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Fractionation of *Acacia seyal* gum by ion exchange chromatography

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ABSTRACT

Acacia gum is a complex gum exudate from trees of selected *Acacia* species (i.e. *A. senegal* and *A. seyal*). It is a continuum of molecular species showing diverse, sugar and protein composition, molar mass and charge density. Numerous studies have been conducted on several aspects of *Acacia senegal* gum (*Asen*), including its fractionation. *Acacia seyal* gum (*Asey*) has been less studied, although it has recently been gaining importance. Certain gum characteristics, such as the protein and polysaccharide composition and the molecular parameters, play a key role in the *Acacia* gums functionality and, hence, in their uses by food, pharmaceutical or materials industries. Our main objective is to obtain a fraction from *Asey* gum with high molar mass and high protein content, allowing future research works and industrial applications. *Asey* gum has been separated by ion exchange chromatography (IEC) into two different fractions, IEC-F1 and IEC-F2, which have been thoroughly characterized. Thus, we have succeeded to recover a protein-rich fraction with high molar mass and high intrinsic viscosity, the fraction IEC-F1. The Mark-Houwink-Sakurada analysis further indicated that fraction IEC-F1 presents more anisotropic conformation compared to fraction IEC-F2. From the partial specific volume (v_s°) and the partial specific adiabatic compressibility (β_s°) coefficients, a more flexible and less hydrated structure in the fraction IEC-F1 compared to *Asey* gum was suggested.

Keywords: *Acacia seyal* gum; fractionation; ion exchange chromatography; polysaccharides; proteins; SEC-MALLS.

1. Introduction

Acacia gum is a complex polysaccharide-based plant gum exudate, obtained from trees of selected *Acacia* species (i.e. *Acacia senegal* and *Acacia seyal*), whom exude this substance to protect trees against external attacks. Much work can be found in literature about several aspects of *Acacia senegal* gum (*Asen*), such as its composition (Islam, Phillips, Sljivo, Snowden, & Williams, 1997; Idris, Williams, & Phillips, 1998; Verbeken, Dierckx, & Dewettink, 2003), polydispersity, structure (Fincher, Stone, & Clarke, 1983; Idris et al., 1998; Renard, Lavenant-Gourgeon, Ralet, & Sanchez, 2006; Sanchez et al., 2008), physico-chemical properties (Phillips, Takigami, & Takigami, 1996; Renard et al., 2006; Mejia Tamayo et al., 2018; Buffo, Reineccius, & Oehlert, 2001; Al-Assaf, Phillips, Aoki, & Sasaki, 2007; Dickinson, 2008). *Asen* has been much more studied over years since it largely dominated the *Acacia* gum commerce with a market share of about 70%. However *Acacia seyal* gum (*Asey*) has been gaining interest and importance in recent years in the *Acacia* gum trade (Rahim, van Ierland, & and Weikard, 2010). *Acacia* gums have been largely used for different purposes in all human history (Sanchez et al., 2018). Currently, this natural substance is employed in a variety of applications such as food, pharmaceutical and other industrial applications (Verbeken et al., 2003). *Asey* is constituted by about 37-44% of galactose, 33-48% of arabinose, 2-3% of rhamnose, 7-13% of glucuronic acid, 6% of 4-*O* methyl glucuronic acid, 1% of protein, and 4% of minerals (Gashua, Williams, Yadav, & Baldwin, 2015; Lopez-Torrez, Nigen, Williams, Doco, & Sanchez, 2015). The arabinogalactan moiety of *Asey* is formed by a core of β -1,3 linked galactose units with branches linked through the 6 position consisting of galactose and arabinose terminated by rhamnose and glucuronic acids (Al-Assaf, Phillips, & William, 2005; Siddig, Osman, Al-Assaf, Phillips, & Williams, 2005; Street & Anderson, 1983). The minor proteinaceous

material is covalently attached to this arabinogalactan moiety (Mahendran, Williams, Phillips, Al-Assaf, & Baldwin, 2008; Siddig et al., 2005). Besides, *Asey* macromolecules present a lower hydrodynamic volume than those from *Asen*, that can be interpreted as a more compact conformation (Al-Assaf et al., 2005; Flindt, Al-Assaf, Phillips, & Williams, 2005; Elmanan, Al-Assaf, Phillips, & Williams, 2008; Lopez-Torrez et al., 2015).

