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Physicochemical, interfacial and emulsifying properties of a non-conventional exudate gum (*Prosopis alba*) in comparison with gum arabic

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ARTICLE INFO

ABSTRACT

Physicochemical and emulsifying properties of a novel exudate gum from *Prosopis alba* (G) were assessed in comparison with the well-known arabic gum (GA).

Compositional analysis, intrinsic viscosity and structural characterization as well as gum emulsifying properties (evaluated in terms of mean droplet size distribution of o/w emulsions, ζ-potential, polidispersity and creaming indexes), were performed and related to the dynamic interfacial tension and rheological behavior of the films formed at oil/water interface. Both gums closely resemble in composition with carbohydrates representing the higher fraction (62 and 65% d.b. for G and GA, respectively). The major difference was observed in the protein content which was higher in *P. alba* gum and largely explains its better interfacial properties. FTIR analysis further supported that the gums share essentially a similar chemical nature.

P. alba gum was able to stabilize emulsions better than GA based on its lower droplet size distributions, higher ζ-potential, higher interfacial film elasticity and lower values of the polidispersity and creaming indexes during storage. Furthermore, the formation of stronger viscoelastic films at the oil/water interface as well as the charge distribution of the adsorbed G fractions could contribute to understand the higher stability of G emulsions.

Present results provide the first approach to the composition of *P. alba* gum and evidence its similar or even better emulsifying properties than GA.

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1. Introduction

In recent years, there has been a growing interest in the search for feasible new sources of biopolymers to be used in the food industry (Dickinson, 2003; Mirhosseini & Amid, 2012). Among them, exudate gums exhibit interesting emulsifying and stabilizing properties (Acedo-Carrillo et al., 2006; Alftrén, Peñarrieta, Bergenståhl, & Nilsson, 2012; Pérez-Mosqueda, Ramírez, Alfaro, Rincón, & Muñoz, 2013) which make them suitable for their inclusion into foods, especially oil-in-water (o/w) emulsions systems. They have been widely used as stabilizer/emulsifier in many emulsion-based foods like ice-creams, sauces, dressings (Dickinson, 2009) and also in many beverages containing flavor oils or non-flavor oils, such as omega-3 fatty acids, included to provide special nutrients (Li & Nie, 2015). The selection of an appropriate gum is based on certain criteria such as solubility, suspension ability, emulsifying/stabilizing properties, natural or synthetic, and relative cost among others. Most natural gums are safe for oral

consumption and are preferred over analogous synthetic gums due to their non-toxicity, low cost and availability (Rana, Rai, Tiwary, Singh, Kennedy, Knill & Rana, 2011).

Arabic gum (GA), a natural arabinogalactan-protein type poly-saccharide obtained from *Acacia Senegal,* is one of the oldest and well known exudate gum widely used in food and non food ap-plications (Islam, Phillips, Sljivo, Snowden, & Williams, 1997; Williams & Phillips, 2009). Although Arabic gum is an effective emulsifier/stabilizer agent, the current search on new sources of gums relies on its variable provision due to political and climatic factors in the primary producing countries which has led to spikes in the price and availability of the ingredient (López-Franco, Higuera-Ciapara, Goycoolea, & Wang, 2009; Mahfoudhi et al., 2014; Sibaja-Hernández, Román-Guerrero, Sepúlveda-Jiménez, & Rodríguez-Monroy, 2015).

Several studies have shown that the exudates of certain *Prosopis* specimens, commonly referred as mesquite gum (MG), exhibit functional properties comparable or even superior to those from GA (Orozco-Villafuerte, Cruz-Sosa, Ponce-Alquicira, & Vernon-Carter, 2003; Vernon-Carter, Pedroza-Islas, & Beristain, 1998). The varia-tions in sugar compositions, nitrogen content and molecular weight among MG samples suggest a significant structural diver-sification, depending mainly on its botanical origin and to a lesser extent to environmental factors such as climatic and soil conditions (López-Franco, Gooycolea, & Lizardi-Mendoza, 2014).

Prosopis genus is widely spread in arid and semiarid regions of South America, especially at the north-east region of Argentine, where the natural occurrence and wide dispersion of *Prosopis alba* make it one of the main specimens in the economic and the ecological scene. *P. alba* tree, locally known as "algarrobo blanco", has been traditionally used as wood source. Actually its pods and seeds have also received special attention as raw material for manufacturing animal feed, flour, cacao substitutes, regional bev-erages and foods, and as polysaccharides source (Cardozo et al., 2010). Under environmental stress condition, *P. alba* excretes a viscous gum which gets hard over the bark. Although, several *Prosopis spp.* have recently been studied as new sources of gums (López-Franco et al., 2012), no studies were conducted to date on the characterization and potential applications of *P. alba* exudate gum (G).

The objective of present work was to evaluate the physico-chemical and functional properties (interfacial activity and emul-sifying properties) of *P. alba* exudate gum in comparison with the well-known GA. Results may contribute to establish possible food applications of a novel non-conventional gum, with the added benefit of employing a natural resource that has not yet been exploited, and could have a positive impact on the development of regional economies.

2. Materials and methods

2.1. Materials

P. alba exudate gum (G) was purified from air-dried exudations of native and protected trees located in the central zone of the province of Chaco, in the north eastern Argentine. The trees were botanically identified by the IBONE (Botanical Institute of the Northeast, Corrientes, Argentine). The samples were manually collected in drought periods of summer and winter seasons (when exudation increases after ripening of the fruits) and included nat-ural exudations (on the main trunk and branches) and also exu-dations produced by mechanical damages (due to agricultural practices and other types of wounds). They had a bitter taste, slightly sweet odor and variable colors (from clear amber to dark reddish brown). The collected exudates were dispersed in water

(20%) at 75 °C under constant stirring for 1 h. The suspension was clarified by filtration (Whatman No. 4, Uppsala Sweden) and the resultant solution was frozen at −40 °C and freeze-dried (Rificor, Model L-I-E300-CRT, Buenos Aires, Argentine). Arabic gum (*Acacia senegal*) was purchased from Sigma—Aldrich Corporation (St Louis, MO, USA) and used as control sample. Refined fish oil used as disperse phase was cordially provided by GHION (Mar del Plata, Argentine) and it was used without further purification. This oil was selected due to its nutritional value (over 35% of ω-3 poly-unsaturated fatty acids) and current importance in the develop-ment of emulsion-based healthy foods. All other chemicals used were of analytical grade and double distilled water was used in all experiments.

