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Structural characterization and emulsifying properties of polysaccharides of *Acacia mearnsii* de Wild gum

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ABSTRACT

Polysaccharides (GNF) from *Acacia mearnsii* de Wild gum exudates, collected from trees growing in the south of Brazil, were characterized (^{13}C and HSQC NMR, GC–MS, colorimetric assays). A commercial gum arabic (GAC) was analyzed similarly and compared with GNF. There were differences, consistent with distinct behavior in tensiometry tests and as emulsion stabilizer. GNF had a higher protein content than GAC, with small differences in the monosaccharide composition, the greater one being the lower uronic acid content of GNF (4%), compared with GAC (17%). GNF had a much broader molecular mass distribution, M_w/M_n, and a lower M_w. GNF was more efficient in lowering the surface tension of water and saline solutions and was more efficient in emulsifying castor oil droplets. Results were discussed taking into account structural and molecular differences between the studied gums. It was concluded that polysaccharides from *A. mearnsii* de Wild are candidates as substitutes of currently commercialized arabic gums (*Acacia senegal* and *Acacia seyal*) having, depending on their application, improved properties.

1. Introduction

Acacia gum, also known as gum arabic, occurs as a neutral or slightly acidic salts of complex polysaccharides with some calcium, magnesium and potassium ions (Williams & Phillips, 2000). It is the most industrially used gum as a protective colloid and emulsifier (Fang, Al-Assaf, Phillips, Nishinari, & Williams, 2010). It is obtained from exudates of injured trees, *Acacia senegal* and *Acacia seyal*, the two species of acacia that are commercially exploited, mainly in Africa and Asia. Brazil is among the countries that imports acacia gum for use in various products. In 2011, Brazil has imported over 1300 tons of gum arabic and between January and April 2012, has spent more than 2 million US dollars in imports (Brazilian Ministério do Desenvolvimento, 2012 Indústria e Comércio Exterior" – MDIC – Alice Web).*Acacia mearnsii* de Wild (black wattle) is a species native to Australia, which was introduced to Brazil with seeds coming from South Africa (Stein & Tonietto, 1997). In the south of Brazil, its cultivation has spread throughout the State of Rio Grande do Sul, so that the acacia production chain has become an important economic activity with considerable social and environmental impacts in this region. The main economic interest in black wattle plantations is related to the extraction of tannins from barks and trunks. As a consequence, there is large availability of wood and gum exudates as side products. Acacia wood has been used for energy production directly, or as derived charcoal. As a family based activity, thousands of charcoal productions furnaces are distributed throughout more than 10,000 small properties in Rio Grande do Sul. The wood is also useful for other industries such as pulp and paper, rayon, shavings, plates, fibers, parquet and wood chips (Beck-Pay, 2012). The gum exudates are not commercially exploited in Brazil, despite the extensive planted areas. In order to achieve the required quality for the tannin industry, acacia trees mature for harvest at five to seven years (Stein & Tonietto, 1997). However, the gum exudates are not collected and left to degrade in the environment.

The use of arabic gums dates back to the second millennium BC by Egyptians who used them as adhesives and ink stabilizers. Nowadays, its use is extended to cosmetics, pharmaceutics, lithography and foods. The properties of gum exudates are affected by the age of the tree, amount of rainfall, season of exudation and type of storage (Aspinall, Carlyle & Young, 1968). The structural characterization of many arabic gums has been extensively described,

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mainly for the species *A. seyal* and *A. senegal* (Al-Assaf, Phillips & William, 2005; Aspinall & Young, 1965; Aspinall, Young, Charlson, & Hirst, 1963; Renard, Lavenant-Gourgeon, Ralet, & Sanchez, 2006; Tischer, Gorin, & Iacomini, 2002; Williams & Phillips, 2000). To the best of our knowledge, there are three investigations dealing with the structural characterization of *A. mearnsii* polysaccharides from exudates, all of them employing chromatographic methods (Aspinall et al., 1968; Kaplan & Stephen, 1967; Stephen, 1951). Since none of these dealt with *A. mearnsii* gum carbohydrates collected in Brazil, this subject is now investigated. We believe this is relevant, given the great potential of Brazil to commercially explore local *A. mearnsii* polysaccharide from the gum exudate.

Many food products contain both polysaccharides and proteins. Proteins have an essential role as emulsifying and stabilizing agents, whereas polysaccharides are mainly used for thickening and emulsifying. The overall stability and texture of food colloids depends on the functional properties of their ingredients, with the nature and strength of the protein–polysaccharide interactions (Corsi, Milchev, Rostiashvilli, & Vilgis, 2007).

The main objectives of our investigation are: (i) to isolate and characterize the polysaccharides from crude acacia gums from *A mearnsii* de Wild, grown in Brazil, RS; (ii) to determine their solution behavior in different environments directed to their application as an emulsion stabilizer and (iii) to compare the results with those from a commercial spray-dried gum (mixture of *A. seyal* and *A. senegal*).

Our results may contribute to add value to the Brazilian acacia tree production chain, in view of any future commercial exploitation of *A. mearnsii* gums, since understanding the solution properties of hydrocolloids is essential for their application in foods and other products. No previous report was found in the literature dealing with emulsifying capacity of *A. mearnsii* gum polysaccharides collected in Brazil.

2. Materials and methods

2.1. Collection of gum exudates and isolation of their polysaccharides

The gum exudates of *A. mearnsii* de Wild were harvested from a private property in São Leopoldo, Vale do Rio dos Sinos (State of Rio Grande do Sul, Brazil). The botanical herborized *A. mearnsii* de Wild material is deposited in the *Herbarium Anchieta* (PACA), in São Leopoldo, under tipping number PACA 107,063. The Vale dos Sinos is located between 29° and 30° south parallel, with altitudes ranging from 60 to 600 m. The soils, according to Streck et al. (2002), were classified as planosoil. The regional climate is Cfa, according to the climatic classification of Koeppen (Moreno, 1961). The temperature of the warmest month is above 22 °C, the average annual rainfall 1649 mm and average annual temperature 19.5 °C, according to the weather station of Campo Bom (29° 41′S and 51° 03′W, 25.8 m altitude). The relative humidity varies little over the year, with an average ranging from 72% to 86%.

