Impact of chemical composition of xanthan and acacia gums on the emulsification and stability of oil-in-water emulsions

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ABSTRACT

Xanthan gum (GX) and acacia gum (GA) are widely employed in food industry, indeed xanthan gum is used for its thickening properties of aqueous solutions and acacia gum for its emulsifying ability. The present work aims to study the effect of GX–GA mixtures on the stability of oil-in-water (o/w) emulsions; attention is particularly focused on the impact of the chemical structure of each gum. Emulsion stability has been evaluated by monitoring the evolution of droplet size and viscometric properties over time when submitted to accelerated ageing conditions. On the one hand results show that the higher the ArabinoGalactanProtein (AGP) content the more stable the emulsion as observed when GA is used alone. On the other hand, we proved, unexpectedly, that the most viscous aqueous phase does not exhibit the best emulsion stability. Besides, we clearly evidenced the presence of specific interactions between GX and GA in both emulsion and aqueous solution, these interactions being governed by the gums chemical composition.

1. Introduction

An emulsion is conventionally defined as a thermodynamically unstable dispersion of given liquid in another one, each being immiscible with the other, usually oil and water. In the formulation industry, both phases are often complex systems containing ingredients such as flavour, additive, salt, antioxidant, weighing agent. Hence emulsifiers and thickening agents are generally added to improve emulsion stability, i.e., to make it kinetically stable (Dickinson, 2003). Emulsifiers contain hydrophilic and hydrophobic region thus they adsorb rapidly at the oil–water interface and consequently stabilize the emulsion through steric and/or electrostatic interactions. Proteins, such as sodium caseinate, are the most commonly used emulsifier in food industry (McClements, 2004; Tcholakova, Denkov, Ivanov, & Campbell, 2006). Due to their texturing properties, polysaccharides and their mixtures are widely employed to control the rheological properties of oil-in-water-emulsions, thus also contributing to emulsion stability. The main stabilizers employed in food emulsions are xanthan gum, galactomannans, and native or modified starch since they are good thickening agents of aqueous solutions. Their efficiency for enhancing both the viscosity and the stability of emulsion depends on the polymer concentration and structure (McClements, 1999). Both emulsifier and thickening agent are usually combined to take benefit of their respective properties to improve emulsion stability. It has been proved that under certain conditions, such mixtures exhibit some synergistic interactions, commonly leading to enhance the functional properties compare to the one of each agent taken separately. This has been clearly demonstrated for a series of protein/polysaccharide mixtures (Benichou, Aserin, & Garti, 2002; Benichou, Aserin, Lutz, & Garti, 2007; Dickinson, 2003; McClements, 2006). Only few polysaccharides actually own a surface activity at the oil–water interface and hence suitable as emulsifier: one can cite acacia gum and modified starch (Dickinson, 2009; Williams, 2007). In the present study, xanthan and acacia gums are combined to improve o/w emulsion stability due to their respective well-known stabilizing and emulsifying properties.

Xanthan gum (GX) is an anionic exocellular polysaccharide produced by aerobic fermentation of sugars by the bacterium of *Xanthomonas campestris*. Its main chain consists of a linear 1, 4-linked β-D-glucose backbone substituted on every two unit with a charged trisaccharide side chain. The latter side chain is composed by a D-glucuronic acid unit linked between two D-mannose units. Pyruvic acid and acetate groups may be present as substituents on the mannose residues. About one half of the terminal D-mannose contains a pyruvic acid residue linked via keto group onto the C4 and C6 positions while acetate groups may be bonded to the D-Mannose directly linked to the main chain at the

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O6 position (Jansson, Kenne, & Lindberg, 1975). Variation of both the bacteria strains and the fermentation conditions used during the production process strongly affects molecular weight, pyruvilation and acetylation degree of xanthan gum and thus its physico-chemical properties (Casas, Santos, & García-Ochoa, 2000; García-Ochoa, Santos, Casas, & Gomez, 2000; Ross-Murphy, Shatwell, Sutherland, & Dea, 1996). In aqueous solution, xanthan gum exhibits an ordered secondary double-helix strand structure at low temperature which is sensitive to temperature. Indeed GX presents an order-to-disorder transition at a specific temperature named conformational temperature (T_m), leading to a strong decrease of its rheological properties (Morris, Rees, Young, Walkinshaw, & Darke, 1977). T_m depends on several parameters such as the acetyl and pyruvate rate and the ionic strength of the solution (Milas & Rinaudo, 1979). The xanthan branching structure and its rigid conformation explain its higher rheological characteristics when compare to most of the other commonly available polysaccharides. Therefore GX is frequently used in the food industry for its great solubility in cold water and its high thickening properties at very low concentration. Viscosity of xanthan solution viscosity is influenced by the acetate and pyruvate contents, the higher acetate% the lower the viscosity while the viscosity is increased with increasing pyruvate rate. We should mention that the pyruvate effect on the viscosity is much more pronounced than the acetate one (Flores-Candia & Decker, 1999; Hassler & Doherty, 1990).

Acacia gum (GA) is an exudate from of *Acacia Senegal* trees and is a complex acidic branched polysaccharide. Indeed acacia gum is described as a heteropolysaccharide containing about 2% of polypeptide. Actually, GA can be fractionated in three fractions which differ from their molecular weight and chemical composition. The main fraction which represents about 88% (in weight) of the total gum is named ArabinoGalactan fraction (AG) according to its chemical composition and has a weight-average molecular weight ($\overline{M}_W$) around 3.10^5 g mol^{-1}. The second fraction ($\sim$10% of the total gum) corresponds to the ArabinoGalactanProtein fraction (AGP), its weight-average molecular weight is about $1.5\,10^6$ g mol^{-1} and owns a greater protein fraction (11%). The AGP fraction has a hyper branched structure (wattle blossom-type structure) where the polysaccharide chains are covalently bonded to the protein backbone. The minor fraction (<2%) consists of a GlycoProtein fraction (GP) and has the lowest M_W ($2.5\,10^5$ g mol^{-1}) and the highest protein content (45%) (Idris, Williams, & Phillips, 1998; Randall, Phillips, & Williams, 1989). The proportion and the composition of these three fractions can differ depending on the geographical origin of the gum. The Acacia gum is mainly used as emulsifier and stabilizer in beverage industry. Its emulsifying capacity is attributed to the AGP fraction (Dickinson, Murray, Stainsby, & Anderson, 1988; Randall, Phillips, & Williams, 1988; Al-Assaf, Phillips, Aoki, & Sasaki, 2007). Indeed the hydrophobic protein chains combined to the hydrophilic polysaccharide fragments confer an amphiphilic character to the molecule. The hydrophobic portion adsorbs strongly onto the surface of oil droplets while the hydrophilic ramifications limit droplets aggregation and coalescence by steric and/or repulsive electrostatic forces (Dickinson, 2003, 2009; Dickinson, Elverson, & Murray, 1989; Garti & Leser, 2001).