2.2. Physicochemical characterization

2.2.1. Chemical analyses

Total nitrogen determination was performed using the Kjeldahl method and the total protein content was calculated using a con-version factor of 6.25 (Alftrén et al., 2012; Anderson & Farquhar, 1982). The inorganic matter, determined by the content of remaining ashes, was obtained according to the AOAC Official Method 923.03 (2000). The samples (1 g of the dehydrated gum) were incinerated in a furnace at 650 °C for 3 h, and the ash content was calculated by the weight difference of the sample before and after incineration. The phenol—sulphuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) was used for the deter-mination of total carbohydrates. Results were expressed as g of glucose/100 g gum in dry basis (d.b.). The total water content of the samples was determined gravimetrically by difference in weight before and after drying 1 g of the purified gum in a vacuum oven at 105 °C until reaching constant weight (4 h) (44—16 of American Association of Cereal Chemists, 2000). These drying conditions were adequate to determine the water content in the studied sys-tems with a confidence interval of 6% for a 95% certainty.

2.2.2. Tannins determination

The tannin content of the gums was evaluated using the color-imetric method described by Anderson and Morrison (1989). Briefly, 50 μl of ferric chloride solution (9%) was added to 5 ml of an aqueous gum solution or to solution of tannic acid, used as refer-ence standard. The absorbance was immediately measured at 430 nm in a spectrophotometer (Evolution 600 UV—Vis, Thermo Fisher Scientific, Waltham, MA, USA). The absorption given by each gum solutions at 430 nm was used as the reference point from which the absorption was measured, after the addition of the ferric chloride solution; the absorption at 430 nm given by the ferric chloride solution was subtracted as a 'blank' value. Tannin content was expressed as percentage of tannic acid equivalent in dry gum.

2.2.3. Specific optical rotation

The specific optical rotation ($[\alpha]^{25}_{589}$) of the aqueous gum dis-persions (2.5% w/v) was determined with a polarimeter (POLAX-D, Atago USA Inc., Bellevue, WA, USA) using a 10 cm length cell and a Na lamp.

2.2.4. Intrinsic viscosity

Gum solutions at different concentrations ($10—40 \text{ gL}^{-1}$) in 0.1 M NaCl were prepared to obtain relative viscosities from about 1.2 to 1.8 in order to assure a good accuracy and the linearity of extrap-olation to zero concentration. Viscosity was measured at 25 °C using a Cannon—-Fenske capillary viscometer Size 50, K = 0.004422 (Cannon Instrument, State College, PA, USA). The intrinsic viscosity was obtained by combined application of Hug-gins and Kraemer equations:

$$\eta_{sp}/c = [\eta] + k1[\eta]^2 c \tag{1}$$

$$\ln(\eta_r) = [\eta] + k2[\eta]^2 c \tag{2}$$

where $\eta_{sp} = [(\eta-\eta_s)/\eta_s]$, η_r is the relative viscosity (η/η_s), and η and η_s are the viscosities of the solution and the solvent, respectively, $[\eta]$ is the intrinsic viscosity of different concentrations of polyelectrolytes at fairly high ionic strength (0.1 M NaCl), over the concentration range of 0.001–0.01 g mL^{-1}; The density of the gum solutions was measured at 25 °C using a digital vibrating tube density meter (DS 7000, Krüss Optronic GmbH, Hamburg, Germany).

2.2.5. Fourier transform infrared analysis (FT-IR)

FT-IR measurements were performed in a FT-IR NICOLET iS5 (Thermo Scientific, Madison, USA). Powdered gum samples were studied by a single-bounce NICOLET iD3 ATR system (Thermo Scientific, Madison, WI, USA) of ZnSe crystal and with an incident angle of 45°. The spectrum of each sample was obtained by taking the average of 16 scans at a resolution of 4 cm^{-1}. The spectra were acquired between 600 and 4000 cm^{-1}. Spectral analysis was performed using the Thermo Scientific OMNIC software (Madison, WI, USA).

2.3. Interfacial properties

The interfacial behavior of the aqueous gum suspensions at 2 and 5% w/v, was characterized by using the pendant drop method in an interfacial tensiometer (PAT-1, SINTERFACE Technologies, Berlin, Germany) as it has already been indicated in the literature (Bellesi, Pizones Ruiz-Henestrosa, & Pilosof, 2014; Pizones Ruiz-Henestrosa, Martinez, Carrera Sánchez, Rodríguez Patino, & Pilosof, 2014; von Staszewski, Pizones Ruiz-Henestrosa, & Pilosof, 2014). The dynamic interfacial tension (σ) at the fish oil-water interface was measured by forming an aqueous drop at a constant volume of 12 μl into a thermostated glass cuvette, containing the oil phase at 20.0 ± 0.1 °C, the silhouette of this drop is cast onto a CCD camera and digitized. The digital images of the drop are recorded over time and fitted to the Young–Laplace equation in order to obtain the interfacial tension with an accuracy of ±0.1 mN/m. Interfacial tension of the fish oil–water system in the absence of the gums was initially measured (24 mN/m) as control. Although a slight decrease of this value was observed (data not shown) probably due to the existence of surface-active molecules in the commercial fish oil, no further purification of the oil was performed in order to evaluate the interfacial behavior of the gums in real o/w interfaces.