Asen has been demonstrated as a continuum of macromolecules which shows varying protein/sugar ratios, molar masses and charge density (Siddig et al., 2005; Renard et al., 2006). Consequently, the fractionation of *Asen* was done by different methods such as the anion-exchange chromatography (Osman et al., 1995), the size exclusion chromatography (SEC) (Ray, Bird, Iacobucci, & Clark 1995) or the hydrophobic interaction chromatography (HIC), which was the most used fractionation procedure (Randall, Phillips, & Williams, 1989; Osman, Menzies, Williams, Phillips, & Baldwin, 1993; Ray et al., 1995; Renard et al., 2006). Using HIC, three main AGP fractions can be obtained from *Asen*: (i) a dominant fraction in percentage with low protein content and low mean molar mass (HIC-F1), (ii) a fraction presenting a high molar mass and rich in protein (HIC-F2) and, finally, (iii) a fraction showing high molar mass and the highest protein content (HIC-F3) (Renard et al., 2006). In a previous work, we presented the successful fractionation of *Asen* by Ionic Exchange Chromatography (IEC) from which two fractions were obtained: one protein-poor fraction with low molar mass and one protein-rich fraction with high-molar mass showing a great tendency to aggregate (Apolinar-Valiente et al., 2019). In comparison with *Asen*, fractionation of *Asey* has been notably less reported. Siddig et al. (2005) undertook both GPC and HIC to compare gums from *Asey* and *Asen* varieties. Using HIC fractionation, they found for *Asey* practically the same class of fractions than those found for *Asen*. On the other hand, they found a fourth fraction with low molar mass and rich in protein when *Asey* was

fractionated by GPC, which was not observed in *Asen*, concluding that separation of *Asey* by HIC was not complete.

Trying to develop a deeper understanding about the not greatly studied characteristics and functional properties of *Asey* gum, we considered that it would be useful to recuperate a fraction showing higher M_w together with higher protein content than those found in starting *Asey* gum. Previously, we succeeded in achieving an *Asen* fraction showing these two characteristics, as compared to the starting *Asen* gum (Apolinar-Valiente et al., 2019). Thus, our main objective was to obtain a high M_w and high-protein amount fraction from *Asey* using IEC. This technique separates macromolecules in accordance with the proportion of anionic groups (mainly carboxylate groups from acid sugar residues) interacting with the positively charged sites on the gel. The retention could also be affected by other parameters, such as the molecular size and the structure of the macromolecule (Medved, Ivanov, & Shpigun, 1996), as well as the type and the hydrophobicity of the gel (Ohta, Tanaka, & Haddad, 1997).

Asey has been therefore fractionated by IEC through the use of DEAE Sephacel gel as stationary phase. Two main fractions were recovered, one of which was rich in protein with very high molar mass. These fractions were subsequently characterized in terms of biochemical composition (amino acid and sugar residues), structure (weight-average molar masses M_w , number-average molar mass M_n , polydispersity index M_w/M_n , intrinsic viscosity $[\eta]$) and volumetric properties (partial specific volume v_s° and partial specific adiabatic compressibility β_s° coefficients and hydrodynamic volume from intrinsic viscosity).

2. Material and Methods

2.1. Material

Spray-dried Acacia gum type from *Acacia seyal* trees (Lot: OF110724) was provided by ALLAND & ROBERT Company – Natural and organic gums (Port Mort, France).

2.2. Preparation of *Acacia seyal* gum (*Asey*) dispersions

Asey dispersions were prepared by weight (wt %). Known amounts of *Asey* powder (650 g, corresponding to 629 g in dry terms) were dispersed in water (6500 mL) and gently stirred for 24 h at room temperature (20 °C). The pH of dispersions was subsequently adjusted at 3.5 using HCl 1 N, HCl 0.1 N, or NaOH 0.5 N solutions.