Xanthan (GX) and acacia (GA) gums mixtures are classically used for food emulsions (i.e., industrial salad dressing) to take benefit of thickening and emulsifying properties of xanthan and acacia gums respectively. Nevertheless, only few studies on emulsion stability are reported (Mirhosseini, Tan, Hamid, & Yusof, 2008a; Mirhosseini, Tan, Hamid, & Yusof, 2008b; Mirhosseini, Tan, Hamid, Yusof, & Chern, 2009; Taherian, Fustier, & Ramaswamy, 2008). Recently, Paraskevopoulou, Boskou, and Kiosseoglou (2005) compared the stability of oil-lemon juice Greek salad sauce containing xanthan gum as stabilizer and acacia gum or propylene glycol alginate as emulsifiers in various combinations. They showed that emulsions

Table 1
Xanthan samples characteristics.

	[η]^a (dL/g)	Protein content^b (%)	DSac^c (%)	DSpy^c (%)	Tm^d (°C)
GX1	19.36	4.7 ± 0.5	13 ± 1	52 ± 1	53 ± 1
GX2	19.06	2.9 ± 0.5	72 ± 1	61 ± 1	48 ± 1

[a] determined at 25 °C by ball viscometry in NaCl 0,01 M.
[b] measured using Bradford method (Bradford, 1976).
[c] measured by NMR (method described by Rinaudo et al., 1983).
[d] measured by polarimetry in NaCl 0.01 M (method described by Capron, 1996).

containing xanthan gum and acacia gum appear more stable against coalescence but less stable against creaming. In addition, the authors found that the optimal GX and GA concentrations were 0.3% and 1% respectively. However from our knowledge no work has been reported on the impact of the chemical composition of gums on the emulsification and stability of model emulsions.

The present work aims to study the impact of GX–GA mixtures on o/w emulsions stability, and focuses on the chemical composition and structure of each gum. For this purpose, on the one hand; two acacia gums (GA1 and GA2) have been selected for their respective high and low AGP% contents. On the other hand, two xanthan gums (GX1 and GX2) were chosen for their distinct acetate and pyruvic groups contents. Four emulsions have been made by crossing the four gums. Then different sunflower oil-in-water emulsions (GA1–GX1, GA1–GX2, GA2–GX1, GA2–GX2) have been obtained at fixed GX and GA concentrations i.e., 0.3% and 1% respectively (optimal conditions according to Paraskevopoulou et al., 2005). To assess the effects of chemical composition of both polysaccharides on the formation and the stability of emulsions, the present study is divided in three separate parts. In the first step, the rheological properties of GA–GX mixture bulk aqueous phase is presented and discussed, then the initial flow behaviour and emulsifying properties of the corresponding emulsions are described, and finally the emulsions stability against accelerated ageing conditions is considered.

2. Materials and methods

2.1. Materials

The two acacia gums (GA1 and GA2) are gift from Alland & Robert (Port Mort, France). The two xanthan gums (GX1 and GX2) were kindly provided by Degussa (Baupte, France). Physicochemical properties of these gums are detailed in Table 1 (GX) and Table 2 (GA).

Ultra pure grade water ("DiamondT2" Barnstead) was used to prepare the aqueous phases. Commercial Sunflower oil purchased from supermarket was used for all emulsions. All the chemical products used for the preparation of buffers solutions (citric acid, $C_6H_8O_7$; sodium hydroxide, NaOH) and salt (sodium chloride, NaCl) were obtained from Merck (Merck Eurolab, France). Sodium azide (NaN$_3$) from Prolabo (Rhône-Poulenc, France) was used as preservative.

2.2. Gums characteristics

Gums intrinsic viscosities were determined using a ball viscosimeter (Anton Paar AMVn, capillary diameter 1.6 mm, Steel ball

Table 2
Acacia gum samples characteristics.

	[η]^a (dL/g)	AGP^b (%)	γ^c (mN/m)
GA1	0.30	14 ± 1	54.4 ± 0.3
GA2	0.213	7 ± 1	61.0 ± 0.4

[a] determined at 25 °C by ball viscometry in NaCl 0.01 M.
[b] measured by SEC/MALLS/RI.
[c] measured by Cahn balance.

diameter 1.5 mm). Solutions were prepared in 0.01 M NaCl and concentration ranges were from 0.005 g/dL to 0.5 g/dL and from 0.3 to 5 g/dL for xanthan and acacia gum respectively. In each case, measurement was performed in the very dilute regime and linear regression of the specific viscosity over concentration was obtained. Gums protein content was measured using the Bradford method (Bradford, 1976), BSA was used as a reference thus protein content are expressed in BSA equivalent.

Acetate and pyruvate contents of xanthan gums were measured by NMR according to the method described by Rinaudo, Milas, Lambert, & Vincendon, 1983.

Transition temperature (Tm) of xanthan gums was measured by polarimetry in NaCl 0.01 M (method described by Capron, 1996).

The AG, GP and AGP percentages were determined using a gel permeation chromatography (GPC) system equipped with a Superose 6 10/300 GL (GE Healthcare). A refractive index (RI) detector (Sopares, France), an ultraviolet (UV) detector (Serie 1100 Helwett Packard, France) at 214 nm and a multiangle laser light scattering (MALLS) detector (Dark V3, Consenxus, Germany) operating at 532 nm were used. The mobile phase was a NaCl solution (0.1 mol/L) and the flow rate was controlled at 0.4 mL/min at ambient temperature. The injection loop volume was 50 μL. The value of the refractive index increment (dn/dc) was 0.142 mL/g as measured in the mobile phase, and mass calibration was carried out using a pullulan standard (Shodex P800). The GA solutions were prepared at 3 g/L in mobile phase 24 h before analysis and filtered (0.45 mm, cellulose ester) 1 h prior to injection.

2.3. O/w emulsion preparation

Emulsions have been prepared according to the process used by Paraskevopoulou et al. (2005). Aqueous solutions of each polysaccharide were prepared from a citrate buffer solution. For this, a small amount of sodium hydroxide solution (30% w/w) is added to the solution of citric acid (10 g/L) to adjust pH to 3.8. NaN$_3$ was then added up to 300 μg/L to avoid bacterial growth. The appropriate amount of NaCl is added to reach a final concentration corresponding to 0.01 M. Xanthan gum and acacia gum are gently dispersed in the buffer solution at room temperature during 1 h with a propeller-type mechanical stirrer in order to obtain stock solutions of GA and GX at 5% w/w and 0.5% w/w respectively. These solutions are then left to rest overnight in the refrigerator to allow complete swelling of the polysaccharides. The four matrices, namely GA1−GX1, GA1−GX2, GA2−GX1 and GA2−GX2 are obtained by mixing 20 mL of acacia solution with 60 mL of xanthan solution during 30 min under moderate mechanical agitation. Then, 20 mL of oil was slowly added to the aqueous matrices under propeller-type mechanical stirring during 3 min at room temperature. The obtained premix (coarse emulsion) was then homogenized with an Ultra-Turrax homogenizer (IKA T25 Digital) under vigorous stirring at 24,000 rpm for 2 min at room temperature, in order to get fine droplet dispersion. Each emulsion was duplicated to check preparation process reliability. The whole protocol for the preparation of emulsion was selected as it allows good reproducibility for the emulsions characteristics on the one hand, and also because the obtained emulsions are not perfectly stabilized, thus allowing the observation of differences in stability when submitted to accelerated ageing conditions on the other hand (see next section).