Furthermore, the interfacial dilatational rheology of the interfacial films was also analyzed by applying sinusoidal oscillations of the interface. Area deformations and periodic automatically controlled sinusoidal interfacial compression and expansion were performed during the formation of the adsorbed layer by decreasing and increasing the drop volume at the desired amplitude and angular frequency. Oscillations were performed at a frequency of 0.05 Hz and each perturbation consisted of six oscillations cycles followed by 10 min constant interfacial area recording. The amplitude of the oscillation was 3% of the initial drop volume in order to guarantee that the rheological parameters are independent of the amplitude. The surface area perturbations lead to a respective harmonic surface tension response. The data obtained were analyzed by using a Fourier transformation, obtaining the corresponding dilatational parameters of the interfacial layer (namely the interfacial elasticity and viscosity) (Bellesi et al., 2014). Rheological parameters (dilatational modulus, interfacial elasticity and interfacial viscosity) were evaluated. The interfacial dilatational modulus value (E), defined as the change in surface tension induced by a small change in surface area, was used in present work for characterizing the viscoelastic properties of the corresponding G or GA interfacial films. This parameter gives useful information about interactions between the adsorbed molecules at the o/w interface (Dickinson, 2009).

All experiments were conducted at least in duplicate.

2.4. Emulsifying properties

2.4.1. Preparation of gum suspensions and fish oil emulsions

Aqueous suspensions of G and GA were prepared at different concentrations in order to obtain emulsions with 2 and 5% w/v of gum. Gums were suspended in double distilled water and left standing overnight with gentle stirring to complete the biopolymers hydration at room temperature. Commercial fish oil was dispersed in aqueous gum suspensions to obtain emulsions of 10% oil volume fraction. Pre-emulsion was performed for 2 min at medium speed with Ultra-turrax (T18 IKA, Staufen, Germany) and final emulsion was carried out at 20,000 rpm for 3 min.

2.4.2. Emulsion droplet size determination

Droplet size distribution of emulsions was determined by static light scattering (SLS) using a Mastersizer 2000 device equipped with a Hydro 2000MU as dispersion unit (Malvern Instruments, Worcestershire, United Kingdom). The pump speed was settled at 1800 rpm. The refractive index (RI) of the disperse phase (fish oil, RI = 1.479) and its absorption parameter (0.001) were used.

Droplet size was reported as $D_{3,2}$ diameter (volume–surface mean diameter or Sauter diameter, Eq. (3)) and $D_{4,3}$ diameter (equivalent volume-mean diameter or De Broucker diameter, Eq. (4)).

$$D_{3,2} = \sum n_i d_i^3 \bigg/ \sum n_i d_i^2 \tag{3}$$

$$D_{4,3} = \sum n_i d_i^4 \bigg/ \sum n_i d^3 \tag{4}$$

where n_i is the number of particles of diameter d_i (Galazka, Dickinson, & Ledward, 1996; Leroux, Langendorff, Schick, Vaishnav, & Mazoyer, 2003).

$D_{3,2}$ provides a measure of the mean diameter of most of the droplets. $D_{4,3}$ is related to changes in droplet size involving destabilization processes so it is more sensitive to fat droplet aggregation (Galazka et al., 1996; Relkin & Sourdet, 2005).

The polidispersity index was calculated by Eq. (5):

$$\text{Polidispersity index} = (D_{0.9} - D_{0.1})/D_{0.5} \tag{5}$$

where $D_{0.1}$, $D_{0.5}$ and $D_{0.9}$ are the diameters at 10%, 50% and 90% of cumulative volume, respectively.

The droplet size and the polidispersity index were reported as average values and standard deviation of duplicates, with ten readings made per duplicate.

2.4.3. ζ-potential measurements

ζ-potential measurements were performed in a dynamic laser light scattering instrument (Zetasizer Nano–ZS, Malvern Instruments, Worcestershire, United Kingdom). The ζ-potential was evaluated from the electrophoretic mobility of the particles. The conversion of the measured electrophoretic mobility data into ζ-potential was done using Henry's equation Eq. (6) (Hunter, 2001):

$$U_e = 2\varepsilon\zeta f(Ka)/3\eta \qquad (6)$$

where U_e is the electrophoretic mobility, ε the dielectric constant, η the sample viscosity and $f(Ka)$ the Henry's function.

Emulsions were previously diluted 1:100 with water and put into disposable capillary cells (DTS1060, Malvern Instruments, Worcestershire, United Kingdom). The reported values are the average and standard deviation of duplicates, with five readings made per duplicate.

2.4.4. Creaming stability

The emulsion stability against creaming was determined by measuring the extent of gravitational phase separation (Acedo-Carrillo et al., 2006; Khan et al., 2015), transferring 10 mL of each emulsion into a graduated tube immediately after the preparation of the emulsion and it was then stored for 2 days at 25 °C. The creaming index (CI) was registered by measuring the serum layer height in the graduated tube over time and thus relating this value with the total volume of the emulsion using the Eq. (7):

$$CI = V_s/V_e * 100 \qquad (7)$$

where V_s is the volume of the serum layer and V_e is the total volume of the emulsion

2.5. Statistical analysis

At least two replicate determinations were performed for each trial. The statistical analysis of physicochemical properties and of the ζ-potential measured for the studied gums were carried out using T-test and ANOVA, respectively. Differences between samples were considered significant at P < 0.05 (interval of confidence of 95%). The statistical analysis and data fitting were performed through GraphPad Version 4 (GraphPad, Software Inc., San Diego, CA, USA).

3. Results and discussion

3.1. Physicochemical characterization of the gums

The physicochemical analysis of *P. alba* exudate gum (G) and arabic gum (GA) are presented in Table 1. The water content of the freeze-dried G was slightly higher than that of commercial GA but lower than the maximum (15%) accepted for the use of GA for food or pharmaceutical applications (Joint FAO/WHO Expert Committee on Food Additives, 1999). Generally, gums displayed a similar content of carbohydrates, tannins, pH value, inorganic matter and intrinsic viscosity. Carbohydrates represented the higher fraction in

Table 1
Physicochemical properties of *Prosopis alba* (G) and *Acacia senegal* (GA) exudate gums.

