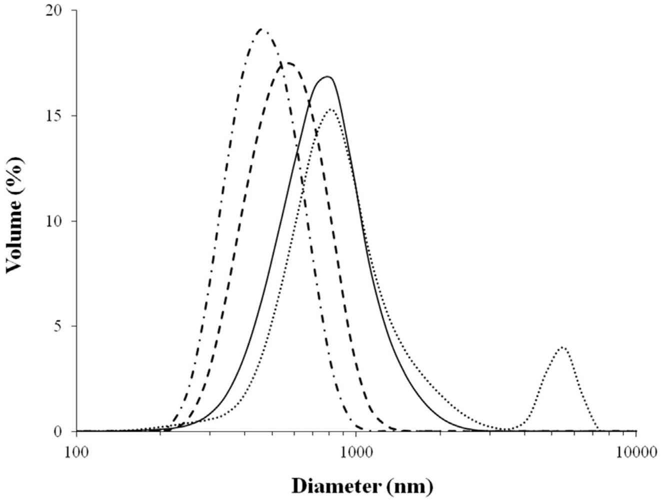


Fig. 1. Particle size distribution of 10% w/w corn oil emulsions stabilized with BG at 5% w/w (dotted line), 10% w/w (solid line), 20% w/w (dash-dot line) and GA at 20% w/w (dashed line).

GA20 presented a similar distribution to that of BG20 and the parameters obtained were not significantly different between these two distributions. Therefore, it could be said that BG presents emulsion forming properties similar to those of GA.

Results could be explained taking into account several phenomena. First, at low BG concentration (BG5), the molecules available would not be enough to completely cover the oil-water interface created during the emulsification process and a re-coalescence phenomenon might be taking place resulting in large droplets (Jafari, Assadpoor, He, & Bhandari, 2008b). Re-coalescence occurs when after the disruption the freshly formed droplets are subjected to a motion that leads to collisions, and if the interface is incompletely covered by emulsifier molecules, a fusion or coalescence of the droplets take place (Floury, Legrand, & Desrumaux, 2004; Perrier-Cornet, Marie, & Gervais, 2005).

On the other hand, the population of diameter around 4894 nm observed in BG5 distribution would correspond to the flocs observed in the micrographs. This droplet aggregation is consistent with bridging flocculation that occurs when the emulsifier is insufficient to completely cover all the particle surface, so some of the polymer molecules become attached simultaneously to more than one particle (Dickinson, 1998). The elastic bridge formed keeps the droplets to a short distance i.e. flocculated, and also the low steric repulsion due to low number of adsorbing polymer molecules favors the approach between droplets (McClements, 2005).

In BG10 and BG20 emulsions, a sufficient number of polymer molecules were present to at least produce a saturation coverage of the fresh interface formed. Then, a steric stabilization of the colloidal dispersion occurred and re-coalescence was prevented, which resulted in lower droplet sizes (Lobo & Svereika, 2003). Several authors agree that in some cases a smaller droplet size is not because of an efficient droplet disruption, but rather a result of better stabilization of the disrupted droplets and prevention of re-coalescence (Floury et al., 2004; Jafari, Assadpoor, Bhandari, & He, 2008a). Regarding avoidance of droplet re-coalescence in BG10 and BG20 emulsions, other factors have to be considered. High BG concentration would be increasing the viscosity of the intervening continuous liquid phase which restricts droplets movement preventing the approach, and slows down the fluid draining between two drops at the time of collision preventing re-coalescence (Khouryieh, Puli, Williams, & Aramouni, 2015). Some authors also propose that if sufficient excess of polymer is present, a gel-like network could be formed in the aqueous phase amongst the particles (Acedo-Carrillo et al., 2006; Dickinson, 1998; Valdez et al., 2006). This network immobilizes the oil droplets preventing droplets to approach and coalescence. These might be the reasons why BG10 and BG20 did not shown large particles or flocs.

Two regimes have been proposed regarding the effect of emulsifier type and concentration on droplet size: (a) emulsifier-poor regime in which droplet size strongly depends on initial emulsifier concentration, and (b) emulsifier-rich regime in which droplet size does not depend on emulsifier concentration and is determined by interfacial tension and by the energy input (Jafari et al., 2008a; Lobo & Svereika, 2003). In this work, no significant

Table 1
Z-average diameter, polydispersity index (PdI) and peak mean of oil-in-water emulsions stabilized with BG and GA solutions.