2.4. Emulsion stability

To evaluate the emulsion stability versus time, two accelerated ageing techniques were employed; centrifugation and heat exposure. After the second homogenization step, each emulsion was separated and transferred into 30 mL vials (for heat exposure test)

and centrifuge tubes (sterile, graduated, capacity 15 mL) both sealed with plastic caps. On the one hand, emulsions were then stored at 50 °C for 20 days and on the other hand, emulsions were centrifuged at 4025 g during 15 min at 25 °C (Sigma, 3-30K). The evolution of the droplets size distribution and viscosity were determined prior and after ageing test by granulometric and rheological measurements respectively. Emulsion stability was also determined by mean of microscopic observation.

2.5. Rheological measurements

The rheological properties of the emulsions and gums solutions were determined at 25 °C using a stress controlled rheometer (AR 2000, TA instruments) equipped with a cone-and-plate geometry (acrylic, angle 2°, diameter 60.0 mm, 52 μm gap). Temperature was controlled using a Peltier module. To achieve thermal equilibrium at 25 °C, the sample was left at rest in the measurement system for 2 min prior to any analysis. The apparent viscosity (η) was determined versus the imposed shear rate ($\dot{\gamma}$) from 0.005 to 100 s^{-1}. 10 points per decade were measured, the whole scanning time being 2 min. Thus solvent evaporation was neglected within this period. Zero shear viscosity (η_0) was determined by fitting the experimental viscosity curve using the Carreau model. Measurements were carried out twice for each sample in order to check reproducibility. Finally the reported zero shear viscosity was calculated as the average of the corresponding results.

$$\frac{\eta - \eta_\infty}{\eta_0 - \eta_\infty} = \frac{1}{\left[1 + (\lambda\dot{\gamma})^2\right]^{d/2}} \tag{1}$$

With η_∞ the infinite shear rate viscosity, λ is the relaxation time of the system and reflects the transition from newtonian to shear thinning behaviour and d is the power law index.

2.6. Particle size measurements

The droplet size distribution and the average drop diameter were measured with a laser diffractometer equipped with the Hydro MU large volume manual sample dispersion unit (Mastersizer 2000 Malvern Instruments) at ambient temperature. The refractive indices used are 1.46 for sunflower oil and 1.33 for water. Emulsions were diluted in a ratio of 1/10 v/v with a solution of Sodium Dodecyl Sulfate SDS (1%) and gently mixed for 10 min. A sufficient quantity of that mixture was then dispersed in 400 mL of deionized water (dispersion unit) to be in the correct range of obscuration of the optical system. Emulsions dilutions were duplicated. Three measurements were carried on each emulsion sample and the average droplet diameter D[4,3] ($= \sum n_i d_i^4 / \sum n_i d_i^2$) reported was calculated as the average of all measurements.

2.7. Microscopic observation

Samples were examined under the microscope with and without any previous dilution in a SDS solution. A drop of emulsion was deposited on a microscope slide and then covered with a cover slip. The structural feature of selected emulsion sample is observed using a Leica DMLP optical microscope equipped with Leica DC300 digital camera system. Images were analysed using the Leica IM2000 software.

2.8. Applied statistics

Data are presented as means ± standard deviations in table (or error bars in graph). Data were tested using one-way ANOVA and

least significant difference tests to determine the effect of the GA−GX mixture or ageing treatment. A probability value < 0.05 was considered to be significant. In tables from 3 to 6 letters in index (or above graph bars for Fig. 4) relate to the significance of the difference between values in a same column. So two values labelled with the same letter are not significantly different (from a statistical point of view). In the opposite, two values labelled with two different letters are significantly different and thus the tested parameter has a significant effect on the reported data.

3. Results and discussion

3.1. Viscometric characterization of GA and GX aqueous solutions and mixtures

To better understand the influence of acacia and xanthan gums chemical composition on the emulsions flow properties, the flow behaviour of these gums and their combinations in the buffer solution is first considered. To this end, gum(s) concentrations are identical to those utilized in the aqueous phase in emulsion: 1% w/w for GA solutions and 0.3% w/w for GX solutions and 1% w/w GA + 0.3% w/w GX for mixtures. The obtained viscosity curves for GA and GX solutions are in good accordance with the literature (Cuvelier & Launay, 1986). On the one hand, GX solutions exhibit a pseudoplastic behaviour, with a Newtonian plateau at low shear rate followed by a shear thinning behaviour. In this case, the pronounced loss in viscosity of GX is classically interpreted as the consequence of a progressive breakdown of aggregates combined with the alignment of xanthan molecules in the shear field. On the other hand, GA solutions almost behave as newtonian fluids as the viscosity remains nearly unchanged in a large range of shear rate. The constant and low apparent viscosity is mainly explained by the highly connected, fairly compact, structure of the GA (Li et al., 2011). When both GA and GX gums are mixed, the resulting mixtures exhibit a translucent homogeneous aspect, with no phase separation visible to the naked eye. Fig. 1 shows the viscosity curves of GX2 solution and GA1−GX2 mixture (highly sheared solution and corresponding emulsion are plotted as well). GA1−GX2 mixture exhibits a pseudoplastic behaviour quite similar to that observed for GX2 solution but with a much higher zero shear viscosity. The same trend is observed with all the systems studied here. Table 3 summarizes the characteristic parameter of the Carreau's model for the four mixtures studied. Letters in index relate to the significance of the difference between values in a same column as explain above. When comparing those parameters one can conclude that only the zero shear viscosity is significantly different from a system to another. This indicates the presence of interactions between both gum in the linear regime. These interactions being broken down by shearing this explain the similar behaviour in the shear thinning regime. Thus, in the following we will focus the rheological discussion only on η_0 which is characteristic of the sample behaviour at rest.

In order to compare the rheological properties of the whole solutions and mixtures, values of zero shear viscosities (η_0) are reported in Fig. 2. For each solution and mixture, reproducibility of the zero shear viscosity reported was assessed as the average of two flow measurements conducted on two different gum samples.