	G	GA
Moisture (%)	6.35 ± 0.04^b	5.10 ± 0.01^a
Ashes (% d.b.)	2.78 ± 0.03^a	3.49 ± 0.01^b
Nitrogen (% d.b.)	2.21 ± 0.05^b	0.21 ± 0.01^a
Protein (% d.b.)	13.81 ± 0.33^b	1.37 ± 0.04^a
Carbohydrates (%d.b.)	61.62 ± 0.09^a	65.20 ± 0.58^b
Tannins (% d.b.)	0.20 ± 0.00^a	0.21 ± 0.01^a
pH (sol 5%)	4.81 ± 0.01^a	4.99 ± 0.01^b
Optical rotation, $[\alpha]^{25}_{589.3}$	$+46.46 \pm 1.53^b$	-58.79 ± 0.60^a
Intrinsic viscosity, $[\eta]$ (L g^{-1})	$1.77 \cdot 10^{-2} \pm 0.03 \cdot 10^{-2b}$	$1.59 \cdot 10^{-2} \pm 0.03 \cdot 10^{-2a}$

Mean $\pm$ SD values followed by different letters within the same row are significantly different according to T-test at P $\leq$ 0.05.

both samples (62 and 65% d.b. for G and GA, respectively). The obtained tannin values were lower than values previously reported for mesquite gums (0.49%) (Goycoolea, Calderón de la Barca, Balderrama, & Valenzuela, 1997), and even lower than the tannin content of MG (0.35%), classified as a high quality exudate gum (Goycoolea et al., 1997; López-Franco et al., 2012). This indicates that the purification process was adequate and no further purification steps are required.

Gums solutions (5% w/v) exhibited slightly different pH values, being G more acidic than GA. This could be related to a naturally higher content of uronic acids as determined for other *Prosopis* exudate gums (López-Franco et al., 2012).

The inorganic matter of G and GA measured as ash content was within the legislation limits (up to 4% d.b.) (Joint FAO/WHO Expert Committee on Food Additives, 1999). The inorganic content has been attributed for other *Prosopis* exudates gums to the presence of various types of cationic species associated with high molecular mass fractions of the gums (López-Franco et al., 2012).

The specific optical rotation has been used as a diagnostic parameter related to gums composition. The obtained values (Table 1) showed remarkable distinction between the studied gums. G dispersion showed dextrorotatory activity, while GA exhibited levorotatory activity as expected for gums from *A. Senegal* (López-Franco et al., 2012). G behavior was according to data reported for others *Prosopis* exudate gums (Anderson and Morrison, 1989), being the value obtained for G (+46.5) lower than those reported for MG (+62 to +75) (López-Franco et al., 2012; Orozco-Villafuerte et al., 2003).

The intrinsic viscosity [η] has been widely used to analyze the relationships between structure-property, the effects of the environment and the stability of polymers in solution (Sibaja-Hernández et al., 2015). [η] is defined as the limiting value of the specific viscosity/concentration ratio at zero concentration (Sibaja-Hernández et al., 2015) and it can be recognized as the volume occupied by 1 g of polymer solution at infinite dilution (P.A. Williams, 2008). It was obtained by the combined application of Huggins and Kramer equations for gum suspensions, using an isoionic dilution method with the presence of a low NaCl concentration (Frollini, Reed, Milas, & Rinaudo, 1995), where the polyelectrolyte gums behave like neutral polymers in the absence of intermolecular interactions. The obtained [η] values (Table 1) were similar for G and GA and close to values reported for GA ($1.8 \cdot 10^{-2}$ L g^{-1}) by other authors (Gómez-Díaz, Navaza, & Quintáns-Riveiro, 2008; López-Franco et al., 2012). However, [η] value obtained for G was considerably higher than that of gums from other species of *Prosopis* which were between $0.83 \cdot 10^{-2}$ and $0.99 \cdot 10^{-2}$ L g^{-1}, this indicate the presence or large molecular size polymers in G which make it more similar in terms of macromolecular structure to GA.

The major difference between G and GA gums was observed in the protein content. G presented higher protein content than GA, being the obtained values 13.81 and 1.37%, respectively (Table 1). Protein content determined for GA was in the range reported previously for this gum, around 1.75 and 3.73% (Alftrén et al., 2012; López-Franco et al., 2012; Mhinzi, 2004), and for other gums obtained from different *Prosopis* species (2.1 and 6.8%) (Alftrén et al., 2012; Trejo-Espino, Rodríguez-Monroy, Vernon-Carter, & Cruz-Sosa, 2010). Protein content in exudate gums and its fractions has been related to their emulsification ability and their interfacial properties (Román-Guerrero et al., 2009; Vernon-Carter, Beristain, & Pedroza-Islas, 2000). Román-Guerrero et al. (2009) found that a fraction of MG with a protein content of 16.29% presented the highest interfacial viscosity and instantaneous elastic modulus. The hydrophobic nature of the proteinaceous fractions explains the adsorption capacity to the interface and the viscoelastic film

formation at the drop interface (Dickinson, 2009). Some reports have shown that a relatively high protein/polysaccharide balance is required to provide a better adsorption at the interface, with enhanced viscoelastic properties and good steric and electrostatic repulsion energy that increase the stability of emulsions (Papageorgiou et al., 2010; Sibaja-Hernández et al., 2015).