Emulsion	Day 1			Day 7		
	Z-average (nm)	PdI	Peak mean* (nm)	Z-average (nm)	PdI	Peak mean* (nm)
BG5	783.8 ± 34.2^c	0.34 ± 0.07^b	741.5 ± 77.0^b 4894 ± 501.5^c	1115.5 ± 4.9^d	0.30 ± 0.11^b	1080.0 ± 99.0^b 5207 ± 188.0^c
BG10	674.4 ± 11.2^b	0.20 ± 0.03^a	760.3 ± 48.2^b	754.7 ± 20.1^c	0.19 ± 0.02^a	801.4 ± 44.0^a
BG20	529.6 ± 13.2^a	0.10 ± 0.04^a	576.1 ± 14.2^a	700.0 ± 4.7^b	0.12 ± 0.02^a	754.2 ± 4.0^a
GA20	546.8 ± 26.5^a	0.12 ± 0.01^a	599.6 ± 32.5^a	621.1 ± 7.1^a	0.15 ± 0.04^a	696.4 ± 43.0^a

Data expressed as average ± standard deviation ($n = 3$). Different superscript letters in the same column indicate statistical difference according to LSD tests ($p < 0.05$). *Based on size distribution by intensity.

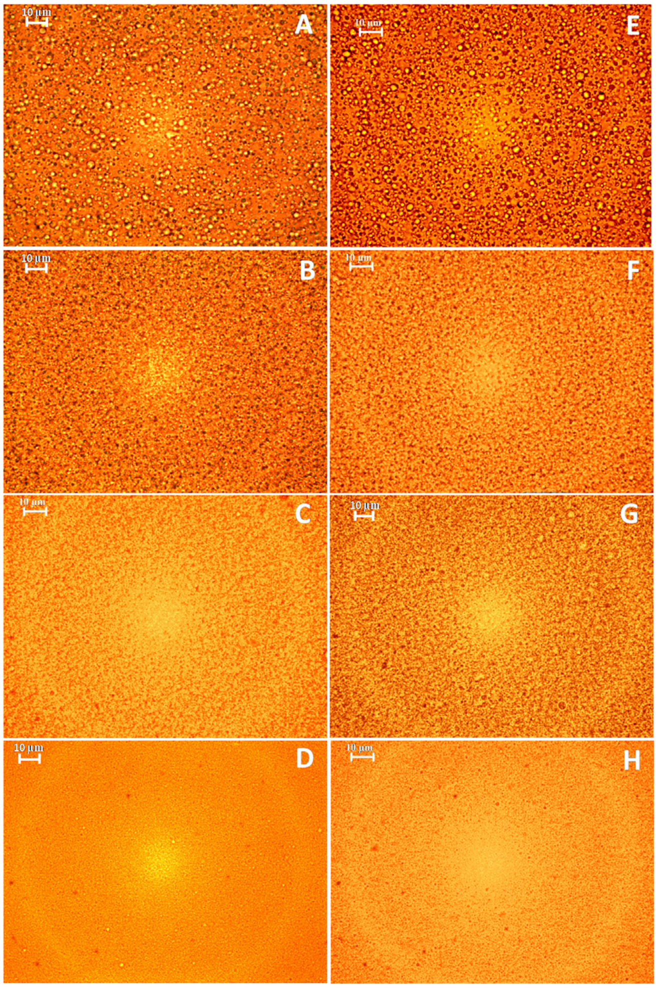


Fig. 2. Micrographs of 10% w/w corn oil emulsions stabilized with (A) 5%. (B) 10%. (C) 20% w/w of BG and (D) 20% w/w GA solutions just after being prepared and (E). (F). (G) and (H) the corresponding emulsions at 7 days of prepared. Scale bar equal to 10 μm.

differences in energy input were founded (data not shown) while a strong relationship between emulsion droplet size and BG concentration was observed, suggesting a state of emulsifier-poor regime despite the high gum concentration.

In contrast to these findings, Bertuzzi et al. (2012) observed that droplet size of corn oil emulsions increased at BG concentrations above 5% w/w. Besides, droplet sizes of 5%, 10% and 20% w/w BG emulsions obtained by these authors were higher than the ones obtained in this work (21,300, 42,800 and 45,600 nm, respectively). Such differences could be due to the greater dispersed-phase volume fraction used in that work (20% w/w) as (i) increasing oil proportion might increase emulsion viscosities and could suppress the formation of eddies responsible for breaking up droplets; (ii) emulsifier concentration might be insufficient to completely cover the oil droplets; and (iii) the rate of droplet coalescence might be increased (McClements, 2005). On the other hand, Bertuzzi et al. (2012) prepared emulsion by one rotor-homogenizer step while in this work two steps of homogenizer and ultrasound were applied to obtain emulsions. The additional ultrasound step increased the total energy given to the system leading to more oil droplet deformation and disruption, hence, droplet size decreased (Jafari, He, & Bhandari, 2007b). Moreover, previous studies comparing different emulsification systems have shown that sub-micron emulsions could not be created by the rotor-stator systems (Jafari et al., 2007a; Perrier-Cornet et al., 2005). Authors state that in rotor-stator systems the main force acting is shear stress, while in ultrasound emulsification the main reason for droplet disruption is cavitation phenomenon which is a more efficient and gives higher energy density (Jafari et al., 2007a).