Immediately after collection, the crude gum was stored at −4 °C and further submitted to a procedure adapted from that described by Simas et al. (2008). A trunk gum sample (40 g) was stirred overnight in water (2 L) at 25 °C to give a dispersion with insoluble fragments. After sedimentation, the supernatant was removed and, to the remaining material, water (1 L) was added and left stirring overnight at 25 °C. The resulting supernatants were concentrated under reduced pressure using a rotary evaporator, dialyzed against Milli-Q water (48 h) through a membrane with a 12–14 kDa M_r cut-off (Spectra/Por® Cellulose Ester) and freeze-dried. The resulting powder was named GNF and the total yield relative to the crude gum was 80%.

For comparison studies, a commercial acacia gum (GAC) obtained from Sigma–Aldrich (G9752), which is generally referred in the label as "gum arabic from acacia tree" was used in the present study. Prior to use, the powder was solubilized in Milli-Q water, dialyzed as described above and freeze-dried.

2.2. Polysaccharide molecular weight and homogeneity determination

Molecular parameters, M_w and polydispersity ($PD = M_w/M_n$), were determined using a Viscotek GPC/SEC equipament, Model 270 Triple Detector (refractive index, viscosity concentration and light scattering detectors) equipped with a Shodex OHPak SB-806 HQ GPC aqueous column – plate number ≥ 12,000, exclusion limit (Pullulan) 20,000 g mol^{-1} and attached to a UV detector. Injections were run at 30 °C and at a 0.6 mL min^{-1} flow rate.

GAC or GNF (1 g L^{-1}) was solubilized in 0.1 mol L^{-1} $NaNO_3$ (also used as eluent), followed by filtration through cellulose acetate Millipore membranes with nominal pore sizes of 0.45 μm. The *dn*/*dc* value was obtained under the same experimental conditions and resulted in 0.1360 cm^3 g^{-1}. Data were analyzed with the help of OmniSEC software (Viscotek).

2.3. Chemical characterization

Polysaccharide samples (2 mg) were hydrolyzed with 1 mol L^{-1} TFA (trifluoroacetic acid) (1 mL) for 8 h at 100 °C. This procedure was carried out in hermetically sealed vials in an oven (Simas et al., 2004; Simas-Tosin et al., 2009). The product was successively reduced with $NaBH_4$ (Wolfrom & Thompson, 1963a), acetylated with Ac_2O – pyridine (1:1, v/v) (Wolfrom & Thompson, 1963b), and the resulting alditol acetates were examined by GC–MS. This was performed with a Varian model 3800 gas chromatograph coupled to a Saturn 2000R mass spectrometer using a DB-225 capillary column (25 m × 0.25 mm i.d.). Temperature used was 50 °C during injection, then programmed at 40 °C min^{-1} to 220 °C (constant), with He as carrier gas. Protein and uronic acid contents of the polysaccharide were determined by colorimetric methods described by Hartree (1972) and Filisetti-Cozzi and Carpita (1991) respectively. ^{13}C NMR and HSQC spectra were obtained from solutions in D_2O at 50 °C, using a 400 MHz Bruker DRX Avance spectrometer equipped with a 5 mm inverse probe. Chemical shifts were expressed in δ ppm relative to an internal standard of acetone (δ 30.2 for ^{13}C and δ 2.224 for ^{1}H). Carboxy-reduction of fraction GNF was carried out by the 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide method (Taylor & Conrad, 1972), $NaBH_4$ being used as the reducing agent. This procedure was carried out twice and after that uronic acid amount was lower than 1%, according to colorimetric assay.

Methylation of fractions GAC, GNF and carboxy-reduced GNF (GNF-CR) was carried out according to Ciucanu and Kerek (1984). 5 mg of each fraction were solubilized in Me_2SO followed by addition of powdered NaOH and CH_3I. Each mixture was agitated strongly for 30 min and then left for 18 h. The per-*O*-methylated products were extracted with $CHCl_3$ from aqueous solutions and were hydrolyzed with 50% (v/v) H_2SO_4 (0.5 mL) at 0 °C for 1 h, followed by dilution to 5.5% (v/v). The solution was maintained at 100 °C for 8 h (sealed vials, oven heated) (Saeman, Moore, Mitchell, & Millet, 1954). The methylated fraction GNF-CR was hydrolyzed with 45% formic acid (v/v) at 100 °C for 16 h. After hydrolysis, solutions were successively neutralized ($BaCO_3$), reduced with $NaBH_4$ and acetylated, as described above, to give a mixture of partially *O*-methylated alditol acetates, which were analyzed by GC–MS (DB-225 capillary column with 25 m × 0.25 mm i.d.). Temperatures employed were 50 °C during injection then programmed at 40 °C min^{-1} to 215 °C (constant), this temperature being maintained at 215 °C for 40 min. The partially-*O*-methylated alditol

acetates were identified by their typical retention times and electron impact spectra (Sassaki, Gorin, Souza, Czelusniak, & Iacomini, 2005).

2.4. Surface tension measurement of polysaccharide solutions under distinct environments

Critical adsorption concentration (cac) and critical micelle concentration (cmc) were determined *via* tensiometry analysis. For tensiometry measurements, amounts of dry GAC or GNF were dissolved in an appropriate volume of Milli-Q water to give final concentrations from 0.05 to 25% (w/v). The prepared solutions were gently agitated for 2–3 h and left at 4 °C overnight to allow complete hydration of polysaccharides (Renard et al., 2006). The solutions were analyzed within 4 h. The same procedure for the preparation of the solutions was carried out using 0.1 mol L^{-1} NaCl, 0.2 mol L^{-1} NaCl, or 0.1 mol L^{-1} $CaCl_2$ as solvents.

The surface-tension measurements and data analysis were performed at 24 °C using Data Physics OCA15 plus tensiometer and SCA20 software. A 500 μL Hamilton syringe was used with needles with outer and inner diameters of 1.65 mm and 0.91 mm, respectively. The total needle length was 38.1 mm. Employed was the pendant drop method, which consists of observation of the profile of a drop of one fluid that falls into another through the edge of a needle. The profile is taken as under the condition of mechanical equilibrium between the gravity and surface tension. A video camera captured the image and software was used to analyze the drop profile according to mathematical models. The Laplace–Young model evaluates the surface tension value according to the equation below:

$$\gamma = \left(\frac{1}{R_1} + \frac{1}{R_2}\right) \cdot \Delta P, \quad (1)$$

where γ corresponds to the surface tension, given in mN m^{-1}, R_1 and R_2 are the curvature radii and ΔP is the pressure difference at the interface. At the time of measurement, both curvature radii had reached equilibrium values. The results were the average of ten measurements.