As expected, both GA solutions own an identical low viscosity, much lower than that of GX solutions. When comparing GX zero shear viscosity of both xanthan gums, one can observe that GX2 solution has a much higher viscosity than GX1 solution. From a general point of view, pyruvate and acetate groups rates are known to affect the viscometric properties of xanthan solutions (Flores-Candia & Decker, 1999; Hassler & Doherty, 1990) i.e., increasing acetate groups rate decreases GX viscosity while
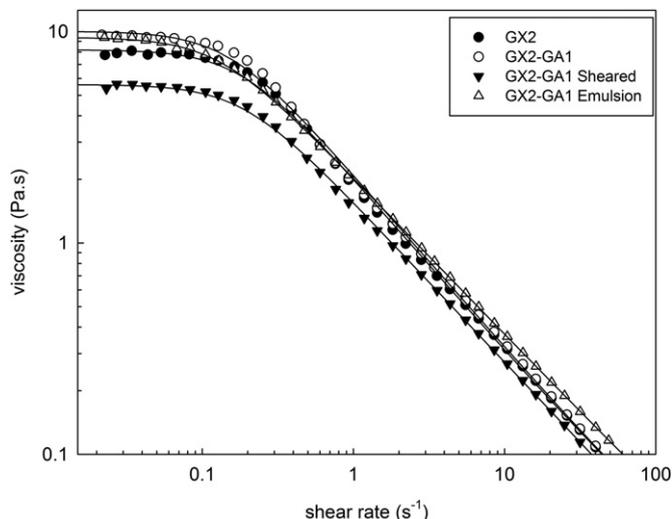

Fig. 1. Viscosity curves of solution 0.3% w/w xanthan gum GX2 and the corresponding mixtures containing both 1% w/w acacia gum GA 1 and 0.3% w/w xanthan gum GX2.

increasing pyruvate conducts to a higher viscosity. In the present study, GX2 has higher rate of both acetate and pyruvate (see Table 1), thus both affect the viscosity in opposite ways. In these conditions, one can not predict the higher viscosity observed for GX2. Nevertheless, even if the acetate rate difference between GX1 and GX2 is much higher than pyruvate rate, the viscosity of GX2 is still greater than the one of GX1. This means that the effect of pyruvate groups on the viscosity is much more pronounced than acetate one which is consistent with previous finding (Flores-Candia & Decker, 1999; Hassler & Doherty, 1990).

When examining the GX−GA mixtures zero shear viscometric properties, one can observe that the corresponding values are mainly influenced by the xanthan gum sample used as GX1 based mixtures have a lower viscosity compared to GX2 based ones. In addition, it is important to stress on that η_0 of all GA−GX mixtures appears significantly higher than the sum of the zero shear viscosity of each gum measured separately. Therefore, as mentioned above, such an observation permits evidencing interactions between GA and GX gums, without, at this stage, any plausible indication concerning the mechanism involved.

The viscosity improvement in mixtures can be expressed as the percentage of the excess of the actual value from the expected "theoretical" one of mixtures in the absence of interactions between gums. The "theoretical" viscosity (of non-interacting systems) can be estimated as the sum of zero shear viscosity of each gum measured separately at the same concentrations as in mixture. This excess attains 18−20% for the mixtures containing GA1 while it is limited to 11−12% for mixtures made with GA2, thus this allows us to illustrate the probable key role of the GA structural characteristics in the mechanism involved in GA/GX interactions. Due to the lack of previous works and the absence of publications (to our knowledge) related to GA−GX systems, it is not possible, at this stage, to propose any explanation for this marked viscosity

Table 3
Rheological characteristics of mixture solutions.

Sample	η_0 (Pa. s)	λ (s)	d
GA1−GX1	7.82[a] ± 0.66	5.94[a] ± 0.48	0.809[a] ± 0.010
GA1−GX2	7.36[a] ± 0.60	5.85[a] ± 0.34	0.805[a] ± 0.017
GA2−GX1	10.03[b] ± 0.79	6.27[a] ± 0.50	0.827[a] ± 0.004
GA2−GX2	9.36[b] ± 0.31	6.09[a] ± 0.11	0.819[a] ± 0.015

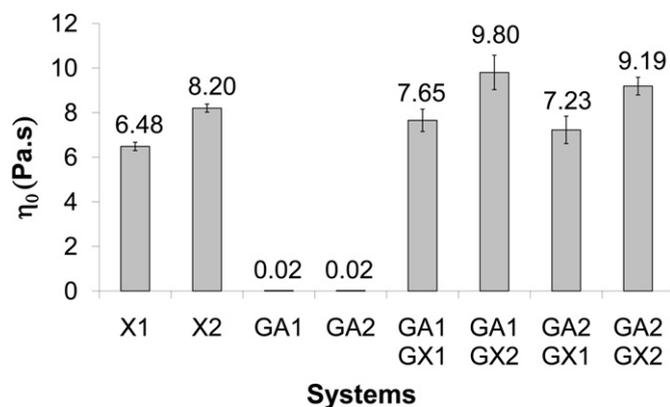Different letters indicate significant differences ($P < 0.05$).

Fig. 2. Zero shear viscosity (η_0) of aqueous solutions with 1% w/w acacia gum, 0.3% w/w xanthan gum and the corresponding mixtures containing both 1% w/w acacia gum and 0.3% w/w xanthan gum.

increase; such a phenomenon is described for several poly-saccharide combinations, and is often attributed to synergistic mechanisms (Launay, Cuvelier, Michon, Langendorff, & Katsuyoshi, 2000). A study is currently underway in our laboratory to determine whether the viscosity increase observed for GA–GX mixtures, may be attributed to specific attractive or repulsive interactions between both polysaccharides.

3.2. Initial emulsions properties

As described in the experimental section, 20% v/v o/w emulsions were obtained by addition of sunflower oil to the GA–GX aqueous solutions characterized in the previous section. Emulsifying process used was chosen and optimized in order to get easy, efficient and reproducible homogenization protocol thus permitting to focus the study on the lonely gum characteristics effect on the emulsion properties. In all cases, emulsions were stored at 4 °C immediately

after preparation. In order to investigate the effect of structural variations of both GA and GX gums on the initial properties of emulsions, granulometric and viscometric analysis were carried out 24 h after fabrication (t_0), using laser diffraction and rheology respectively. To ensure correct interpretation of granulometric properties between gums and with ageing, droplets average-size and distribution were measured three times on two distinct emulsions for each system studied here (6 measurements in total). It is well established that xanthan molecules reduce creaming phenomena by increasing the viscosity of the continuous phase and/or by creating a 3D network of aggregated particles depending on the xanthan concentration (Parker, Gunning, Ng, & Robins, 1995). Thus this makes difficult to visualize fully individualized droplets. Therefore, in order to determine the effective individual droplets size, corresponding to the deflocculated state, prior to particle size analysis, each emulsion was diluted in a ratio of 1/10 v/v in an aqueous solution containing 1% (w/w) Sodium Dodecyl Sulfate (SDS), and then gently stirred for 10 min using a magnetic stirrer to insure a fully disaggregated state. In parallel, all emulsions were studied by optical microscopy in order to determine the emulsion aspect before dilution. Both the distribution of droplet size and photomicrographs of emulsions with and without dilution in the SDS solution are represented in Fig. 3. Since SDS is highly surface active it may be suspected to exclude GA from the droplet surface or to create mixed layer at the interface. Nevertheless, Drakos and Kiosseoglou (2008) used similar protocol with yolk-based emulsion and proved that SDS molecules do not change emulsion stability. In the present study measurement are realized quite immediately (10 min) after dilution thus avoiding change in droplet stability. Additionally, one can clearly see on photomicrographs in Fig. 3 that individual droplet size does not change before and after dilution in SDS thus confirming the ability for SDS to efficiently deflocculate the droplets as well.