2.3. Ion exchange chromatography (IEC)

IEC was performed at room temperature on DEAE Sephacel (Sigma Aldrich, St. Louis, Mo) column (54 x 20 cm), and fractions IEC-F1 and IEC-F2 were obtained following the methodology described by Apolinar-Valiente et al. (2019). The use of buffer was excluded because our work is focused not only from a laboratory point of view but also from the possibility of an industrial production perspective. The experience was conducted in triplicate, and the results were reproducible. Therefore, we considered that although the use of a buffer for controlling pH is the more scientifically rigorous choice, not using buffer eases the laboratory procedure and it opens the possibility of future industrial food applications.

2.4. Yariv detection

β -glucosyl Yariv reagent has been widely used to identify AGPs (Osman et al., 1993; Paulsen, Craik, Dunstan, Stone, & Bacic, 2014). Petri dishes containing 1% agarose gel in 10 mM Tris buffer, pH 7.3, with 0.9% NaCl and 1 mM CaCl₂ were used. Consequently, β -D-Glucosyl Yariv reagent (Biosupplies, Victoria, Australia; 40 μ L, 1 mg·mL⁻¹) was delivered to a central well, placing fractions IEC-F1 and IEC-F2 in equidistant peripheral wells. The Petri dishes were left overnight at 25°C. We used arabinogalactan from larch wood (Sigma Aldrich, St. Louis, Mo) as negative control.

2.5. Amino acid analysis

Total amino acids were analysed with a Biochrom 30 analyser (BIOCHROM 30, Cambridge, UK). Amino acid composition of samples was determined after acid hydrolysis (6 N HCl) and heating (110°C, 24 h). Norleucine was used as internal standard.

2.6. Sugar composition as trimethylsilyl derivatives

The neutral and acidic sugar composition was determined after solvolysis with anhydrous MeOH containing 0.5 M HCl (80 °C, 16 h), by GC of their per-O-trimethylsilylated methyl glycoside derivatives (Doco, O'Neill, & Pellerin, 2001; Apolinar-Valiente et al., 2014).

2.7. Glycosyl-linkage compositions.

The glycosyl-linkage compositions of the partially methylated alditol acetates were determined by gas chromatography-electron ionization-mass spectrometry (GC-EI-MS) as previously described (Lopez-Torrez et al., 2015).

2.8. Molar mass distribution and intrinsic viscosity.

Fractions IEC-F1 and IEC-F2 from *Asey* were analysed by size exclusion chromatography (SEC) as described by Apolinar-Valiente et al. (2019). The SEC line was constituted, depending on the analysis, by an OHPAK SB-G guard pre- column followed by four columns (OHPAK SB 803, 804, 805 and 806 HQ, Shodex) for *Asey* and fraction IEC-F2 and by an OHPAK SB-G guard pre- column followed by one column (OHPAK SB 805 HQ, Shodex) for fraction IEC-F1. In the latter, only one Shodex column was used in order to decrease anomalous elution of high M_w hyperbranched macromolecules. The AGPs were eluted with filtered (0.1 μm filter, Millipore) solution (0.1 M LiNO_3 + 0.02% NaN_3) at a flow rate of 1 $\text{mL}\cdot\text{min}^{-1}$ and 30°C. The samples were dissolved in filtered Milli-Q water (1 $\text{mg}\cdot\text{mL}^{-1}$), stirred gently (24 h) and centrifuged (10 000 rpm, 10 min). Subsequently, 75 μL of IEC-F2 fraction and *Asey* and 10 μL IEC-F1 fraction were injected. Intrinsic viscosity was determined using an online viscometer (Viscostar II Wyatt, Santa Barbara, CA, USA) and a differential refractometer (Optilab T-Rex, Wyatt, Santa Barbara, CA, USA).. The refractive index increment values (dn/dc) were determined as 0.155, 0.158 and 0.155 $\text{mL}\cdot\text{g}^{-1}$ for *Asey*, IEC-F1 and IEC-F2, respectively.