2.5. Emulsifying capacity of the polysaccharides under distinct environments

Evaluated was the emulsifying capacity of the polysaccharides from *A. mearnsii* fraction (GNF) in comparison with a commercial acacia gum (GAC). Also, the influence of the environmental conditions (water *vs.* saline solution) was studied. Firstly, solutions of GNF or GAC were prepared in Milli-Q water and 0.1 mol L^{-1} NaCl, at 1 wt%. At this concentration, both solutions have virtually the same viscosity (data not shown). At 2 mL of each solution, 0.2 mL (10%, v/v) of castor oil was then added to under gentle stirring at a rate of 0.033 mL at each 5 s. After that, the systems were left stirring for an additional 10 min. In this way, oil-in-water emulsions were formed, with an estimated oil droplet sizes of the order of micrometers. The emulsions were followed from minutes to 24 h after cessation of the agitation in terms of their turbidity (measured as a decrease of the transmittance, %T). Turbidity measurements were performed with a double beam Shimadzu model UV-240-1PC spectrophotometer at a wavelength of 600 nm (Lee & McClements, 2010) using 1 cm path length quartz cuvettes at 20 °C. Pictures were taken with a digital camera (Sony DSC-W120, 7.2 megapixels).

Dynamic light scattering experiments were also performed, in order to follow the emulsified oil droplet sizes and their distribution. Measurements were carried out for the first 10 min after cessation of the agitation. A Microtrac Flex–Nanotrac 150 analyzer was employed, equipped with a 0.1–2 mL volume sample cell and a single diode laser (Class IIIb) of 780 nm wavelength with a nominal power level of three milliwatts. Run time was 30 s. The refractive index of castor oil was taken as 1.47.

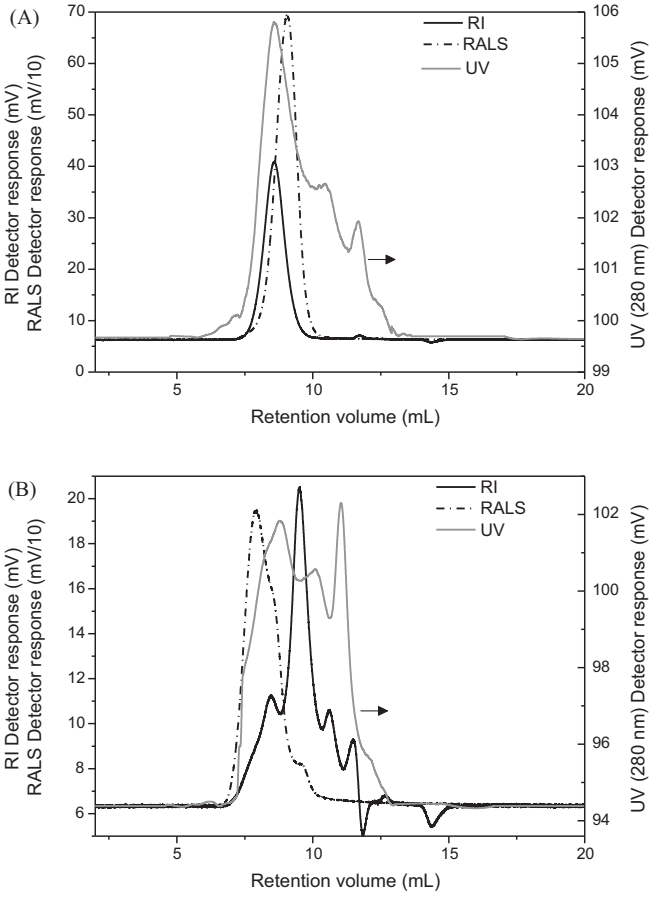

Fig. 1. GPC/SEC elution profiles of samples GAC (A) and GNF (B) using refractive index (RI), light scattering (RALS) and UV (280 nm) detectors.

3. Results

3.1. Characterization of polysaccharides of A. mearnsii de Wild gum

The GPC/SEC elution profiles of the gum arabic samples GAC and GNF, using light scattering (LS), refractive index (RI) and UV (280 nm) detectors, are shown in Fig. 1. Light scattering is sensitive to the concentration and molecular mass while the refractive index depends on concentration only. The UV detector is currently employed to determine the protein content of each eluted fraction (Al-Assaf et al., 2005; Padala, Williams, & Phillips, 2009; Renard et al., 2006; Williams & Phillips, 2000, chap. 9). The GAC profile displays a homogeneous distribution (RI and RALS) with a polydispersity $M_w/M_n = 1.7$ while that of GNF has a very broad distribution with polidispersity of 8.4. Although not satisfactorily resolved, GNF seems to contain three (by LS) or even four (by RI) distinct populations. The UV absorbance profiles contain three distinct peaks for GAC and GNF that can arise from arabinogalactan-protein complexes (first peak) and glycoproteins (second and third peaks) (Renard et al., 2006). UV absorbance peaks differ when GAC is compared with GNF, evidencing differences in protein composition. The first peak from GAC was the most in contrast with GNF. Additionally, GAC was found to interact well with Yariv's reagent, whereas GNF did not (Supplementary material/Fig. S1), confirming the GPC/SEC results. According to the RI response, the second peak in the GNF profile, which eluted at approximately 8.3 mL, did not

Table 1
Monosaccharide composition, total sugar and protein content of polysaccharides from acacia tree gum exudates.

Polysaccharide fraction	Monosaccharide composition (%)[a]						Total sugar (%)	Protein (%)[c]
	Rha	Ara	4-Me-Glc	Gal	Glc	Uronic acid[b]		
GAC	13	31	–	39	tr.	17	95	4
GNF	7	43	–	46	tr.	4	95	7
GNF-CR	7	40	4[d]	42	7[d]	tr[e]	–	nd[f]

[a] Percentages of monosaccharides. Analyzed with a DB-225 column by GC–MS after acid hydrolysis, reduction and acetylation.
[b] Determined by the colorimetric methods of Filisetti-Cozzi and Carpita (1991).
[c] Determined by the colorimetric methods of Hartree (1972).
[d] Mass spectra of 4-Me-glucitol acetate and glucitol acetate from fraction GNA-CR were added of two mass units, indicating that these derivatives were from 4-Me-GlcA and GlcA respectively, which were present in the original fraction (GNA).
[e] Traces (≤1%).
[f] Not determined.

give rise to a signal on UV detection and represents the main contribution to the sample concentration, corresponding to the arabinogalactan fraction. Weight average molecular weights, M_w were 93.2×10^4 g mol^{-1} for GAC and 31.8×10^4 g mol^{-1} for GNF.

The GPC/SEC results point out important differences regarding the homogeneity and protein distribution among fractions of GNF when compared to GAC.