As expected, the o/w emulsions are presented as spherical globules of oil dispersed in the aqueous continuous phase. Emulsions obtained without dilution in the SDS solution (Fig. 3A) present a wide droplet size distribution, from 0.5 µm to 40 µm.
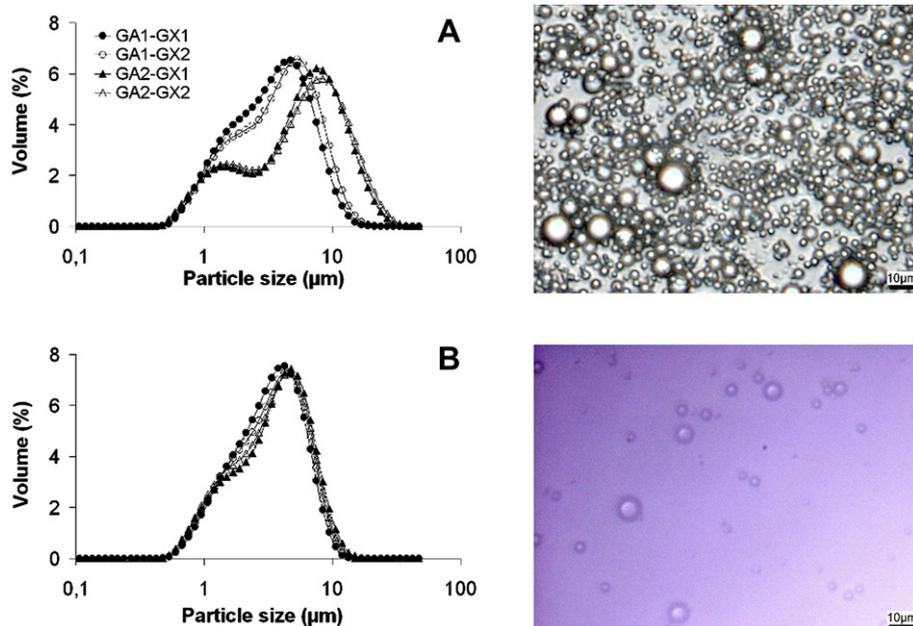

Fig. 3. Influence of gums mixtures on the particle size distribution of 20% v/v sunflower o/w emulsions freshly prepared (left panel), emulsions without (A) and with dilution in solution SDS (B). The right panel shows micrograph of emulsion obtained with GA2–GX1 without dilution and after dilution with SDS (A and B respectively). The bar length corresponds to 10 µm for each emulsion.

When looking at the corresponding photograph (right panel of Fig. 3A) one can observe a broad droplet size distribution nevertheless none of them exceed ∼15 μm of diameter. This means that the larger sizes measured by laser diffraction correspond to droplet aggregates. This phenomenon is clearly shown on the photograph of Fig. 3A. It is important to point out that emulsions containing GA2 are much more prone to flocculation than the one prepared with GA1. As the droplet size distribution is expressed in volume, one should notice that the numbered fraction of small droplets is actually much greater than the one corresponding to larger ones (for a similar volume fraction) which explains the apparent distribution difference between laser diffraction and photograph. However, when diluted in the SDS solution, emulsions exhibit a narrower droplet size distribution between 0.5 μm and 15 μm but remains non gaussian (Fig. 3B). In addition, one can also observe that the droplets size distribution mainly consists on small ones attesting the homogenizing process efficiency. Again, the corresponding microscope image (Fig. 3B) confirms the presence of individualized droplets attesting that SDS leads to aggregate breaking down. This breaking down can't be attributed to a simple dilution effect since in both cases (with and without dilution in SDS solution) emulsions are dispersed in a large volume of milliQ water (see the materials and methods section). Fig. 3B allows comparison of the droplet size distribution of emulsions prepared with every gums mixtures used. First, all emulsions exhibit the same overall size distribution, i.e., a relatively narrow non gaussian distribution. However, distributions of emulsions obtained with GA2 present a pronounced shoulder (between 1 and 5 μm) whatever the GX used. This clearly illustrates the emulsifying ability of each GA used. It is well established that GA ability to stabilize lipophilic−hydrophilic interfaces is attributed to the AGP fraction; the more the AGP content the highest (i) the emulsifying properties and (ii) droplet individualizing ability (Al-Assaf et al., 2007; Randall et al., 1988). Our results are consistent with this trend as illustrated by the narrower droplets distribution of GA1 based emulsions compared to GA2 ones, since GA1 contains about twice AGP than GA2 (see Table 2). Secondary, the GX impact appears much more limited as for a given GA−GX combination, only a slight shoulder in the range of 1−5 μm is observed (for GA1 based emulsions), thus allowing to distinguish the actual GX effect. In all cases, the GX impact is low but significant when considering the good reproducibility of measurements. More generally, the whole observations clearly illustrate the influence of both GA and GX gums on the emulsifying abilities.

Fig. 4 shows the initial average diameter D[4,3] of the o/w emulsion prepared from the four different GA/GX combinations. Those mean diameters D[4,3] are obtained from Fig. 3B. The difference in shape of distributions (shoulder + shift) of droplet size of emulsions prepared with GA1 and GA2 directly impacts D[4,3]. Indeed, emulsions prepared with GA1 have a lower average diameter (3.53 and 3.64 μm for GX1 and GX2 respectively) than the one obtained with GA2 (4.18 and 3.76 μm for GX1 and GX2 respectively). Based on the applied statistics analysis (letter above graph bars), no significant difference related to xanthan gum used is observed on D[4,3] for the different emulsions prepared with GA1, while, for emulsions containing GA2, the one prepared with GX1 owns a significantly larger D[4,3] than the one with GX2. Basically, the droplets size resulting from the mechanical homogenization process depends on the required time for the emulsifier to adsorb at the surface of the oil droplet, and the droplet−droplet collisions frequency due to the intense mechanical agitation as well (McClements, 1999). Obviously, this collision frequency depends also on the continuous phase viscosity, the more viscous the lower the frequency. Nevertheless as previously established in the present paper, GA−GX mixtures exhibit a marked pseudoplastic
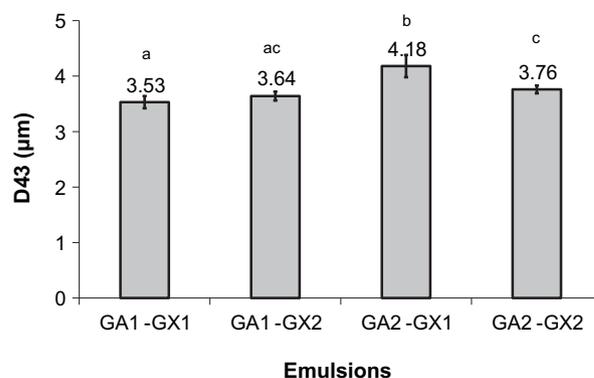

Fig. 4. Influence of GA−GX combinations on the initial average droplet diameter D[4,3] of 20% o/w emulsions.

behaviour thus leading to a strong decrease of the viscosity when submitted to high shear. Consequently, since during high-shear homogenization procedure, the viscosity of the aqueous phase is reduced in such a way that η becomes roughly identical whatever the xanthan used thus the collision frequency as well. This may explain the limited effect of xanthan gum on the droplets formation.