3.2. Emulsions rheological characterization

The apparent viscosity (η_{app}) as function of the shear rate ($\dot{\gamma}$) of the emulsions stabilized with BG and GA are shown in Fig. 3. By looking this curves, it is clear that increasing BG content from 5% w/

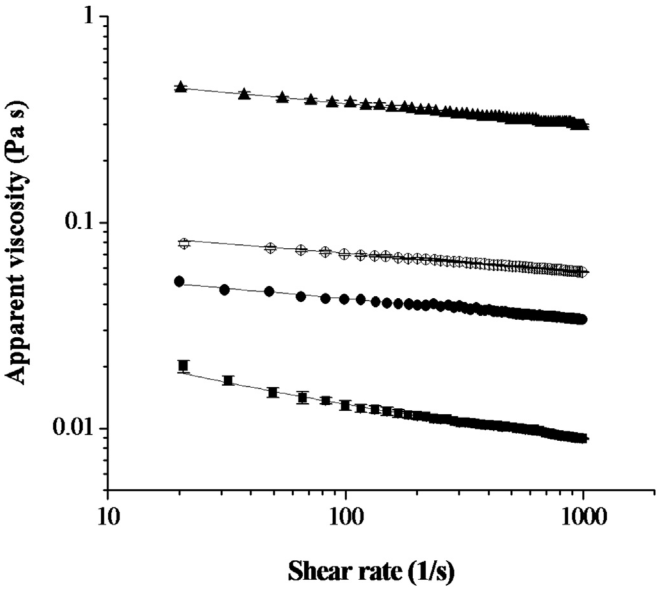


Fig. 3. Apparent viscosity vs shear rate ($\dot{\gamma}$) data of corn oil emulsions stabilized with BG solutions at 5% w/w (■), 10% w/w (●), 20% w/w (▲) and GA solution at 20% w/w (○). Lines are fits to the Sisko rheological model.

w to 20% w/w increased emulsion η_{app} and GA20 apparent viscosity was lower than η_{app} of BG20 in all the $\dot{\gamma}$ range. The dependence of emulsion η_{app} on hydrocolloid concentration has been previously observed in different systems (Dokić, Krstonošić & Nikolić, 2012; Domian, Brynda-Kopytowska, & Oleksza, 2015; Jafari, Beheshti, & Assadpoor, 2012). The increased viscosity is claimed to result from the fact that hydrocolloid addition increases viscosity of the emulsion aqueous phase and reduces the mean size of the dispersed phase droplets (in concordance with previous section data), which is reflected in the increased viscosity of the whole system (Domian et al., 2015).

On the other hand, all the emulsions exhibited shear thinning Non-Newtonian behavior with a tendency to Newtonian plateau at high-shear rate. BG5 exhibited a pronounced shear-thinning at low shear rates, while BG10, BG20 and GA20 viscosities declined very slightly in all $\dot{\gamma}$ range. This interesting behavior was also found by others authors in GA and other hydrocolloids dispersions, were the shear-thinning behavior was more pronounced at low polymer concentration (Li et al., 2009; Mothé & Rao, 1999; Sanchez, Renard, Robert, Schmitt, & Lefebvre, 2002). The authors attributed this anomalous behavior to the presence of micro-aggregates, when shearing is applied the aggregates formed by association would be disassociated into smaller entities, therefore showing Newtonian behavior. At higher concentration, BG10 and BG20, the effect of micro-aggregates on emulsion viscosity might be attenuated by the high continuous phase viscosity so the shear-thinning is less noticeable.

When data contain both a power law and the infinite shear rate Newtonian regions, the Sisko model (Sisko, 1958) can be used to describe the data:

$$\eta_{app} = k_s \dot{\gamma}^{(n_s - 1)} + \eta_\infty \tag{2}$$

where, η_{app} is the apparent viscosity (Pa s), $\dot{\gamma}$ is the shear rate (s^{-1}), η_∞ is the infinite-shear viscosity (Pa s), k_s is the consistency index, and n_s is the flow behavior index (Mothé & Rao, 1999). In this work, the log η_{app} vs. log $\dot{\gamma}$ data was well described by Sisko rheological model as can be checked by comparison with the continuous lines in Fig. 3, which corresponds to the fitting equation with the actual viscosity data. In addition, all the squared of the correlation indexes (R^2), corresponding to the plot of the viscosity predicted by Sisko model versus the experimental viscosity data, were higher than 0.98 (Table 2), suggesting the suitability of the model for the emulsions studied in this work. In Table 2, Sisko model parameters obtained by data no linear fitting are presented. It can be observed that increasing BG concentration, parameters k_s and η_∞ have a tendency to increase, indicating an increase in emulsion viscosities. Furthermore, the parameter n_s was less than 1 in all cases reflecting a pseudoplastic behavior. Similar shear-thinning flow behavior has been already reported for gum exudate aqueous dispersions (Mothé & Rao, 1999; Muñoz et al., 2007; Sibaja-Hernández, Román-Guerrero, Sepúlveda-Jiménez, & Rodríguez-Monroy, 2015) and emulsions (Lupi, Gabriele, de Cindio, Sánchez, & Gallegos, 2011; Wang et al., 2011; Yang & Li, 2013), and the same rheological model was used.

Table 2
Sisko model parameters for oil-in-water emulsions stabilized with BG and GA.

Emulsion	k_s (Pa s)	n_s	η_∞ (Pa s)	R^2
GB5	0.04 ± 0.00^a	0.65 ± 0.02^b	0.0058 ± 0.0003^a	0.993
GB10	0.08 ± 0.00^a	0.53 ± 0.01^a	0.0316 ± 0.0004^b	0.998
GB20	0.59 ± 0.05^b	0.88 ± 0.02^d	0.0295 ± 0.0057^b	0.987
GA20	0.08 ± 0.00^a	0.73 ± 0.05^c	0.0478 ± 0.0009^c	0.998

Data expressed as average ± standard deviation ($n = 3$). Different superscript letters in the same column indicate statistical difference according to LSD tests ($p < 0.05$). k_s is the consistency index, n_s is the flow behavior index, η_∞ is the infinity shear viscosity and R^2 is the coefficient of determination.

The mechanical spectra of the corn oil emulsions obtained using small-deformation oscillatory measurements ($\gamma = 0.05$) are shown in Fig. 4. According to Valdez et al. (2006), disruption of any structuring bonds holding the droplets together are likely to be reduced using this type of measurements. It can be seen in BG5 and BG10 mechanical spectra that G′ values exceed those of G″ over most of the frequency range. This behavior could correspond to flocculated emulsions with characteristics of a concentrated colloidal system where droplets tend to arrange as a network (Lorenzo, Zaritzky, & Califano, 2010). Some authors have called this behavior as “gel-like network” or weak-gel structure (Valdez et al., 2006). In contrast, the mechanical spectrum of BG20 showed a particular behavior in which G″ > G′ until a critical frequency where moduli present a crossover and from which the system showed characteristics of a network. In this emulsion, the arrangement of the droplets could be delayed by the higher continuous phase viscosity and the low droplet size.

Similar behavior has been observed in orange oil-in-water emulsions stabilized with mesquite gum (Valdez et al., 2006). Authors suggested that the weak-gel phenomenon arises from an electrosteric interaction between the charged oil spheres dispersed in the continuous aqueous phase where a weak gel network is set up involving protein and polysaccharide molecules of the gum attached to the oil droplets. Previous studies by Dickinson and Pawlowsky (1995), have demonstrated that polysaccharide-protein interactions have effects on the o/w emulsions rheology, as thickening effects and gel-like structures developments in the emulsions were found.

Then, these findings support what was suggested in the previous section, the high viscosities and a network might be preventing re-coalescence in BG10 and BG20, and that is one of the reasons of low droplet sizes and absence of flocculation in these emulsions.

On the other hand, in all cases a strong dependence of G′ and G″ on frequency was observed throughout the entire frequency range studied. G′ increased linearly with frequency and was independent of BG concentration, while G″ showed a quadratic increase with frequency and also increase with BG concentration.

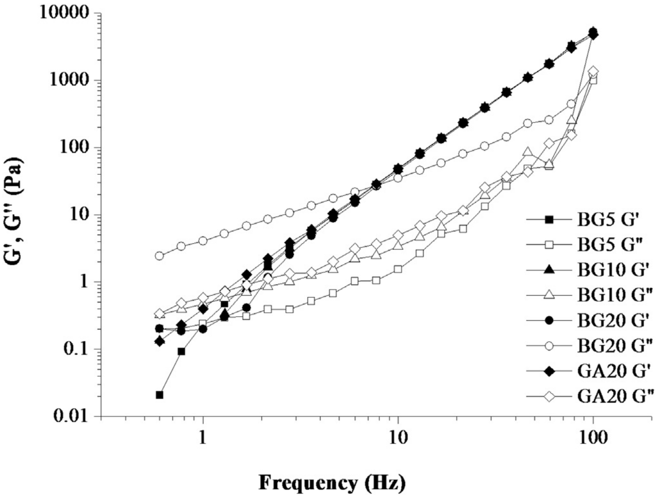


Fig. 4. Frequency dependence of the storage, G′, and loss, G″, moduli of oil-in-water emulsions stabilized with BG and GA solutions.