The polysaccharide fractions GAC and GNF both contained 95% of total carbohydrate (Table 1) and were composed of Rha, Ara, Gal and uronic acids in 13:31:39:17 and 7:43:46:4 molar ratios, respectively (Table 1), consistent with arabinogalactan-like structures. The protein content of *A. mearnsii* gum fractions and GAC fraction is in the range of 7% and 4%, respectively, suggesting the presence of arabinogalactan-proteins (AGPs) in all samples. Carboxy-reduction of GNF provided material (GNF-CR) with glucose and its 4-*O*-methyl derivative in a molar ratio of 1.8:1, indicating the presence of glucuronic acid and 4-*O*-methylglucuronic acids as acid components in GNF. This is in agreement with Aspinall et al. (1968), who also found GlcA and 4-Me-GlcA in *A. mearnsii* gum exudates collected in Jamaica. According to Tischer et al. (2002), who studied the same commercial gum arabic examined herein, GAC has glucuronic acid, but not its 4-Me-derivative, as acid monosaccharide component.

According to ^{13}C NMR and HSQC spectra, those of GAC and GNF (Fig. 2) had noteworthy similarities. Both anomeric regions (δ 110.0–98.0 for ^{13}C/δ 4.400–5.400 for ^{1}H) revealed at least 8 signals, indicating the high structural complexity of the samples. ^{13}C signals between δ 109.5 and 106.4 can be attributed to α-L-Ara*f* units. In GAC spectra (Fig. 2A) signals at δ 109.5/5.191 and δ 108.1/5.029 were from 3-*O*-substituted and non-reducing end of α-L-Ara*f* units (Tischer et al., 2002). The presence of these units was confirmed by methylation data (Table 2). The additional α-L-Ara*f* signals in GNF spectrum (Fig. 1B) indicated that α-L-Ara*f* units are present in another chemical environmental. Signal at δ 107.4/5.012 and δ 66.8/3.803 can be assigned respectively to C-1/H-1 and C-5/H-5 of 5-*O*-substituted Ara*f* units (Petkowicz, Sierakowski, Ganter, & Reicher, 1998). It is in agreement with methylation data of GNF, which show 14% of 2,3-Me$_2$-Ara derivative from 5-*O*-substituted units (Table 2). Signals at δ 103.6/4.620 (for GAC) and δ 103.6/4.638 (for GNF) can be assigned to C-1 of β-Gal*p* main-chain units. Those at δ 102.9/4.419 (for GAC) and δ 102.9/4.412 (for GNF) were from β-Glc*p*A units, according to Delgobo, Gorin, Tischer, and Iacomini (1999). Comparing our results with the results obtained from the same commercial arabic gum (Tischer et al., 2002), other GAC C-1/H-1 signals (Fig. 2A) at δ 100.6/4.737, δ 99.9/5.085 and δ 99.1/4 could be assigned to α-Rha*p*, α-Gal*p* (and/or β-Ara*p*), and β-Ara*f*, respectively. The low frequency signal C-1 at δ 16.5, in both GAC and GNF spectra, characterizes -CH$_3$ (C-6) groups of Rha units (Gorin & Mazurek, 1975). The signal at δ 174.9, which was evident only at GAC spectrum (Fig. 2A) was attributed to —*C*O$_2$H of uronic acids units (Gorin & Mazurek, 1975). A signal at δ 61.2 was attributed to non-substituted C-6 of Gal*p* units and, since the signal is slightly broadened, it may be superimposed on that of non-substituted C-5 from α-L-Ara*f* units (Delgobo et al., 1999). The signal at δ 59.8, evident in GNF spectrum, is characteristic of —OCH$_3$ groups of 4-*O*Me-Glc*p*A units.

Methylation analysis of carboxy-reduced GNF (GNF-CR) (Table 2) revealed the presence of 2,3,4,6-Me$_4$-Glc (7%) and 2,3,6-Me$_3$-Glc (6%) alditol acetates, which were not formed from GNF, indicating that Glc*p*A (and 4-Me-Glc*p*A) units were non-reducing end and 4-*O*-substituted in GNF. Typical ions with *m*/z plus two units (*m*/*z* 207, 163, 147 and 131) (Supplementary material/Fig. S2) in the spectrum of 2,3,4,6-Me$_4$-Glc confirmed that this derivative arising from Glc*p*A (and 4-Me-Glc*p*A) units, since it were carboxy-reduced with NaBH$_4$. The same was observed in mass spectrum of 2,3,6-Me$_3$-Glc, which contained ions with *m*/*z* 235, 175, 115 and 101 (Fig. S2), indicating the presence of 4-*O*-substituted Glc*p*A units. Many studies on the structure of gum arabic showed 4-*O*-substituted Glc*p*A units as well as non-reducing end units (Aspinall & Young, 1965; Aspinall et al., 1963; Smith, 1940). This was also observed by Tischer et al. (2002) studying free, reducing oligosaccharides from commercial gum arabic. On the other hand, Aspinall et al. (1968), found GlcA and 4-Me-GlcA only in non-reducing end units in polysaccharide from *A. mearnsii* gum exudate collected in Jamaica.

3.2. Solution behavior of *A. mearnsii* polysaccharides: Surface tension properties

The solution surface properties of *A. mearnsii* de Wild polysaccharides and GNF fraction, were studied in comparison to those of commercial gum arabic polysaccharides (GAC). As the concentration of a surface active molecule increases in an aqueous solution, the physical properties of water are modified and the surface tension (γ) decreased (Myers, 1999). This phenomenon is generally related to the amphiphilic character of the molecule. The opposite is true for adding highly water soluble substances, for example strong electrolytes.

As the concentration of GNF in pure water increases, the surface tension of the solution decreases. At the concentration range where a sharp decrease of γ is observed, it is possible to assign, taking the first and second inflexion points, the critical adsorption concentration (cac), which corresponds to the beginning of the adsorption phenomena, and the critical micelle concentration (cmc). At concentrations higher than cmc, micelles exist in solution.