On the opposite, when considering GA effect, D[4,3] being significantly lower for GA1 emulsions than GA2 ones with GX1 (this difference does not seem that significant for GX2 based emulsion at t_0), it is obvious that acacia gum structural properties play a key role on the emulsifying process. Actually in the literature, it is well established that the protein-rich AGP fraction predominantly adsorbs at the oil−water interface, and therefore strongly influences the emulsifying and interfacial properties of GA gums (Fauconnier et al., 2000; Randall et al., 1988; Ray, Bird, Iacobucci, & Clark, 1995). Consequently, the emulsifying properties of gums increase with the percentage of the AGP fractions (Castellani et al., 2010; Savary, Hucher, Bernadi, Grisel, & Malhiac, 2010). In the present work, as presented previously, AGP content of GA1 is twice the one of GA2. Therefore, during homogenization process, GA1 is more able to coat and stabilize the oil−water interface due to its higher AGP content compared to GA2 and thus leads to smaller droplet sizes. The present observations are fully consistent with the surface tensions measured for each GA and with literature (see Table 2).

Viscosities of both emulsions and corresponding aqueous phase containing GA−GX mixtures were studied as a function of shear rate ranging from 0.005 to 100 s^{-1}. In order to take into account the high-shear effect related to the emulsifying process, each gum aqueous phase was submitted to the same rotor-stator shear using the ultra-Turrax prior to rheological measurement. As expected, corresponding flow curves are characteristic of a shear-thinning fluid preceded by a newtonian plateau at low shear rate (curves are shown in Fig. 1 for GA1−GX2 system).

Table 4
Zero shear viscosities of aqueous solutions before and after shearing and of emulsions.

Sample	η_0 (Pa. s)		
	Aqueous phase	Sheared aqueous phase	Emulsion
GA1−GX1	7.82[a] ± 0.66	3.42[a] ± 0.03	6.46[a] ± 0.16
GA1−GX2	7.36[a] ± 0.60	5.68[b] ± 0.08	9.69[b] ± 0.41
GA2−GX1	10.03[b] ± 0.79	3.23[c] ± 0.04	6.37[a] ± 0.24
GA2−GX2	9.36[b] ± 0.31	5.12[d] ± 0.09	9.21[b] ± 0.31

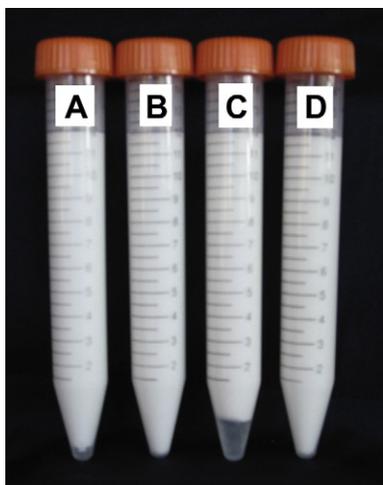Different letters indicate significant differences ($P < 0.05$).

Fig. 5. Photographs of bottle test emulsions after 20 days storage at 50 °C. From left to right, the systems are exposed in the following order: GA1–GX1 (A), GA1–GX2 (B), GA2–GX1(C) and GA2–GX2 (D).

Table 4 presents the zero shear viscosities for each gums solutions and emulsion obtained by fitting viscosity curves with the Carreau model. Results for non sheared solutions are also plotted for comparison. First one can observe that η_0 after shearing is much lower than before the high-shear treatment for every aqueous phase. The viscosity is divided by about a factor 2.2 for solutions containing GX1 while by a factor 1.75 for GX2 solutions without any significant effect of the GA used. This clearly shows that the solution viscosity is mainly governed by the xanthan gum and that GX2 is less affected by the high-shear procedure than GX1. Furthermore, aqueous phase as well as corresponding emulsions can be separated in two distinct groups according to the xanthan gum used: all solutions and emulsions prepared with GX1 own a lower zero shear

viscosity compared to GX2 based ones. In addition, no significant difference related to the nature of acacia gum used can be observed.

When comparing the viscosity of emulsions with the corresponding aqueous phase, one can observe that the former are more viscous. Such a viscosity increase is related to the fine dispersion of oily droplets which strongly interact with the aqueous phase (Tadros, 1994). Nevertheless when looking at the data more carefully, we should notice that the viscosity increase between emulsion and the corresponding solution depends mainly on the xanthan gum used i.e., the viscosity is multiplied by a factor about 1.9 between emulsions and solutions prepared with GX1 while the factor is about 1.7 for GX2. We may speculate that during the high-shear homogenization process GX1 is more destructured than GX2 (Milas, Lindner, Rinaudo, & Borsali, 1996) thus the "internal" groups are exposed to the surface of the polymeric chain as observed for proteins (Aymard, Nicolai, Durand, & Clark, 1999). Those groups may be suspected to interact with GA. Nevertheless the lack of experiments does not allow us to discuss more in detail about that point. This will be address in a forthcoming paper. As a first concluding remark, the whole results presented in this first section suggest that at the initial stage of emulsions, the droplet size is mainly governed by the acacia gum while the viscosity is controlled by the xanthan gum.

3.3. Emulsions stability

Once prepared, all emulsions exhibit a fairly good stability over time when stored at rest at room temperature. Thus all emulsions were submitted to accelerated ageing conditions, namely centrifugation on the one hand and heat exposure on the other hand (see the materials and methods section). The stability of the different emulsions was evaluated by following the evolution particle size evolution and flow properties after ageing.