3.3. Emulsions stability

Emulsion destabilization often results from various physical processes: coalescence or flocculation (particle aggregation) leading to an increase in particle size, and particle migration leading to creaming or sedimentation (Domian et al., 2015; Mengual, Meunier, Cayré, Puech, & Snabre, 1999). Measurements of Backscattering (BS) light flux by Turbiscan provide information of the real condition of the emulsion and the course of destabilization processes earlier than they become noticeable by human eye (Mengual et al., 1999). Fig. 5 shows BS profiles in function of sample height for the emulsions determined directly after preparation (0 d) and on the following seven days (1–7 d). Profiles obtained for BG5 emulsion showed simultaneously a sharp decrease of BS at the bottom of the test tube and an increase BS at the top (Fig. 5A). Since BS intensity is a function of the volume of dispersed phase (number of droplets) located at a specific height of the tube and the particle diameter (Mengual et al., 1999), the sharp decline of BS in the bottom represents a decreasing concentration of droplets in this part of the tube indicating a clarification process. While the increase of BS in the upper part corresponds to an increasing concentration of droplets reflecting a creaming process. Moreover, the decrease of BS level in the middle part of the tube indicates an increase in droplet sizes due to flocculation or coalescence processes. In concordance, BG5 showed the highest CI (14.42%) (Table 3) which is an indirect parameter about the extent of droplets aggregation, the higher the CI, the faster the droplets move, the larger the flocs formed, and therefore the more droplet aggregation has occurred (Sun, Sundaram, & Mark, 2007). The delay time determined for BG5 showed that system destabilization was very fast reaching 5% of clarification in just one hour (Table 3). Droplet aggregation was also evidenced by the increase in BG5 droplet size measured by DLS after seven day of storage (Table 1). The maximum of the major peak in BG5 profile increased around 34% although the PdI remained constant. These findings were visible in BG5 micrographs that showed a greater amount of large droplets at day 7 than at day 1 micrograph (Fig. 2B). BG5 instability was probably due to a lack of absorbed molecules at the emulsion interface at this BG concentration.

Table 3
Creaming index (CI) and delay time of oil-in-water emulsions stabilized with solutions of GA and BG at different concentrations.

Gum	Concentration (% w/w)	CI (%)	Delay time (h)
BG	5	14.42	1
	10	1.97	26
	20	0.77	–
GA	20	0.85	168

Increasing BG concentration led to an increase in emulsion stability as can be seen in the BS profiles in Fig. 5. BS profiles of BG10, BG20 and GA20 remained almost constant over the 7 days of study indicating the great stability of these emulsions. Also, CI of these emulsions decreased drastically comparing with BG5 (Table 3). BG20 presented the lowest CI and the delay time was not measurable over the 7 days, demonstrating that this emulsion was the most stable, even more stable than GA20. Droplet sizes measurements performed after seven days of storage indicated increments of around 13% and 32% in the droplets diameters for BG10 and BG20, respectively, which was undetectable in microscopic observations. The increased stability observed in these emulsions could be related to the increase of adsorbed molecules at the interface which produce a higher steric repulsion of droplets and prevent droplets aggregation. Another reason for the high stability would be related to the high viscosity of BG10 and BG20, as observed in the previous section, or even more, to the gum network formation which immobilizes the oil droplets and prevents them from coming close together (Acedo-Carrillo et al., 2006; Khouryieh et al., 2015).

The mechanism of interfacial stabilization of BG might be based on the polysaccharide-protein complexes present in its