Knowing the slope of the curve γ *vs.* ln c, and through the Gibbs adsorption isotherm (Hunter, 1993; Myers, 1999), the surface excess concentration of the adsorbed species (Γ) at the interface can be determined using the relation:

$$\Gamma = -\frac{1}{RT}\frac{d\gamma}{d\ln c} \quad (2)$$

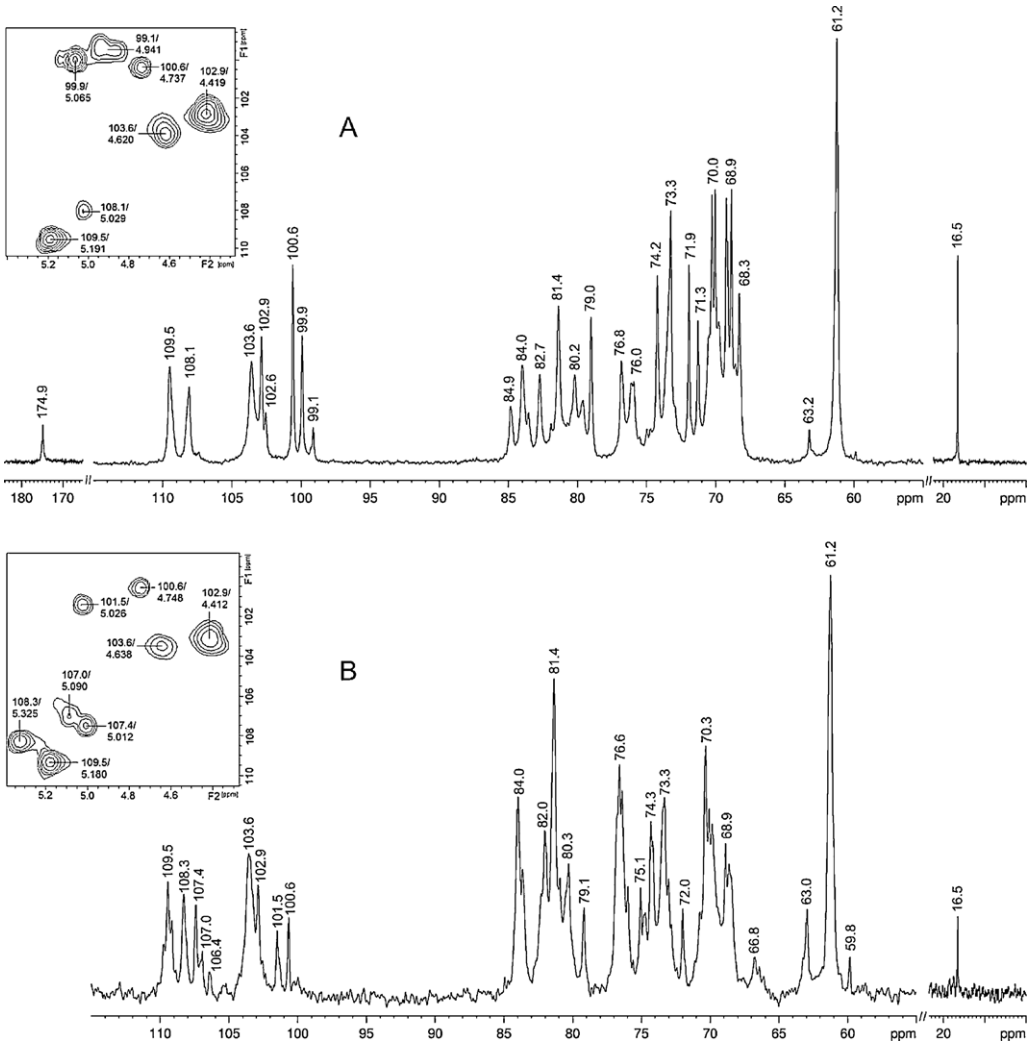

Fig. 2. ^{13}C NMR and HSQC (anomeric region) spectra of samples: (A) GAC and (B) GNF (D_2O, at 50 °C). Chemical shifts are expressed as δ ppm.

The molecular area corresponding to the absorbed substance at the interface can therefore be obtained:

$$A = \frac{1}{N_A \Gamma} \quad (3)$$

where N_A is Avogadro's number.

The adsorption behaviour of GNF and GAC was studied in pure water and in saline solutions. The shapes of the curves are displayed in Fig. 3. Calculated cac and cmc values, as well as the corresponding

Table 2
Partially *O*-methylalditol acetates formed on methylation analysis of fractions GAC, GNF and GNF-CR.

Partially *O*-methylated alditol acetates[a]	Linkage types	(mol%)		
		GAC	GNF	GNF-CR
2,3,5-Me_3-Ara	Ara*f*-(1→	24[b]	20	17[b]
2,3,4-Me_3-Ara	Ara*p*-(1→	3	tr.	tr.
3,5-Me_2-Ara	→2)-Ara*f*-(1→	3	5	3
2,5-Me_2-Ara	→3)-Ara*f*-(1→	18	13	7
2,3,4,6-Me_4-Glc [b]	Glc*p*-(1→	–	–	8
2,3-Me_2-Ara	→4)-Ara*p*-(1→→5)-Ara*f*-(1→	tr.	14	10
2,3,4,6-Me_4-Gal	Gal*p*-(1→	18	7	6
2-Me-Ara	→3,4)-Ara*p*-(1→→3,5)-Ara*f*-(1→	–	tr.[c]	tr.[c]
2,4,6-Me_3-Gal	→3)-Gal*p*-(1→	4	6	5
2,3,6-Me_3-Glc [d]	→4)-Glc*p*-(1→	–	–	6
2,3,4-Me_3-Gal	→6)-Gal*p*-(1→	3	–	4
2,6-Me_2- Gal	→3,4)-Gal*p*-(1→	3	6	3
2,4-Me_2-Gal	→3,6)-Gal*p*-(1→	15	20	20
2-Me-Gal	→3,4,6)-Gal*p*-(1→	9	9	11

[a] Eluted successively from a DB-225 capillary column at 215 °C.
[b] The 2,3,5-Me_3-Ara derivative overlapped with 2,3,4-Me_3-Rha, arising from fractions GAC and GNA-CR.
[c] Traces (<1%).
[d] Mass spectra corresponding to these derivatives contained typical ions with *m*/*z* of plus two units, indicating that they are from Glc*p*A (and 4-Me-Glc*p*A) units.