Centrifugation tests were performed in order to rush the emulsion in such a way that the evolution of each GA/GX system
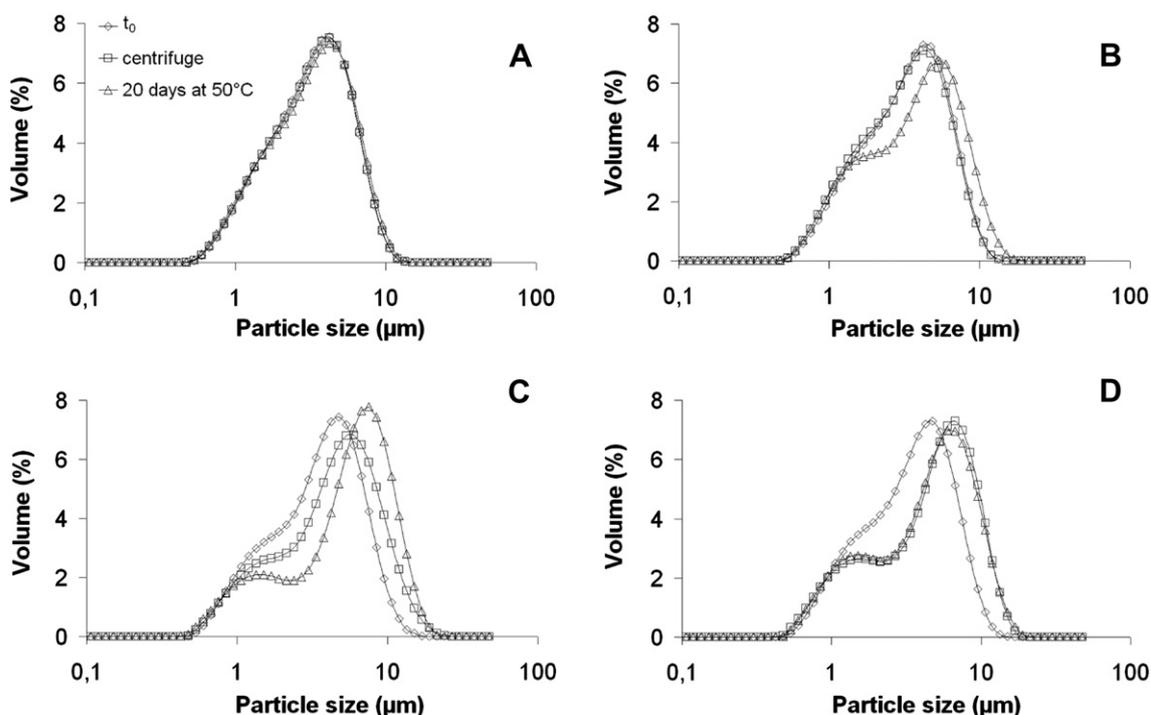

Fig. 6. Droplet size distribution freshly prepared (◇), after centrifugation (□) and heat exposure of each emulsion (△). The systems are exposed in the following order: GA1–GX1 (A), GA1–GX2 (B), GA2–GX1 (C) and GA2–GX2 (D).
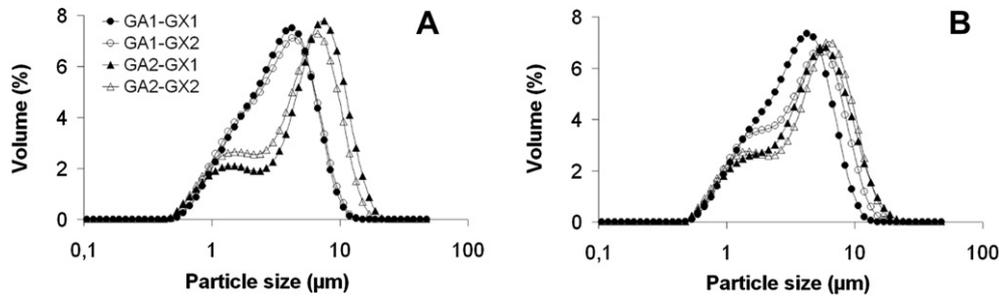
Fig. 7. Influence of the ageing treatment on the droplet size distribution of the 20% v/v sunflower o/w emulsions; centrifugation (A) and heat exposure (B).

allows highlighting stability differences. First, from a macroscopic point of view, no marked phase separation was observed after centrifugation, except a slight creaming (not shown). Xanthan gum at such concentration improve emulsion stability against creaming proving that xanthan creates a strong enough network to avoid (at least to limit) droplet diffusion through this network (Hemar, Tamehana, Munro, & Singh, 2001; Moschakis, Murray, & Dickinson, 2006). On the opposite, with regard to emulsions undergoing heat exposure (50 °C) for a period of 20 days, the bottle test results allow evidencing differences between the GA–GX systems as presented in Fig. 5.

First, both emulsions containing GX1 show an aqueous phase separation at the bottom of the tube, this phenomenon being more pronounced for the emulsion with GA2–GX1 and to a lesser extent for the emulsion with GA1–GX1. On the other hand, no oil phase separation, resulting from droplets coalescence, was noticed for all emulsions. Since xanthan gum does not adsorb directly onto the droplet surface (Hemar et al., 2001), such an aqueous phase separation is usually attributed to repulsive interactions between the stabilizer and the oil droplets which are at the origin of depletion of the stabilizer from the region between two droplets. As a consequence the xanthan concentration in this region is lower than in the bulk phase, thus creating an osmotic pressure gradient which make the solvent (water) flowing out this region (Tesch & Schubert, 2002). Nevertheless, one has to consider that here AGP fraction of GA is adsorbed at the droplet surface resulting a decrease of the repulsive interactions between oil and xanthan gum by creating a "protecting" layer between both. The higher the AGP content the denser the adsorbed layer on the drop surface, thus leading to weaken repulsions. Since GA1 contains twice AGP compared to GA2 this may explain the better stability observed for GA1–GX1 emulsions compared to GA2–GX1 based one.

In order to investigate the ageing effect on the structure of each emulsion, the particle size distribution and average diameter D[4,3] were measured and compared to the initial stage. The particle size distributions of emulsions after centrifugation and after 20 days of storage at 50 °C are shown in Fig. 6.

3.3.1. Centrifugation tests

Regarding the results corresponding to centrifuged samples, emulsions can be separated into two groups according to the evolution of the particle size distribution. On the one hand, we should notice that for GA1 emulsions, droplet size distribution remains the same than at the initial stage whether combined with GX1 or GX2 thus GA1 emulsion are very stable versus centrifuge. On the other hand emulsions prepared with GA2 are more affected by ageing since the droplets size distribution broadens towards larger drop sizes, and the initial shoulder is much more accentuated thus leading to the emergence of bimodality. In addition, Fig. 6 allows evidencing that the bimodality and expansion of the size distribution of the emulsion prepared with GA2–GX1, is more

pronounced than the one prepared with GA2–GX2. The increase in droplet size is usually attributed to two mechanisms; droplets coalescence or Ostwald ripening or both (Dickinson, 1992, chap. 1). In our cases, the shift towards larger droplet size is accompanied with the appearance of a shoulder in the range of small sizes thus meaning that volume fraction of such small droplets (~5 μm) decreased. That observation shows that Oswald ripening seems predominant for our emulsions. The evolutions of drop sizes distributions allow concluding that the emulsions prepared with GA1 exhibit better stability than emulsions prepared with GA2 after centrifugation with a limited effect of the xanthan gum used.

3.3.2. Heat exposure tests

One can observe that all droplet size distribution curves markedly change with the exception of the emulsion prepared with GA1–GX1 that remains unchanged when compared to the initial stage. Consequently, GA1–GX1 system appears the most performing combination to stabilize oil-in-water emulsions. Except for this last gum combination, each of the other emulsions show a broader droplet size distribution, shifted to larger droplets with accentuation of the shoulder towards a bimodal distribution. In addition, distribution curves allow pointing out that both the bimodality and the size distribution enlargement are more pronounced for the emulsion prepared with GA2–GX2 than with GA2–GX1 system, thus demonstrating that GX2 appears as the least efficient suspending agent. This is also the case for emulsions prepared with GA1.