2.9. Partial specific volume and partial specific adiabatic compressibility

Volumetric properties of biopolymers [partial specific volume (v_s°) and partial specific adiabatic compressibility (β_s°)] can be calculated from measurements of density and sound velocity (Mejia Tamayo et al., 2018), which were simultaneously measured (25° C) using a DSA 5000M sonodensimeter (Anton Paar, France), as previously described (Mejia Tamayo et al., 2018). Dispersions were dialyzed overnight against sodium acetate buffer (10 mM, pH 5) to reach isopotential equilibrium, centrifuged (12 000 rpm, 30 min, 20°C) to remove insoluble materials and degassed 15 min to remove dissolved air (300 Ultrasonik bath, Ney, Yucaipa, CA, USA). Measurements were duplicated.

2.10. FTIR spectral acquisition.

Spectral acquisitions were performed on dry gum samples using a Vertex 70V Fourier-transform mid-spectrometer equipped with an ATR Diamond cell (SPECAC, Smyrna, GA, USA). Each recorded spectrum was the average of 128 repetitions from 900 to 1800 cm^{-1} with a 4 cm^{-1} spectral resolution. A micrometric screw applying constant pressure ensured good contact between the sample and the crystal. The spectra were acquired and analysed using OPUS Software version 7.5. A sample-less spectrum was recorded between two polysaccharide fractions to monitor the stability of the background. Each spectrum was subjected to linear standardisation at absorbance values from 0 to 1 for the respective wave numbers of 1800 and 1025 cm^{-1} for proper spectra exposure and interpretation.

3. Results and discussion

The aim of the present study was the fractionation of *Asey* using IEC in order mainly to recover one AGPs fraction (IEC-F1) with high molar mass and high protein content. It is necessary to mention that, in our view, it was also important to characterize our material of departure in order to show that it corresponded to a “classical” *Asey*. All the information about *Asey* will be discussed in the corresponding section.

3.1. Fractionation of Asey by IEC: yield of the recuperation

The IEC fractionation procedure allowed recovering two fractions, IEC-F1 and IEC-F2, which correspond respectively to 1.4% and 82.6% of the initial gum. The losses of material can occur during the fractionation, concentration, diafiltration and spray-drying steps; nevertheless, we consider that the total yield (84.0% of initial gum) was satisfactory. Supplementary data (Fig. 1) shows the precipitation of both fractions IEC-F1 and IEC-F2 by Yariv’s reagent, corroborating that they belong to AGP family. Fractions from *Acacia senegal* gum obtained by different fractionation techniques, such as preparative GPC (Qi, Fong, & Lamport, 1991), HIC (Osman et al., 1993) or IEC (Apolinar-Valiente et al., 2018), were also found to interact with Yariv reagent.

3.2. Fractions IEC-F1 and IEC-F2: composition and structure

Knowing that our principal aim is to isolate an AGPs fraction presenting high molar mass and high content of protein, we will look more closely at the results of the fraction IEC-F1.

Regarding *Asey* and IEC-F2, their data are also exhibited in the different Tables and Figures, being similar both of them.

3.2.1. Sugar composition

Table 1A presents the neutral sugar and uronic acid composition of *Asey*. We found high content of arabinose (48.5%) and galactose (34.2%), whereas rhamnose only represents 3.2%. Concerning the uronic acids, glucuronic acid and 4-O-methyl glucuronic acid show values of 7.7% and 6.4%, respectively. This sugar composition corresponds with data largely reported in literature (Biswas, DeVido, & Dorsey, 2003; Flindt et al., 2005; Siddig et al., 2005; Andres-Brull et al., 2015; Lopez-Torrez et al., 2015; Gashua, Williams, & Baldwin, 2016). When characteristic ratio Ara/Gal of *Asey* was calculated, it presents a value of 1.4, which is in general also coherent with values found in literature (Siddig et al., 2005; Andres-Brull et al., 2015; Lopez-Torrez et al., 2015). It can be noted that this ratio could notably vary depending on the origin of the *Asey* gum (Biswas et al., 2003; Flindt et al., 2005).