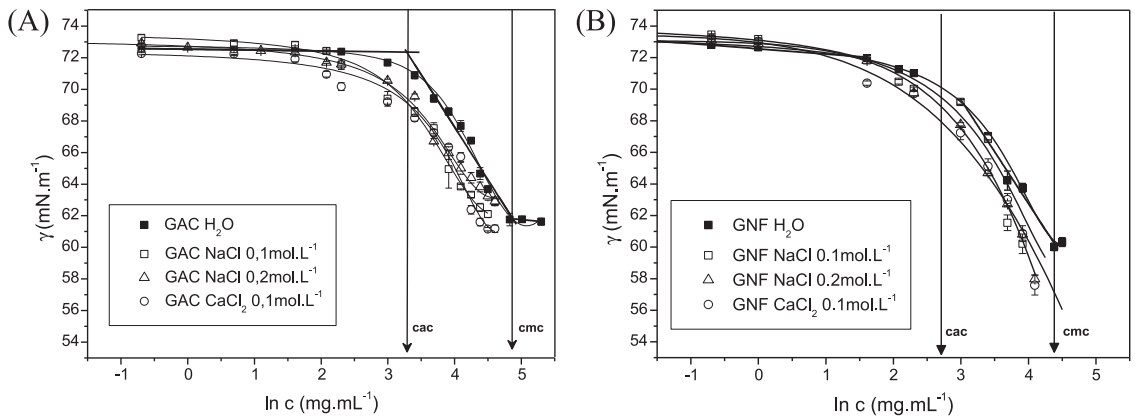

Fig. 3. Surface tension (γ) as a function of concentration, determined for (A) GAC and (B) GNF in Milli-Q water, at 24 °C.

surface tension at cac and cmc were evaluated according to the data depicted in Figure 3 and summarized in Table 3. The molecular area of GAC and GNF molecules at the interface is also presented.

It is known that gum arabic has proabsortive effect on water and on electrolyte solutions and for this reason there are many studies on health benefits of gum arabic on treatment of diarrhea (Ali, Ziada & Blunden, 2009). GAC tends to adsorb in the surface solution, forming a monolayer and later, at higher concentrations, form aggregates within the solution (Myers, 1999; Hunter, 1993). The interfacial activity of gum arabic is attributed to their amphiphilic nature since the polysaccharide fractions are highly water soluble, containing hydroxyl and acid moieties whereas the protein rich fraction is responsible for their hydrophobic behavior (Al-Assaf et al., 2009; Al-Assaf, Sakata, McKenna, Aoki, & Phillips, 2009).

As depicted in Fig. 3, both samples, GNF and GAC, show surface active behavior, that is, they act as surfactants in water as well as in saline solutions. However, some differences arise. Regarding the shape of the curves, it is possible to accurately determine the cmc for GAC under all experimental conditions (aqueous and saline solutions). This is not the case for GNF, whose cmc values could only be estimated since the surface tension continues to decrease, even at high concentrations.

Analyzing the data from Table 3, the polysaccharides in water adsorb at the interface and aggregate in solution at higher concentrations than in saline solutions. Salts are typical tensoionic substances, that is, they increase the surface tension of water when in solution. Even under these conditions, GAC and GNF decreased the surface tension. However, the assigned values of γ_{cac} and γ_{cmc}, namely the surface tension at cac and cmc, respectively, are nearly the same regardless of the sample (GAC or GNF) and the ionic strength of the solution (pure aqueous or saline solution). In the case of saline solutions, the radius of the cation and its concentration do not strongly influence the critical concentrations (cac or cmc) or the surface tension values at those concentrations. Since both samples have a certain amount of uronic acids, screening of the gum charges by the electrolytes promotes early adsorption of the gum at the air/solution interface when compared to pure water. The screening of the charges by electrolytes in solution is a well known phenomenon for low molecular weight surfactants, resulting in decrease of the electrostatic repulsion between dispersed molecules. In addition, in the case of strong electrolytes, their ions are fully solvated in water. Under these conditions, the interaction electrolyte–water is favored towards that of water-gum. At a given gum concentration, in this high ionic strength environment, the polysaccharides molecules start to adsorb at the interface and continue doing so until the available surface area is completely occupied. From this point on, there is balance of two acting forces, the strong steric repulsion due to the overlap of two or more neighboring molecules and the osmotic tension of the solution that tends to expel the molecules from solution. On increasing the gum concentration, any migration of more polysaccharide molecules to the surface becomes very unfavorable and in this situation the additional polysaccharide molecules aggregate in the bulk of the solution as micelles.

In all cases, GNF starts to adsorb at the interface at lower concentrations than GAC (half the concentration), which clearly demonstrates that GNF is a more powerful surface active agent than GAC. It is necessary, to attain the same surface tension value (see cac) one half of the concentration. This behavior can be attributed to the higher protein content of GNF (~7%) when compared to GAC (~4%) (Table 1) and their structural differences (Fig. 2).

The area of GAC and GNF at the fluid interface is around 60–80 Å^2 and was not found to vary systematically under the experimental conditions. It is only possible to conclude that adsorbed GAC and GNF at the interface occupy greater areas when in saline solution when compared to pure water (Table 3). This could be due to lowering of the electrostatic repulsion between molecules, caused by the neutralization of the acid groups, as mentioned above.

Table 3
Physico-chemical parameters regarding the adsorption of GAC or GNF molecules at the interface of aqueous or saline solutions.

Solution	cac (mg mL^{-1})	γ_{cac} (mN m^{-1})	cmc (mg mL^{-1})	γ_{cmc} (mN m^{-1})	Area (Å^2)
GAC H_2O	27.0	71.3	126.5	61.8	59.7
GAC 0.1 M NaCl	14.0	71.2	95.7	62.3	69.2
GAC 0.2 M NaCl	17,1	70.8	103.8	62.0	74.4
GAC 0.1 M $CaCl_2$	18,8	70.2	90.5	61.1	81.0
GNF H_2O	14.2	70.2	81.4	56.7	61.0
GNF 0.1 M NaCl	84	70.4	57.2	59.8	78.2
GNF 0.2 M NaCl	9.5	69.1	61.1[a]	54.1[a]	61.6
GNF 0.1 M $CaCl_2$	7.2	69.2	62.0[a]	56.3[a]	76.0

[a] Estimated values since their direct determination was not possible.