Fig. 7 combines the same results than Fig. 6 for the two ageing process. In Fig. 7A, one can clearly see that, after centrifugation, GA1 is a much better emulsifier than GA2 since droplet size distribution is unchanged compared to t_0. Nevertheless no significant differences are observed between GX1 and GX2 (see Table 5). Fig. 7B presents the droplet size distribution after heat exposure for all emulsions. Except GA1–GX1, every emulsion shows a more or less pronounced bimodality. Thus this result allows us to rank the GA–GX combinations from the most to least stable against heat exposure; GA1–GX1 > GA1–GX2 > GA2–GX1 > GA2–GX2.

Table 5 represents the ageing process effect on the average droplets diameter for the different systems studied. When analysing results according to the ageing process, one can conclude that centrifugation process has no effect on D[4,3] for emulsions

Table 5
Ageing condition effect on D[4,3] droplet diameter.

Ageing condition	D[4,3] μm			
	GA1–GX1	GA1–GX2	GA2–GX1	GA2–GX2
t_0	3.53[a] ± 0.11	3.64[a] ± 0.08	4.18[a] ± 0.20	3.76[a] ± 0.07
Centrifuge	3.63[a] ± 0.03	3.71[a] ± 0.07	6.20[b] ± 0.15	5.88[b] ± 0.09
20 days - 50 °C	3.62[a] ± 0.21	4.24[b] ± 0.09	5.13[c] ± 0.04	5.46[c] ± 0.37

Different letters indicate significant differences ($P < 0.05$).

Table 6
Ageing condition effect on the zero shear viscosity of the emulsion.

Ageing condition	η_0 (Pa. s)			
	GA1–GX1	GA1–GX2	GA2–GX1	GA2–GX2
t_0	$6.46^a \pm 0.16$	$9.69^a \pm 0.41$	$6.37^a \pm 0.24$	$9.21^a \pm 0.31$
Centrifuge	$6.43^a \pm 0.03$	$9.32^a \pm 0.64$	$6.68^a \pm 0.15$	$10.40^b \pm 0.32$
20 days - 50 °C	$5.19^b \pm 0.21$	$7.67^b \pm 0.27$	$5.55^b \pm 0.25$	$7.97^c \pm 0.30$

Different letters indicate significant differences ($P < 0.05$).

prepared with GA1 as it remains unchanged. On the opposite, D [4,3] for emulsions prepared with GA2 show marked increase of droplet sizes (48–56%) thus indicating a lower stability of the corresponding emulsions when submitted to mechanical destabilization. When submitted to high temperature, D[4,3] for emulsion prepared with GA1–GX1 show no change, while the one prepared with GA1–GX2 slightly increases of 0.6 μm (about 15%). On the contrary both emulsions containing GA2 show a pronounced increase of D[4,3], the drop size evolution being more significant for the emulsion containing GX2 (#1.7 μm) compare to GX1 (#0.95 μm).

Zero shear viscosity of the different emulsions measured after centrifugation and heat exposure are reported in Table 6, and are compared to initial results.

Firstly, after centrifugation η_0 remains almost unchanged for every system studied thus proving that extreme mechanical perturbation do not affect significantly the emulsion viscosity, except for GA2–GX2 which exhibits a much higher zero shear viscosity after centrifugation. This viscosity enhancement may be explained by depletion-flocculation phenomenon (Parker et al., 1995), the resulting droplet cluster inducing a higher emulsion viscosity. Nevertheless, there is no explanation why this phenomena is only observed for this specific GA2–GX2 combination. On the opposite, emulsions exhibit a sharp viscosity decrease when submitted to heat exposure as visible in Table 6; this can be attributed to the long lasting heat exposure close to the xanthan molecules transition temperature. However, this decrease depends on the acacia gum sample as the viscosity lowering is about 25% for emulsions prepared with GA1 while it is only 15% with GA2 whatever GX.

Thus we should stress that even if the emulsion viscosity is mainly controlled by the xanthan gum (as presented above) its stability versus heat exposure appears strongly affected by the acacia gum characteristics.

The viscosity loss for GA2–GX based emulsion is the direct consequence of the droplet size increasing. Hence Ostwald ripening is the driving force of GA2 emulsions destabilization. On the opposite we should mention that the viscosities of GA1–GX based emulsions are strongly affected by heat exposure while the corresponding D[4,3] remain constant, thus meaning that the viscosity decreasing is only related to droplets flocculation for these GA1 based emulsions.

To summarize, the whole observations clearly allow establishing the greater interfacial stabilizing ability of GA1 when compared to GA2 performance. This difference of functional properties is observed whatever the xanthan sample used thus independently from the occurrence of interactions between both gums as evidenced by flow properties of gum mixtures in aqueous solutions.

4. Conclusion

The viscometric properties of xanthan − acacia gum aqueous mixtures have been characterized. Afterwards, xanthan and acacia gums were conjointly used to stabilize o/w emulsions, particularly the impact of their chemical composition on the emulsion stability has been investigated. Firstly, viscosity enhancement was observed for GA–GX mixtures compared to pure GX solutions which clearly shows that xanthan and acacia molecules do interact, thus resulting in a so-called synergistic mechanism; furthermore, viscometric results clearly indicate that the extend of interactions between both gums is governed by their structural and chemical characteristics. Secondly, GA–GX associations stabilizing ability for emulsions was tested. For freshly prepared emulsions, results obviously indicate that the viscosity is mainly governed by the xanthan gum structures while the droplet size depends on the acacia gum AGP content. Indeed the higher the pyruvate xanthan rate the thicker the emulsion, while the higher the acacia gum AGP content the better the o-w interface stabilization.

If considering ageing effects, over the fact that, as expected, the rheological properties of emulsions are mainly controlled by the xanthan gum used, their changing's actually depend on both GX and GA used. Unexpectedly, the oil droplet size distribution evolves differently depending on the xanthan composition; in particular we proved that the most thicken emulsion does not exhibit the best stability as could be expected by the Stokes law (droplet diffusion). No doubt that the GA–GX intermolecular interactions play a major role on the gum mixtures stabilizing ability. From our results, we speculate that AGP fraction of acacia gum plays a major role in those interactions since GA1 based emulsion are more stable than those obtained with GA2 which contains about half AGP compared to GA1. Concerning the xanthan gum, its impact on the emulsion stabilization is less pronounced than GA but GX1 based emulsions seem to be a little more stable than those obtained with GX2. On the one hand, the difference in acetate rate is greater than the pyruvate one and on the other hand their behaviour in emulsion only slightly diverges, we can suppose that the emulsion stability should be more related to the pyruvate content. Since only 2 samples have been used in the present study, we cannot, at this stage, unambiguously propose any reliable mechanism for the influence of the GX structural properties. Thus, further investigations are needed to elucidate the mechanisms involved. Work is currently under development in our laboratory to better understand both the nature and the underlying mechanisms of these interactions between GX and GA.

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