Regarding the neutral sugar and uronic acid composition of the IEC-F1 fraction (Table 1A), arabinose appears as its major component (54%), followed by galactose (29%). This corresponds to data previously reported for fractions from *Asey* after separation by different separation methods (Flindt et al., 2005; Siddig et al., 2005). Fraction IEC-F1 shows the greatest value (1.8) for the calculated Ara/Gal ratio. This behaviour could indicate longer side branches of arabinose than the other two studied samples. Lopez-Torrez et al. (2015) suggested that a higher content of long arabinose side chains that may self-organize and interact between them (e.g. hydrogen bonding, steric effect, etc.) could imply a more compact structure. The calculated characteristic ratios are quite similar to those found in *Asey*

fractions by GPC separation (1.1-2.8) (Flindt et al., 2005) and by HIC (around 1.2) (Siddig et al., 2005). In both cases, the different separation technique together with the above mentioned origin influence could explain the variations observed. Regarding glucuronic acid, the highest percentage appears in fraction IEC-F1 (10%). Using IEC separation, the most negative charged macromolecules should be more retained by the positively charged column. It would be logical to think that the first eluted fraction would present less compound showing negative charges, such as glucuronic acid. The opposite is, however, found. Our observation concerning glucuronic acid may be caused by steric hindrance of some groups to interact with the DEAE gel. In the same line, Osman et al. (1995) reported that the order of elution of the fractions separated by IEC does not follow their glucuronic acid amount, suggesting the possibility of a steric disability. Previously, we also proposed this behavior during fractionation of *Asen* by IEC (Apolinar-Valiente et al., 2019).

From the glycosidic linkages reported in Table 1B, the ratio of terminal to branched residues can be calculated. For this purpose, we have calculated the terminal units (TU) as follows:

$$TU = \frac{2.3.4.Rha}{0.69} + \frac{2.3.5.Ara}{0.60} + \frac{2.3.4.Ara}{0.61} + \frac{2.3.4.6.Gal}{0.70}$$

We have calculated the branched units (BU) using:

$$BU = \frac{2.6.Gal}{0.79} + \frac{2.4.Gal}{0.80} + 2x \frac{2.Gal}{0.84} + \frac{3.Ara}{0.70}$$

The coefficients used in these two formulas were reported by Sweet, Albersheim and Shapiro (1975). The TU/BU ratio is higher in fraction IEC-F1 (0.57) compared fraction IEC-F2 (0.47). The highest TU/BU ratio of fraction IEC-F1 could suggest a less branched structure of the carbohydrate moiety. The data given in Table 1B enables us to calculate the content of free OH in the polysaccharide portion, as shown below in section 3.2.6.

3.2.2. Amino acid composition

Table 2A shows the total amino acid content and amino acid composition of *Asey* and the two IEC fractions. The total content of amino acids for *Asey* is 7 mg·g⁻¹ of sample, which is in agreement with those obtained by other authors (Elmanan et al., 2008; Lopez-Torrez et al., 2015; Gashua et al., 2016). Regarding the amino acid composition of *Asey*, hydroxyproline is by far the major amino acid, followed by serine, and in less amount leucine, aspartic acid, proline and threonine, as previously presented in literature (Osman et al., 1993; Flindt et al., 2005; Siddig et al., 2005; Lopez-Torrez et al., 2015). Minor amino acids were isoleucine, lysine, arginine and tyrosine (Table 2A).

Fraction IEC-F1 presents much lower content of hydroxyproline (6%) and serine (8%) amino acids compared to fraction IEC-F2 (34% and 16%, respectively) and *Asey* (32% and 13%, respectively). Mahendran et al. (2008) reported that the link between the protein fraction and the polysaccharide fraction are both *O*-serine and *O*-hydroxyproline residues. Therefore, these results could indicate a lower number of polysaccharide chains linked to proteins in fraction IEC-F1. On the other hand, aspartic acid appears as the most abundant amino acid of fraction IEC-F1 (15%), showing also high amounts of leucine (10%) and valine (9%). When Flindt et al. (2005) separated the *Asey* gum by GPC, they also detected that aspartic acid was the major amino acid in several fractions, whereas hydroxyproline presented low values in some of these or other fractions.