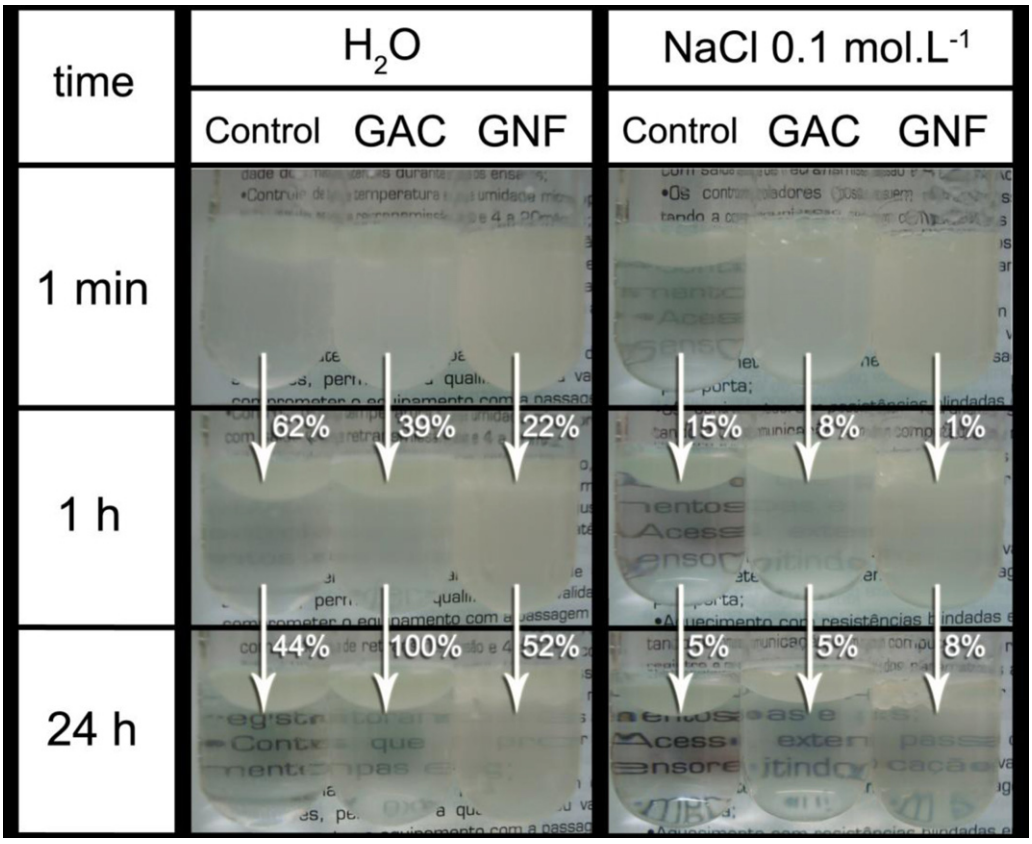

Fig. 4. Images of the prepared emulsions adding 10% (v/v) castor oil at 1 wt% GAC and GNF solutions (water or 0.1 mol L^{-1} NaCl). Arrows point to the decrease in turbidity in %T (λ = 600 nm).

3.3. *Emulsifying properties of polysaccharides of A. mearnsii de Wild*

In order to evaluate comparatively the emulsifying capacity of GAC and GNF, solution of both samples were prepared in water and in 0.1 mol L^{-1} NaCl. Castor oil was added to the solutions and the emulsion stability was followed as a function of time. Control samples with no addition of polysaccharides were also examined. Images of the prepared emulsions are show in Fig. 4 along with the decrease in the transmittance, given in *%T*. In water and saline solution, the absence of polysaccharides causes the emulsion to break up within 24 h. In the case of saline solution, this happened within the first hour. Both GNF and GAC were able to act as emulsion stabilizer in both environments, namely water and 0.1 mol L^{-1} NaCl, during the first hour. In the case of GNF in water, the emulsion remained stable even after 24 h, showing the remarkable emulsifying efficiency of GNF over GAC. Under saline conditions, GNF clearly acted as a stabilizer within the first hour, which was not the case for GAC.

From the perspective of decrease in turbidity (increase of the transmittance), the better performance of GNF over GAC is also evident. Within the first hour, the oil-in-water emulsion stabilized by GNF had an increase of the transmittance of only 22% whereas the one stabilized by GAC decreased 39%. The emulsions prepared in 0.1 mol L^{-1} NaCl demonstrated low values of the decrease in turbidity because the oil droplets had coalesced very rapidly and as a consequence initial values of the transmittance were around two times higher than the emulsions prepared in water. Even so, the GNF was able to stabilize the oil droplets for at least the first hour.

The initial oil droplets sizes and cumulative size distribution of the emulsion prepared in water and in 0.1 mol L^{-1} NaCl, determined by DLS, are depicted in Fig. 5. The observed sizes ranged from 0.01 to 7.0 μm, which do not exclude the possibility of existing bigger sizes droplets in the observed systems. Particularly, the ability to stabilize small oil droplets is valuable information in practice. Emulsification can be understood as a random process in which braking and coalesce occur in a dynamic equilibrium which results in a polydisperse system where small and big drops coexist. The breaking up step is controlled by many factors the stirring energy and surface tension. Generally speaking lower interfacial tensions lead to an increase in the break up step, favoring the formation of small drops.

According to Fig. 5A the profiles of the cumulative distribution of the oil droplets stabilized by GAC or GNF appear as double shaped curves which is not the case for the control emulsion (black line). The results imply that GNF and GAC can stabilize both lower and bigger sized droplets. However, GNF could stabilize particles as low as 50 nm whereas GAC could stabilize droplets not bigger than 200 nm. The size distribution of the oil droplets stabilized by GAC is clearly polydisperse bimodal whereas the size distribution of the droplets stabilized by GNF appears to be trimodal. The average sizes are 0.3 μm and 1 μm for GAC distribution and 0.07 μm, 0.2 μm and 0.9 μm for GNF. When the emulsions where prepared in 0.1 mol L^{-1} NaCl, it was observed the prevalence of small oil droplets which can be explained because the oil droplets coalesce rapidly (Fig. 4) under this environment. The sizes of the emulsified oil droplets were followed by optical microcopy images that showed qualitatively the same results (Suplementary material/Fig. S3.

4. Discussion

Many reports in the literature associate the arabinogalactan protein fraction (AGP) of gum arabic to its interfacial activity which in turn is associated to its emulsifying characteristics (Dickinson &