In order to investigate the similarities and the differences in the chemical structure of G and GA, FT-IR analyses were performed (Fig. 1). The infrared spectrum obtained for both gums showed a wide band centered at 3300 cm^{-1} for −OH stretching, attributable to monosaccharides with free −OH (Quintanilha, Orth, Grein-Iankovski, Riegel-Vidotti, & Vidotti, 2014). Absorption stretch bands for primary (3400–3500 cm^{-1}) and secondary (3310–3350 cm^{-1}) amine groups of protein fraction could not be identified probably due to swamped of these bands by the broad adsorption O−H stretching band (Roque, Bicho, Batalha, Cardoso, & Hussain, 2009). A sharp band attributable to −CH stretching was observed at 2944 cm^{-1} and 2947 cm^{-1} for GA and G, respectively. Salt nature of carboxylic acid groups of GA was reported by the −C=O asymmetrical stretching at max 1616 cm^{-1} with a weaker symmetrical stretching band at 1423 cm^{-1} (Singh & Singh, 2011). The band for −C=O asymmetrical stretching was observed at 1617 cm^{-1} for GA and at 1632 cm^{-1} for G, which are in concordance with the results from Tiwari and Singh (2008). Both samples showed a weaker band at 1415 cm^{-1} which could be attributed to the −C=O symmetrical stretching. In addition to −C=O stretching band, the glucuronic acids have specific vibrations due to −OH bending peaks (Tiwari & Singh, 2008). In GA and G, the −OH stretching band was observed at about 1360 cm^{-1} and it was according to previously values reported for GA (Quintanilha et al., 2014). A band at 1292 cm^{-1} was observed in both samples which could be assigned to C−N stretching (Tiwari & Singh, 2008). The peak observed at 1038 cm^{-1} in GA and 1025 cm^{-1} in G, could be attributed to O−H bending peak according to Singh and Singh (2011). In summary, the spectra for G and GA were comparable. FT-IR analysis further supported that both types of gums share essentially a similar chemical nature although their fine structure and composition, in terms of amount of carbohydrates and proteins, are different (Table 1).

3.2. Interfacial properties
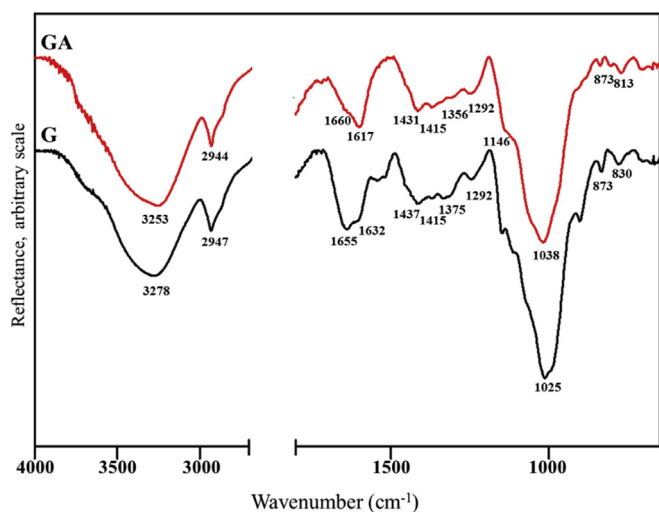
The surface activity of G and GA were characterized by measuring the interfacial tension at O/W emulsion interface. Fig. 2A shows the time evolution of the dynamic interfacial tension (σ) obtained for different bulk aqueous gums concentrations (2 and 5%). As a general trend, it can be observed an initial sharp decrease of σ during the first 600 s which then evolved towards asymptotic values both for G and GA at the studied concentrations. The kinetic of the initial decreased of σ was previously related to the degree of diffusion of the emulsifier to the interface (Pizones Ruiz-Henestrosa, et al., 2014). Then, a steady decrease of σ occurred as the adsorbed molecules tend to reach a quasi-equilibrium state that can be considered when the σ values do not change by more than 0.1 mN/m in 30 min (Seta, Baldino, Gabriele, Lupi, & Cindio, 2014). The decrease of σ with time was also related to the interface molecule concentration and to the conformational changes that it experience (Dickinson, 2011; Graham & Phillips, 1979; MacRitchie, 1989; Pizones Ruiz-Henestrosa, et al., 2014). An effective emulsifier should rapidly adsorb to the surface of the newly formed droplets to reduce the interfacial tension and hence prevent droplets coalescence (Dickinson, 2009). The adsorption of a hydrophilic macromolecular emulsifier at the O/W interface goes through three different stages affecting the measurable interfacial tension: (a) the diffusion from the bulk to the boundary layer at the interface, (b) the molecular adsorption at the interface and the penetration in the oil phase, and (c) the molecular reorganization at the interface (Babak, Desbrières, & Tikhonov, 2005; López-Franco et al., 2012; Martinez, Sánchez, Patino, & Pilosof, 2009). Fig. 2A shows that lower values of σ were reached when using G within all the analyze period which indicates better interfacial activity of P. alba gum at the different stages. The capacity to lower σ values was related to the composition as well as to the molecule concentration and molecular conformation at the o/w interface (Dickinson, 2011;
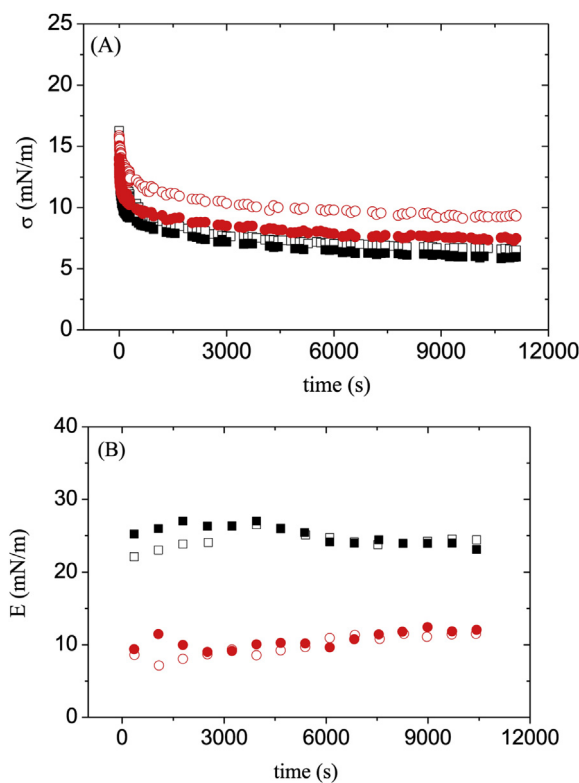

Fig. 1. FTIR spectra of dehydrated *Prosopis alba* exudate gum (G) and arabic gum (GA). Wave number in cm^{-1} is indicated for some bands.