We have calculated the percentages of several amino acid families trying to observe a possible influence of the charges during the separation of fractions by IEC. In particular, we determined the percentages of negatively charged, positively charged, polar (hydrophilic) and non-polar (hydrophobic) amino acid residues (Table 2B). Negatively charged amino acids are

estimated as the sum of aspartic acid and glutamic acid. The sum of histidine, lysine and arginine is considered as positively charged amino acids. Hydrophilic or polar amino acids are calculated as the sum of serine, threonine and tyrosine. Finally, hydrophobic or non-polar amino acids are calculated as the sum of alanine, glycine, isoleucine, leucine, phenylalanine, proline and valine.

The first eluted fraction (IEC-F1) should present a lower content of negative charged macromolecules, which would interact with the positively charged gel. Instead, the IEC-F1 fraction shows the highest percentage of negatively charged amino acids (23%) compared to fraction IEC-F2 (8%) and *Asey* (12%). That could be explained, like previously suggested for the sugars, by the fact that some negatively charged amino acids may be sterically hindered to interact with gel.

Concerning the polarity/hydrophobicity, we have found that fraction IEC-F1 shows the highest percentages for the hydrophobic amino acids (47%) and the lowest value for the hydrophilic amino acids (14%). On the other hand, fraction IEC-F2 presents the opposite trend: the lowest percentage of hydrophobic amino acids (26%) and the highest percentage of hydrophilic amino acids (23%). Therefore, a different hydrophobicity between amino acids from the two obtained fractions separated by IEC has been found.

Regarding the total content of amino acids, fraction IEC-F1 shows clearly the highest value (74 mg amino acid·g⁻¹ of sample), matching with our previous results about IEC-fractionation of *Asen* (Apolinar-Valiente et al., 2019). Literature shows varying protein contents between fractions when *Asey* gum was fractionated by HIC (Siddig et al., 2005) or by GPC (Flindt et al., 2005). Our results are corroborated by the infrared spectra of fractions IEC-F1 and IEC-F2 (Supplementary Figure 2). The absorbance is clearly higher at 1650 and at 1545 cm⁻¹ for fraction IEC-F1, indicating the emergence of Amide I and II vibration bands

of proteins, respectively (Bertrand & Dufour, 2000; Lopez-Torrez et al., 2015). In accord with Barth (2007), they mainly depend on the secondary structure of the polypeptide backbone, being influenced by the nature of the side chains. The whole infrared spectra of our AGP samples (Supplementary Figure 2) resemble, as expected, to the *Asey* spectrum found by Lopez-Torrez et al. (2015) and also to those of AGPs from wine reported by Boulet, Williams, & Doco (2007) in the range between 1800 and 800 cm⁻¹.

3.2.3. Size exclusion chromatograms and M_w distribution

Asey chromatogram (Figure 1A) shows one main peak between 26 and 33 min elution time (RI signal). This profile, together with the obtained M_w distribution as a function of the elution time (Figure 1A), is in coherence with those previously reported by several authors (Gashua et al., 2015; Lopez-Torrez et al., 2015). Figure 1B exhibits the molar mass distribution analysis of *Asey*. It was divided into three selected ranges in order to estimate the relative percentage in small, medium and large molar masses: range 1 (below 5 x 10⁵ g·mol⁻¹), range 2 (between 5 x 10⁵ and 1 x 10⁶ g·mol⁻¹) and range 3 (above 1 x 10⁶ g·mol⁻¹). Thus, we have obtained higher values for range 1 (42%) and 2 (34%) in comparison with the range 3 (24%).

The chromatogram of fraction IEC-F1 presents one major peak with elution time values (RI signal) ranging from 6.4 to 8.9 min (Figure 1A). This population presents only high M_w AGPs (upper than 10⁶ g·mol⁻¹). A second lower but well-defined peak can be deduced between 8.9 and 11 min, showing AGPs with low M_w (below than 10⁶ g·mol⁻¹).