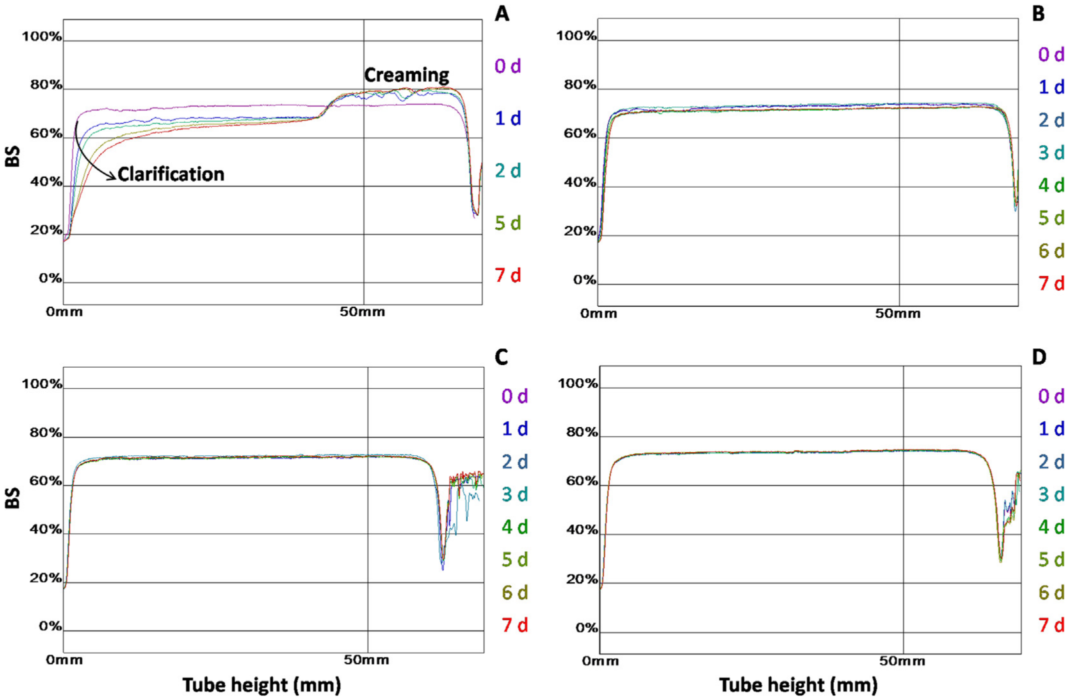


Fig. 5. Backscattering (BS) profiles determined the day of preparation (0 d) and on the following seven days (1–7 d) of corn oil emulsions stabilized with BG solutions at (A) 5% w/w, (B) 10% w/w and (C) 20% w/w and (D) GA solution at 20% w/w.

macromolecular structure (Castel et al., 2016). The complexes confer an amphiphilic character to the gum allowing its adsorption at the oil-water interface. Protein components of the complexes would adsorb at the interface by their hydrophobic residues decreasing interfacial tension, and the polysaccharides would extend out from the surface into the continuous phase increasing viscosity and the steric repulsion between droplets (Picton et al., 2000).

4. Conclusions

The increasing BG content in the emulsion led to a significant decrease in oil droplets sizes and polydispersity, while apparent viscosities and stability were increased. Decreasing droplet sizes were related to a decrease of re-coalescence phenomenon caused by a better interface coverage by BG molecules and a higher emulsion viscosity at higher gum concentration. Then, the rheological characterization demonstrated that emulsion viscosities increase with increasing BG concentration, and showed the possible formation of a network in the emulsions, both phenomena were related to high emulsion stability. On the other hand, a shear-thinning behavior with a tendency to Newtonian plateau at higher shear rates was observed in all emulsions.

Despite BG emulsion showed a droplet size distribution similar to the emulsion stabilized with GA at the same concentration, BG emulsion resulted more stable than the GA one, possible due to the higher viscosity of BG emulsion.

Results of this study confirmed that BG is a good emulsifier/stabilizer of o/w emulsions and could be used in the food industry replacing GA in several applications.

Acknowledgments

The authors are thankful to the projects PICT-2011-1118: “Extensive use of non-wood forest products: purification, characterization and applications of Brea Gum and Espina Corona Gum” and CAI+D, UNL, 501 20110100021 LI: “Utilization of a native polysaccharide for protection of lipophilic compounds” for the financial support.

References

Acedo-Carrillo, J. I., Rosas-Durazo, A., Herrera-Urbina, R., Rinaudo, M., Goycoolea, F. M., & Valdez, M. A. (2006). Zeta potential and drop growth of oil in water emulsions stabilized with mesquite gum. *Carbohydrate Polymers, 65*, 327–336.

AOAC. (1995). *Official methods of analysis*. Arlington. VA: AOAC International.

Bertolini, A. C., Siani, A. C., & Grosso, R. F. (2001). Stability of monoterpenes encapsulated in gum arabic by spray-drying. *Journal of Agricultural and Food Chemistry, 49*, 780–785.

Bertuzzi, M. A., Slavutsky, A. M., & Armada, M. (2012). Physicochemical characterisation of the hydrocolloid from Brea tree (*Cercidium praecox*). *International Journal of Food Scence and Technology, 47*, 776–782.

Bouyer, E., Mekhloufi, G., Huang, N., Rosilio, V., & Agnely, F. (2013). β-Lactoglobulin, gum arabic, and xanthan gum for emulsifying sweet almond oil: Formulation and stabilization mechanisms of pharmaceutical emulsions. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 433*, 77–87.