Fig. 2. Effect of gums concentration on the dynamic interfacial tension, σ (A) and on the binterfacial dilatational modulus, E (B) of G and GA interfacial films adsorbed at the o/w interface as a function of time from pendant drop formation. G suspensions: 2% □ and 5% ■; GA suspensions: 2% ○ and 5% ●.

López-Franco et al., 2012; MacRitchie, 1989; Vernon-Carter et al., 2000).

Fig. 2A also shows that G reduced the values of σ faster than GA. This can be explained by considering a combination of higher amount of protein fraction and a more flexible structure of G than GA, that offer a higher interfacial activity to these components (Dickinson, 2003; Román-Guerrero et al., 2009). These better interfacial properties are known to affect the emulsion droplet size, thus the formation of o/w emulsions with smaller droplet size may be achieved (Mahfoudhi et al., 2014).

In general, the interfacial activity of food biopolymers is mainly controlled by the accessibility of the protein moiety to the interface and the net surface charge of adsorbing molecules. The interfacial properties of gum arabic were related to the arabino-galactan protein content and its molecular weight in the gum sample. In addition, a good correlation was reported between the limiting interfacial tension and the initial protein content of gum Arabic (Dickinson, 2003). Similarly, the high protein content determined in G (13.8%) could contribute to its high interfacial activity, which was better to that of gum arabic.

Considering the effect of gums concentration on the interfacial behavior of G and GA, Fig. 2A shows that σ decreased with increasing the concentration of the gums in the aqueous medium. The higher decreased of σ was observed when increasing GA concentration, while a slight decrease was observed for G, which indicates that the G interfacial film may be almost saturated at these concentrations values (Beverung, Radke, & Blanch, 1999; Möbius & Miller, 1998).

The interfacial dilatational properties of the interfacial film have also been determined using the oscillating pendant drop method, as explained previously, in order to better understand the impact of the gums on the adsorbed layers. These properties are very important because they affect the stability of colloidal systems, such as o/w emulsions (Dickinson, 2001, 2009; Wilde, Mackie, Husband, Gunning, & Morris, 2004). Fig. 2B shows the interfacial dilatational modulus (E) of G and GA films adsorbed at the o/w interface as a function of time from pendant drop formation for different gums concentrations. The values of E were very similar to the values corresponding to the dilatational elasticity, and they were both much higher than the values of the dilatational viscosity (data not shown), which is indicative of a viscoelastic behavior of the film (Ruíz-Henestrosa, Sánchez, & Rodríguez Patino, 2008). It is shown in Fig. 2B that E increased gradually with the adsorption time for both of the gums, which is associated with the adsorption of the surface-active molecules at the o/w interface, the interactions that take place between them and the conformational changes that occur at the interface (Bos & van Vliet, 2001; Damodaran & Song, 1988; Graham & Phillips, 1979). When comparing the values of E for G and GA interfacial films, higher values were observed for G, suggesting the formation of stronger intermolecular network at the interface showing a solid character of the film (Pizones Ruiz-Henestrosa, et al., 2014).

Fig. 2B also reflects the influence of the gum concentration on the interfacial elasticity. The values of E were almost similar with increasing concentration of the gums in the bulk phase, which could indicate that the interactions between the adsorbed molecules may be almost similar.

3.3. Emulsifying properties of exudates gums

3.3.1. Droplet size determination

Fig. 3 shows the volume droplet size distribution for emulsions containing 2% of G (Fig. 3A) or GA (Fig. 3B) after preparation and after storage during 48 h at 25 °C. The droplet size distributions of G emulsions after preparation (Fig. 3A), showed monomodal
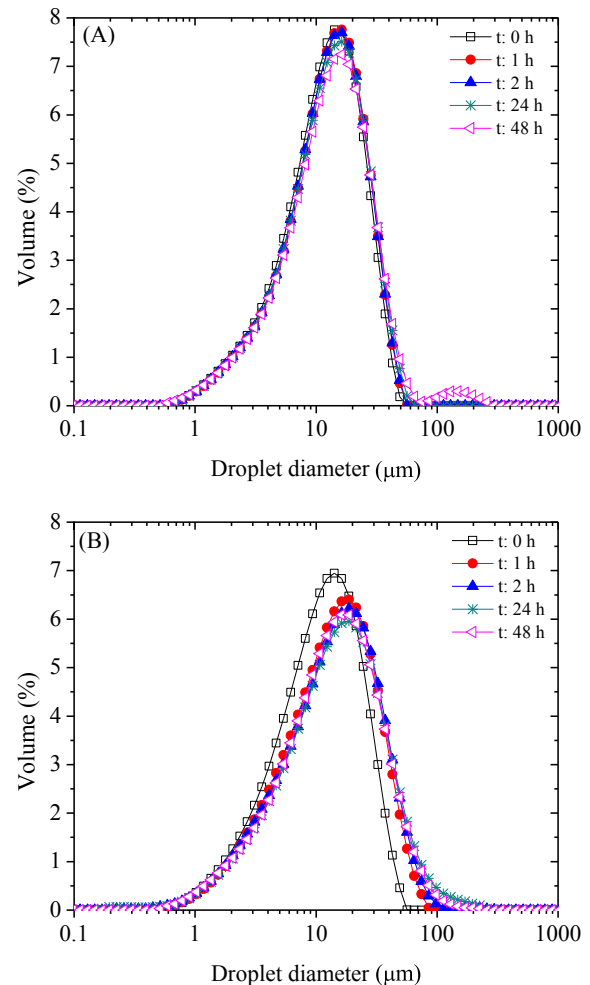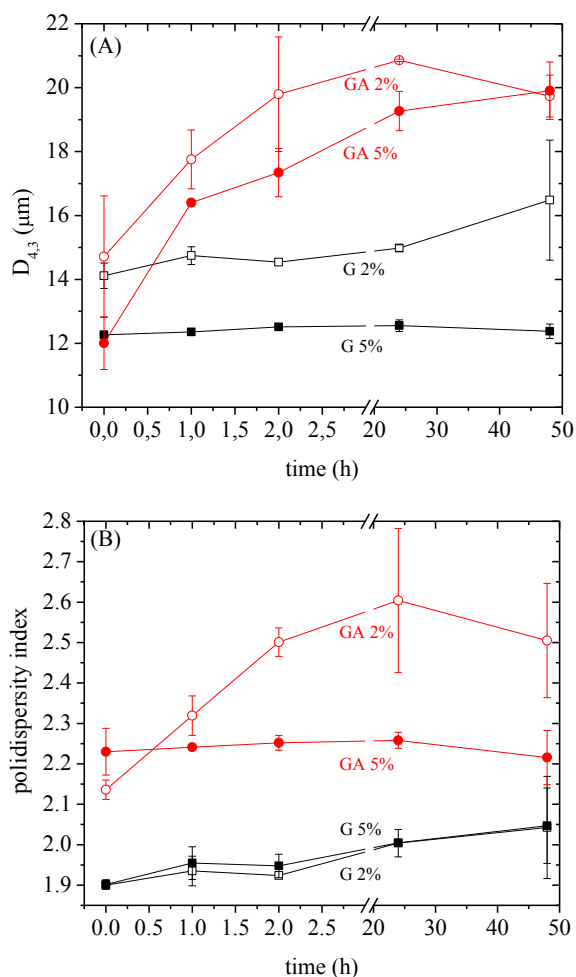