Figure 1B shows that fraction IEC-F1 has much higher percentage of cumulative molar mass in range 3 (82%) compared to ranges 1 (12%) and 2 (6%). As expected, our procedure

enables to obtain high M_w AGP from *Asey*, corresponding to the fraction IEC-F1. However, IEC separation seems not as good for *Asey* as we previously found for *Asen* (Apolinar-Valiente et al., 2019), where we reported 96% of fraction IEC-F1 in the range 3. One likely explanation could be the higher starting percentage of macromolecules from range 3 ($>10^6$ g·mol⁻¹) in the case of *Asey* (24%) in comparison to *Asen* (15%, from Apolinar-Valiente et al., 2019). This high number of macromolecules would result in a steric hindrance which should hinder that macromolecules from ranges 1 and 2 should be fixed to the gel (Osman et al., 1995). In such a way, these macromolecules from ranges 1 and 2 would pass easily through the column and would be eluted as an integral part of fraction IEC-F1. Therefore, the hydrodynamic volume of the macromolecules contributes in the efficiency of IEC separation method. Another explanation, not in conflict with the previous account, could be the higher negative attractive potential interaction in *Asen* compared to *Asey* due to the charged residues content (Lopez-Torrez et al., 2015). This behaviour would explain the lower separation for *Asey* compared to our previous work (Apolinar-Valiente et al., 2019).

3.2.4. Static and dynamic molecular parameters

The static and dynamic parameters of *Asey* and fractions IEC-F1 and IEC-F2 are given in Table 3A. *Asey* shows values of 7.8×10^5 g·mol⁻¹ for M_w and 4.4×10^5 g·mol⁻¹ for M_n . These values are close to those found in literature (Flindt et al., 2005; Elmanan et al., 2008; Lopez-Torrez et al., 2015; Gashua et al., 2016).

Fraction IEC-F2 displays weight-average molar mass (M_w) and number-average molar mass (M_n) of 8.1×10^5 g·mol⁻¹ and 4.7×10^5 g·mol⁻¹, respectively. In contrast, fraction IEC-F1 presents much higher values for M_w (3.1×10^6 g·mol⁻¹) and M_n (1.3×10^6 g·mol⁻¹). Flindt et

al. (2005) and Siddig et al. (2005) also detected differences in M_n and M_w between fractions when *Asey* was separated, respectively, by GPC and by HIC. However, IEC technique allows to obtain a fraction whose M_w is largely greater than the starting *Asey* gum. As mentioned earlier, Siddig et al. (2005) obtained two high protein-content fractions by HIC: the classical fractions HIC-F2 and HIC-F3. These fractions display, respectively, M_w values smaller (–3.8%) and larger (+17%) than the M_w value of starting *Asey* gum. On the other hand, we obtain by IEC a fraction (IEC-F1) whose M_w represents an increase of 283% with regard to the starting *Asey* gum M_w . Concerning the polydispersity data (M_w/M_n), *Asey* presents a value of 1.8, which is also close to values reported by other authors (Lopez-Torrez et al., 2015; Gashua et al., 2016). Fraction IEC-F2 presents similar polydispersity (1.7) than *Asey* (1.8), whereas fraction IEC-F1 shows the highest value for this parameter (2.6).

Asey gum presents a larger intrinsic viscosity ($[\eta]$) value (23 mL·g⁻¹) than those reported previously (12-18 mL·g⁻¹) (Flindt et al., 2005; Elmanan et al., 2008; Lopez-Torrez et al., 2015). These variations may be due to differences in the age of trees (Idris et al., 1998) and/or the origin of the gum (Gashua et al., 2015). Fraction IEC-F2 shows similar values (22 mL·g⁻¹) to *Asey* gum, whereas, as expected, the highest intrinsic viscosity ($[\eta]$) is found for fraction IEC-F1 (36 mL·g⁻¹). This parameter in *Acacia* gum has been suggested as strongly linked to the molar mass as well as to the protein content (Chikamai, Banks, Anderson, & Weiping, 1996). In our case, the $[\eta]$ value (Table 3A) and the amino acid total content (Table 2A) of fraction IEC-F1 are in perfect coherence with these two remarks, which can be explained by the fact that protein-rich macromolecules in *Acacia* gum display generally high M_w .