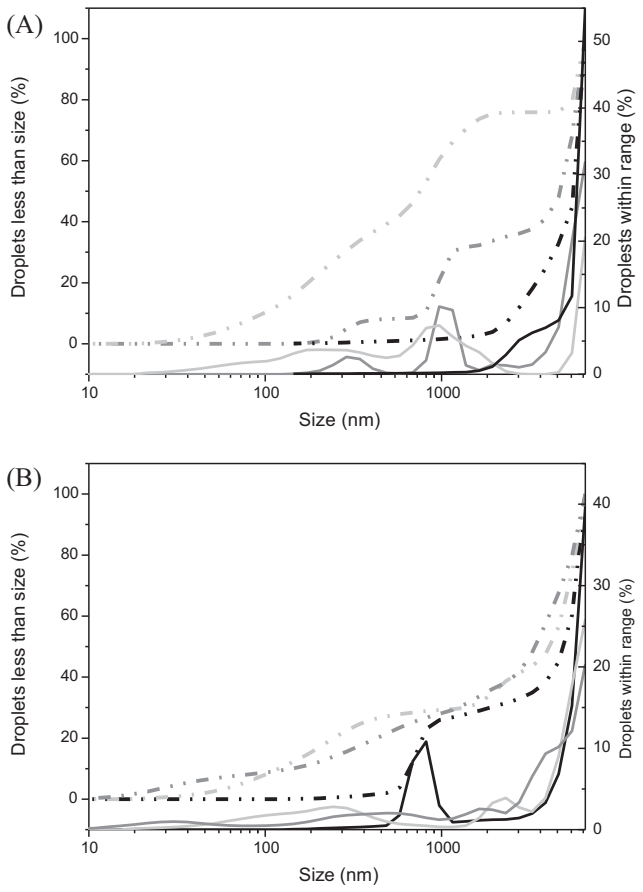

Fig. 5. Particle size distribution and cumulative particle size distribution of the prepared emulsions adding 10% (v/v) of castor oil as a control systems (black lines); 1 wt% GAC (dark gray lines) and 1 wt% GNF solutions (light gray lines) in water (A) or 0.1 mol L^{-1} NaCl (B).

Galazka, 1991; Islam, Phillips, Sljivo, Snowden, & Willians, 1997; Jayme, Dunstan, & Gee, 1999; Li et al., 2009; Randall, Phillips & Williams, 1988; Wang, Wang L-j. Dong, Adhikaric, & Shid, 2011). The arabinogalactan protein complexes adsorb to the interface with the polysaccharide moieties protruding into solution. Hence, the protein rich fraction would be responsible for the gum's emulsifying ability, which is believed to occur mainly by steric repulsion (Jayme et al., 1999; Lee & McClements, 2010). According to Dickinson (2011), for polysaccharides containing determined protein content, there are two factors that contribute to the decrease of the interfacial tension responsible for the emulsion stabilization behavior. One is the molecular weight of the complex polysaccharide-protein and the other is the conformational or structural accessibility of the amino acid groups within the complex. Additionally, not only the overall nitrogen content, but also the distribution of protein between low- and high-molecular-weight fractions appears to play a role on the emulsifying behavior of *Acacia* gum (Dickinson & Galazka, 1991). Castellani, Al-Assaf, Axelos, Phillips, and Anton (2010a) have also attributed the increased ability of lowering the interfacial tension of some gums to molecular weight factors and arabinogalactan-protein content. Lower molecular weight molecules can diffuse at higher rates to the water/oil interface and, if the amino acid groups are relatively available, a rapid reduction of the interfacial tension will occur and molecules would remain adsorbed, giving rise to a very stable emulsion.

An interesting study of Qian, Cui, Wang, Wang, and Zhou (2011) has found that peach gum exudates showed better emulsion capacity and stability than gum arabic and fenugreek. The studied peach gum polysaccharide fractions contained 13–14.5% of uronic acids with no protein detected. Therefore, the emulsifying capacity of peach gum polysaccharides was not associated with protein or protein–polysaccharide complexes. According to the authors, the high molecular weight and high branched substitution of peach gum polysaccharide are the responsible factors contributing to its emulsification properties.

Our results demonstrated that the polydispersity of GNF is very high (8.4) and the average molecular weight is lower than that of GAC. So, comparing GNF and GAC either the polydispersity as well as the molecular weight will favor the GNF emulsifying character over GAC. Although GNF has higher protein content than GAC, GP content in GNF is higher than in AGP. The opposite was found for GAC. Also, according to GPC results, GNF has more than one GP fraction, evidenced by the peaks at higher elution times (Fig. 1). The structural analysis has demonstrated that GNF has a higher degree of branching than GAC, since α-L-Ara*f* units occur in more diverse chemical environments (Fig. 2). Then, it is reasonable to suggest that GNF presents higher number of carboxyl groups of the arabinogalactan branches in its periphery when compared to GAC.

The greater surface activity of GNF over GAC can be correlated to GNF improved capacity on stabilizing castor oil droplets, even in an ionic environment (NaCl, 0.1 mol L^{-1}). Padala et al. (2009), studying gum arabic egg-white proteins, has observed that egg-white protein adsorbs preferentially onto limonene oil droplets. The preferential adsorption of egg-white is attributed to its greater surface activity based on surface tension measurements. As a consequence, the amount of egg-white protein adsorbed was higher than the amount of protein-rich fraction of gum arabic. GNF was also able to stabilize smaller oil droplets, when compared to GAC, which can be also correlated to GNF reduced surface energy. Huang, Kakuda, and Cui (2001) have directly correlated the capacity of fenugreek gum in forming small oil droplets to its lower interfacial energy.

Since the isoelectric point of GNF and GAC is far below the pH studied in this report and the viscosities of the initial solutions were nearly the same (2–3 mPa s) (data not shown here), we can only attribute the distinct behavior of GNF to its structural and molecular features, in accordance to what is described in the literature. GNF higher polidispersity, lower average molecular weight and higher degree of branching will contribute to the interfacial and emulsifying properties. Also, when compared to GAC, we believe that lower molecular weight molecules, from GP fractions, are able to adsorb preferentially onto hydrophobic surfaces, resulting in an enhanced capacity of stabilizing oil droplets. The smaller proportion of charged uronic acids units of GNF (4%) when compared to GAC (17%) can also be considered as favoring the emulsifying capacity of GNF. Castellani et al. (2010b) have found that the excellent interfacial characteristics of gum ghatti are unaffected by pH probably due to the small proportion of charged uronic acid units.

The mechanisms of water-soluble polysaccharides reducing the surface tension of oil in water system are still under debate since it was proved that many factors contributes to the emulsifying capacity and stabilization of O/W interfaces.

5. Conclusion

Polysaccharides from of *A. mearnsii* de Wild gum exudates (GNF) were characterized as heterogeneous, with acidic arabinogalactans and some protein. GP content in GNF is higher than that of AGP. Carboxy-reduction of GNF indicated the presence of glucuronic acid and 4-*O*-methylglucuronic acids as acidic components. The polysaccharides display tensoactive behavior and are able to act as stabilizers of castor oil droplets in aqueous and saline solution. Comparing the results to those obtained similarly taking a commercial arabic gum (GAC), there were structural and molecular

differences, consistent with their distinct behavior in tensiometry tests and as an emulsion stabilizer. It follows, therefore, that polysaccharides from *A. mearnsii* de Wild are candidates for applications as substitutes of currently commercialized arabic gums.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2012.09.041.

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