Bouyer, E., Mekhloufi, G., Le Potier, I., du Foude Kerdaniel, T., Grossiord, J.-L., Rosilio, V., et al. (2011). Stabilization mechanism of oil-in-water emulsions by β-lactoglobulin and gum arabic. *Journal of Colloid and Interface Science, 354*, 467–477.

Castel, V., Zivanovic, S., Jurat-Fuentes, J. L., Santiago, L. G., Rubiolo, A. C., Carrara, C. R., et al. (2016). Chromatographic fractionation and molecular mass characterization of *Cercidium praecox* (Brea) gum. *Journal of the Science of Food and Agriculture*. http://dx.doi.org/10.1002/jsfa.7642.

Dickinson, E. (1998). Stability and rheological implications of electrostatic milk protein-polysaccharide interactions. *Trends in Food Science and Technology, 9*, 347–354.

Dickinson, E., & Pawlowsky, K. (1995). Rheology as a probe of protein-polysaccharide interactions in oil-in-water emulsions. In G. O. Phillips, P. A. Williams, & D. J. Wedlock (Eds.), *Gums and stabilisers for the food industry* (Vol. 8, pp. 181–191). Oxford: IRL Press.

Dokić, L., Krstonošić, V., & Nikolić, I. (2012). Physicochemical characteristics and stability of oil-in-water emulsions stabilized by OSA starch. *Food Hydrocolloids, 29*, 185–192.

Domian, E., Brynda-Kopytowska, A., & Oleksza, K. (2015). Rheological properties and physical stability of o/w emulsions stabilized by OSA starch with trehalose. *Food Hydrocolloids, 44*, 49–58.

Floury, J., Legrand, J., & Desrumaux, A. (2004). Analysis of a new type of high pressure homogeniser. Part B. Study of droplet break-up and recoalescence phenomena. *Chemical Engineering Science, 59*, 1285–1294.

Gu, Y. S., Decker, E. A., & McClements, D. J. (2005). Influence of pH and carrageenan type on properties of b-lactoglobulin stabilized oil-in-water emulsions. *Food Hydrocolloids, 19*, 83–91.

Guzey, D., Kim, H. J., & McClements, D. J. (2004). Factors influencing the production of o/w emulsions stabilized by β-lactoglobulin–pectin membranes. *Food Hydrocolloids, 18*, 967–975.

Ishido, E., Hakamata, K., Minemoto, Y., Adachi, S., & Matsuno, R. (2002). Oxidation Process of Linoleic acid encapsulated with a polysaccharide by spray drying. *Food Science and Technology Research, 8*(1), 85–88.

Jafari, S. M., Assadpoor, E., Bhandari, B., & He, Y. (2008a). Nano-particle encapsulation of fish oil by spray-drying. *Food Research International, 41*, 172–183.

Jafari, S. M., Assadpoor, E., He, Y., & Bhandari, B. (2008b). Re-coalescence of emulsion droplets during high-energy emulsification. *Food Hydrocolloids, 22*, 1191–1202.

Jafari, S. M., Beheshti, P., & Assadpoor, E. (2012). Rheological behavior and stability of D-limonene emulsions made by a novel hydrocolloid (Angum gum) compared with Arabic gum. *Journal of Food Engineering, 109*, 1–8.

Jafari, S. M., He, Y., & Bhandari, B. (2006). Nano-emulsion production by sonication and microfluidization-a comparison. *International Journal of Food Properties, 9*, 475–485.

Jafari, S. M., He, Y., & Bhandari, B. (2007a). Effectiveness of encapsulating biopolymers to produce sub-micron emulsions by high energy emulsification techniques. *Food Research International, 40*, 862–873.

Jafari, S. M., He, Y., & Bhandari, B. (2007b). Production of sub-micron emulsions by ultrasound and microfluidization techniques. *Journal of Food Engineering, 82*, 478–488.

Khouryieh, H., Puli, G., Williams, K., & Aramouni, F. (2015). Effects of xanthan–locust bean gum mixtures on the physicochemical properties and oxidative stability of whey protein stabilised oil-in-water emulsions. *Food Chemistry, 167*, 340–348.

Krstonošić, V., Dokić, L., Nikolić, I., & Milanović, M. (2015). Influence of xanthan gum on oil-in-water emulsion characteristics stabilized by OSA starch. *Food Hydrocolloids, 45*, 9–17.

Li, X., Fang, Y., Al-Assaf, S., Phillips, G. O., Nishinari, K., & Zhang, H. (2009). Rheological study of gum arabic solutions: Interpretation based on molecular self-association. *Food Hydrocolloids, 23*, 2394–2402.

Liu, X. D., Atarashi, T., Furuta, T., Yoshii, H., Aishima, S., Ohkawara, M., et al. (2001). Microencapsulation of emulsified hydrophobic flavors by spray drying. *Drying Technology, 19*, 1361–1374.