Fig. 3. Volume droplet size distributions curves of fish oil emulsions containing 2% of *Prosopis alba* exudate gum, G (A) or arabic gum, GA (B), obtained after preparation (t₀) and after storage at 25 °C for ● 1 h, ▲ 2 h, ✳ 24 h and ◁ 48 h.

distribution with a wide peak from 0.7 to 60 μm. No changes of the distribution were observed over time, except for the last time evaluated (48 h) in which a second peak with a higher size (100–200 μm) was observed. The droplet size distribution of GA emulsion immediately after preparation (Fig. 3B) showed a distribution similar to the observed for G emulsion (Fig. 3A), with a wide peak in the same range and a mean droplet size at around 13 μm, evidencing a similar emulsifying capacity of both gums. However, it is important to highlight that the time evolution of the droplet size distribution of GA emulsion (Fig. 3B) was different since the peak slightly moved toward higher size values (ranging from 0.7 to 200 μm). Assuming that the increase in droplet size is an indicator of the gum's decreased ability to stabilize a given surface area, then G would seem to have better emulsification power than GA. The emulsifying properties of GA have been related to a small fraction of the gum rich in protein (Randall, Phillips, & Williams, 1988). In a similar way, the emulsifying properties of G can also be attributed, mainly, to its protein content (Table 1), which was significantly higher than that of GA.

The $D_{4,3}$ diameter and the polidispersity index, both related to the destabilization processes, are plotted in Fig. 4 for emulsions with different concentrations of G and GA. Both for G and GA emulsions the $D_{4,3}$ diameter was smaller for the more concentrated system (5%), suggesting that a higher concentration of the gum

Fig. 4. $D_{4,3}$ diameter (A) and polidispersity index (B) calculated from the droplet size distribution of emulsions over time for G and GA emulsions containing 2 or 5% of G or GA.

favors the formation of smaller drops during the emulsification process. This effect could be related with the faster diffusion of the molecules when working with gum solutions at 5% of G or GA in the first step of the adsorption compared with the solutions with 2% of G or GA and the higher decrease of σ for the higher gums concentrations (Fig. 2A).

$D_{4,3}$ diameter for 2 and 5% G emulsions (Fig. 4A) practically remains unchanged after 2 days of storage at 25 °C. In contrast, $D_{4,3}$ values for 2 and 5% GA emulsions were found to increase significantly over time, being always higher than $D_{4,3}$ values obtained for G emulsions after 1 h of their preparation. These results are in agreement with the droplet size distributions observed for 2% GA emulsion (Fig. 3B), where a significant increase in the mean droplet size was observed for the stored (1–48 h) emulsions.

The $D_{3,2}$ diameters of both gums were in the range of 5 and 8 μm, being lower for the higher concentration of the gums. It has been reported that higher differences between $D_{3,2}$ and $D_{4,3}$ are indicative of a higher tendency for destabilization (Gu, Decker, & McClements, 2005) and in fact, it is important to mention that GA emulsions showed higher differences between these diameters values than G (Fig. 4).

The polidispersity index also showed higher values for GA emulsions (Fig. 4B) and hence higher tendency for destabilization, since higher $D_{4,3}$ and polidispersity index values promote the creaming and flocculation of the emulsions (Camino &

Pilosof, 2011).

The smaller droplet volume size distributions (Fig. 3) and also of the $D_{4,3}$ and polidispersity index values (Fig. 4) over time for G emulsions, can be related with the higher values of E observed in the interfacial rheology (Fig. 2B) compared with GA emulsions, since a higher E value suggests the presence of stronger interactions between G molecules in the interfacial film, which could be important for the formation of a stable emulsion (Wilde, 2000).