Lobo, L., & Svereika, A. (2003). Coalescence during emulsification 2. Role of small molecule surfactants. *Journal of Colloid and Interface Science, 261*, 498–507.

Lorenzo, G., Zaritzky, N. E., & Califano, A. N. (2010). Comportamiento viscoelástico de emulsiones alimentarias modificado por adición de biopolímeros. In *World congress & exhibition engineering-2010-Argentina*. IPPIA.

Lupi, F. R., Gabriele, D., de Cindio, B., Sánchez, M. C., & Gallegos, C. (2011). A rheological analysis of structured water-in-olive oil emulsions. *Journal of Food Engineering, 107*, 296–303.

McClements, D. J. (2005). *Food emulsions: Principles, practice, and techniques* (2nd ed.). Boca Ratón, Florida, USA: CRC Press.

Mengual, O., Meunier, G., Cayré, I., Puech, K., & Snabre, P. (1999). TURBISCAN MA 2000: Multiple light scattering measurement for concentrated emulsion and suspension instability analysis. *Talanta, 50*, 445–456.

Mothé, C. G., & Rao, M. A. (1999). Rheological behavior of aqueous dispersions of cashew gum and gum arabic: Effect of concentration and blending. *Food Hydrocolloids, 13*, 501–506.

Muñoz, J., Rincón, F., Alfaro, M. C., Zapata, I., de la Fuente, J., Beltrán, O., et al. (2007). Rheological properties and surface tension of *Acacia tortuosa* gum exudate aqueous dispersions. *Carbohydrate Polymers, 70*, 198–205.

Perrier-Cornet, J. M., Marie, P., & Gervais, P. (2005). Comparison of emulsification efficiency of protein-stabilized oil-in-water emulsions using jet, high pressure and colloid mill homogenization. *Journal of Food Engineering, 66*, 211–217.

Picton, L., Bataille, I., & Muller, G. (2000). Analysis of a complex polysaccharide (gum arabic) by multi-angle laser light scattering coupled on-line to size exclusion chromatography and flow field flow fractionation. *Carbohydrate Polymers, 42*(1), 23–31.

Renard, D., Lavenant-Gourgeon, L., Ralet, M. C., & Sanchez, C. (2006). A. Senegal gum: Continuum of molecular species differing by their protein to sugar ratio, molecular weight, and charges. *Biomacromolecules, 7*, 2637–2649.

Sanchez, C., Renard, D., Robert, P., Schmitt, C., & Lefebvre, J. (2002). Structure and rheological properties of acacia gum dispersions. *Food Hydrocolloids, 16*, 257–267.

Sibaja-Hernández, R., Román-Guerrero, A., Sepúlveda-Jiménez, G., & Rodríguez-Monroy, M. (2015). Physicochemical, shear flow behaviour and emulsifying properties of *Acacia cochliacantha* and *Acacia farnesiana* gums. *Industrial Crops and Products, 67*, 161–168.

Sisko, A. W. (1958). The Flow of Lubricating Greases. A relatively simple flow equation is used to fit experimental flow data, and can be applied to other materials as well as greases. *Industrial and Engineering Chemistry, 50*(12), 1789–1792.

Soottitantawat, A., Bigeard, F., Yoshii, H., Furuta, T., Ohkawara, M., & Linko, P. (2005). Influence of emulsion and powder size on the stability of encapsulated d-limonene by spray drying. *Innovative Food Science and Emerging Technologies, 6*, 107–114.

Soottitantawat, A., Yoshii, H., Furuta, T., Ohgawara, M., & Linko, P. (2003). Microencapsulation by spray drying: Influence of emulsion size on the retention of volatile compounds. *Journal of Food Science, 68*, 2256–2262.

Sun, C., Sundaram, G., & Mark, R. P. (2007). Effect of xantham gum on physiochemical properties of whey protein isolate stabilized oil-in-water emulsion. *Food Hydrocolloids, 21*, 555–564.

Valdez, M. A., Acedo-Carrillo, J. I., Rosas-Durazo, A., Lizardi, J., Rinaudo, M., & Goycoolea, F. M. (2006). Small-deformation rheology of mesquite gum stabilized oil in water emulsions. *Carbohydrate Polymers, 64*, 205–211.

Wang, B., Wang, L.-J., Li, D., Adhikari, B., & Shi, J. (2011). Effect of gum Arabic on stability of oil-in-water emulsion stabilized by flaxseed and soybean protein. *Carbohydrate Polymers, 86*, 343–351.

Yang, X., & Li, W. (2013). Some viscous behaviors of styrene acrylic emulsion thickened with konjac glucomannan. *Progress in Organic Coatings, 76*, 1396–1401.