3.3.2. ζ-potential measurements

ζ-potential values for the emulsions immediately after preparation are reported in Table 2. The ζ-potential value was for both gums and concentrations studied below −30 mV, which is the value usually indicated as the limit for significant droplet stabilization by electrostatic repulsion (Guzey & McClements, 2007). In addition it should be noted that absolute ζ-potential values for G emulsions were significantly higher than the obtained for GA emulsions, which shows that the new gum further reduces the tendency to destabilization. Therefore, the obtained results indicate that GA emulsions may tend to destabilize faster than G emulsions, as a consequence of their higher $D_{4,3}$, higher values of the polidispersity index, the lower initial absolute ζ-potential values, and the lower interfacial film elasticity. The better behavior of G emulsions could be attributed to the negative charges in its molecular structure, mainly due to the presence of a high fraction of hydroxyl and carboxyl radicals. The combination of a high molecular weight, with an appropriate carbohydrate/protein ratio and a predominantly negative charge would be critical for improving the stability of disperse systems like emulsions (Sibaja-Hernández et al., 2015).

3.3.3. Creaming stability of the emulsions

The emulsifying ability of gums was also evaluated in terms of their creaming stability, which reflects the phase separation at normal conditions (not accelerated). The creaming stability of the G and GA emulsions was evaluated at 25 °C through the measurement of the height of the separated serum layer as a function of time and this was expressed as the creaming index (CI). This value is known to be related to the extent of the droplet aggregation in an emulsion (Ye & Singh, 2006). Fig. 5 shows the variation of CI for emulsions containing 2% and 5% of the studied gums during storage at 25 °C. It can be observed that all the emulsions creamed immediately after their preparation. GA emulsions showed higher values of CI than G emulsions up to 4 h of storage at both gums concentrations. After that, a similar behavior was observed for all systems, with creaming index reaching a constant value after 24 h of storage.

As indicated in the literature (Carp, Wagner, Bartholomai, & Pilosof, 1997; Dickinson, 2009; Martinez, Baeza, Millan, & Pilosof, 2005; Pizones Ruiz-Henestrosa, et al., 2014), it is important to consider the effect of the bulk viscosity on the stability of colloidal systems such as foams and emulsions. Thus, the better stability observed for G emulsions could be further related to the effect of the hydrophilic components of G increasing the viscosity, which may reduce the movement of the oil droplets and thus their flocculation/coalescence. This fact could also be reflected in the maintenance of the droplet size distribution during storage (Fig. 3A) as well as the better interfacial properties (Fig. 2B) and elastic characteristics observed for the films generated with G, which could make coalescence more difficult (Seta et al., 2014).

Considering that the droplet size distribution in G samples did not change during the examined time period, the dominant mechanism of instability would be gravitational creaming.

Table 2
ζ-potential of emulsions containing 2 and 5% of *Prosopis alba* (G) and *Acacia senegal* (GA) exudate gums.

	G		GA	
	2%	5%	2%	5%
ζ-potential (mV)	-43.50 ± 0.79^c	-45.85 ± 0.35^d	-41.20 ± 1.13^b	$-36,10 \pm 0.28^a$

Mean $\pm$ SD values followed by different letters within the same row are significantly different according to ANOVA at $P \leq 0.05$.

4. Conclusions

Present work investigated the physicochemical and emulsifying properties of *P. alba* exudate gum in comparison with arabic gum. The studied gums exhibited similar compositional profile, functional groups and intrinsic viscosity. The major difference was found in the protein content, which was higher in the *P. alba* gum and largely explains its better interfacial and emulsifying properties.

The interfacial properties of the two gums revealed a higher ability of G to lower the interfacial tension which was well correlated with its better emulsifying capacity.

P. alba gum was able to stabilize emulsions better than GA based on its lower droplet size distributions, higher ζ-potential, higher interfacial film elasticity and lower values of the polidispersity and creaming indexes during storage. Furthermore, the formation of stronger viscoelastic films at the oil/water interface as well as the charge distribution of the adsorbed G fractions could contribute to understand the higher stability of G emulsions.

Present work provides a first study on the composition and properties of *P. alba* gum. Results are promising and allowed considering G as a novel non-conventional exudate gum with similar or even better emulsifying properties than the well-known GA, with the added benefit of taking advantage of a natural untapped resource that would have a positive impact on the development of regional economies.

Fig. 5. Creaming Index CI (%) of emulsions containing G and GA gums in different concentrations, (A) 2% and (B) 5% during storage at 25 °C.

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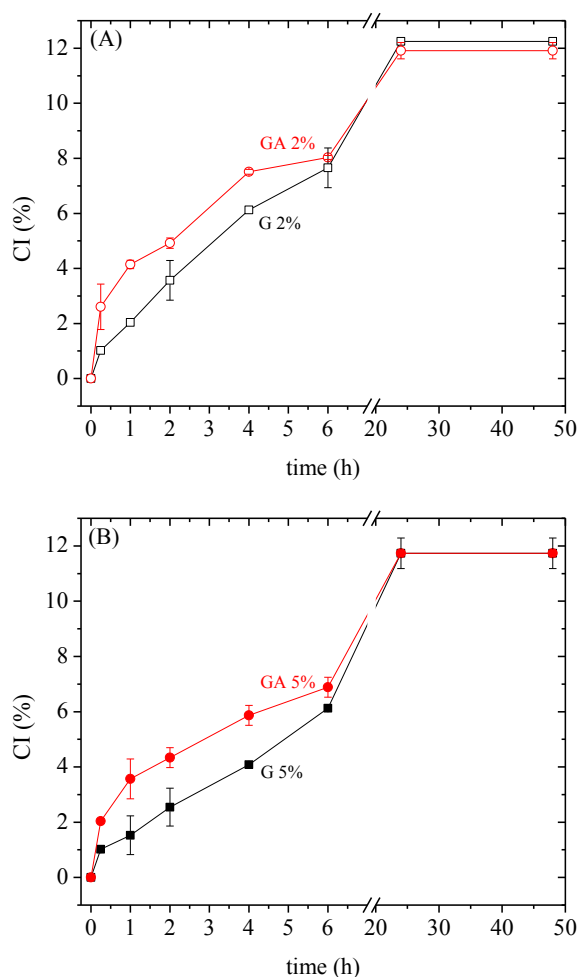
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