The average sphere-equivalent hydrodynamic radius (R_H) of *Asey* gum (13.6 nm, Table 3A) was calculated from the intrinsic viscosity following $R_H = ([\eta] \cdot M_v \cdot 3) / (10 \cdot \pi \cdot N_A)^{1/3}$. In this

formula, $[\eta]$ is the intrinsic viscosity and M_v the viscosity-weighted molar mass. This R_H value was close to the 9.3 nm value previously determined on another *Asey* batch (Lopez et al., 2015). Values in the range 8.4–9.0 nm were also found by Flindt et al. (2005). These values are logically close to the hydrodynamic radius determined on IEC-F2 (\cong 13.4 nm) and smaller than the R_H of IEC-F1 (27.3 nm). Regarding the radius of gyration (R_g), values of about 32 nm were found for IEC-F1, which according to our experience is quite common with high M_w AGP. Only R_g values above 10 nm were considered to calculate R_g , corresponding to about 53% of macromolecules from *Asey*, 61% of macromolecules from IEC-F1 and 53% of macromolecules from IEC-F2.

Figure 2A shows the results of Mark-Houwink-Sakurada plot for IEC fractions, which relates the intrinsic viscosity $[\eta]$ as a function of M_w . These two parameters are linked by the equation $[\eta] = K_\alpha M_w^\alpha$, where K_α is a constant and α an exponent value called hydrodynamic coefficient (Burchard, 1999). The theoretical values for α vary from 0 (sphere) to 1.8 (rod) with 0.5-0.8 intermediate values for flexible polymers depending on the solvent quality (Ross-Murphy, 1994). Mark-Houwink-Sakurada log-log plots from IEC-F1 and IEC-F2 can be described by one single slope (exponent of the power law), indicating one single conformation for these AGP in solution. Slope values were 0.31 for IEC-F2 and 0.39 for IEC-F1. Generally, slope values within 0.3-0.5 are classically found for hyperbranched structures (Callaghan & Lelievre, 1985; Lelievre, Lewis, & Marsden, 1986; Millard, Dintzis, Willett, & Klavons, 1997; Rolland-Sabaté, Mendez-Montevalvo, Colonna, & Planchot, 2008; Li, Lu, An, & Wu, 2013). The higher slope value for fraction IEC-F1 (0.39) would indicate a more anisotropic shape, a different density or a different affinity for solvent.

The ratio of geometric to hydrodynamic radius ($\rho = R_g \cdot R_H^{-1}$), also called asymmetry (Adolphi & Kulicke, 1997) or anisotropy parameter (Mansfield & Douglas, 2013), is a

structural parameter. It can be affected by macromolecular flexibility and polydispersity (Adolphi & Kulicke, 1997) but also by particle homogeneity and density. Only R_g values above 10 nm were considered to calculate ρ ratio, corresponding to about 53% of macromolecules from Asey, 61% of macromolecules from IEC-F1 and 53% of macromolecules from IEC-F2. In the case of fraction IEC-F1, this ratio remains constant around 0.9-1.1 (Figure 2B), which is close to the values found with dendrimers or hyperbranched polymers (Lopez-Torrez et al., 2015). On the other hand, the ρ ratio for IEC-F2 increases with M_w from 0.65 to 0.90, which would indicate that AGP with high M_w are more anisotropic than low M_w AGP since the theoretical ratio for hard homogeneous sphere is around 0.8.

3.2.5. Thermodynamic volumetric parameters

Partial specific volume and partial specific adiabatic compressibility coefficient are thermodynamic parameters which can be related to solvent-solute and solute-solute interactions. These two volumetric properties have been used to describe the structure and flexibility changes of macromolecules when submitted to changes in their environment (Gekko & Hasegawa, 1986; Hoiland, 1986; Gekko & Yamagami, 1991). The partial specific volume (v_s°) can be defined as the change of the system volume due to the addition of an infinitesimal amount of the solute, whereas the partial adiabatic compressibility coefficient (β_s°) is defined as the change of the system pressure caused by the addition of an infinitesimal amount of the solute (Gekko & Hasegawa, 1986; Hoiland, 1986; Gekko & Yamagami, 1991; Mejia Tamayo et al., 2018). Both of them depend mainly on the intrinsic contribution of the solute and its hydration. The first one concerns the constitutive volume of

the molecule itself, that is to say, the atoms forming the molecule (van der Waals volume) and the cavities (voids) within the molecule. On the other hand, the hydration contribution concerns the solute-solvent interactions, and specially the changes in physical properties of water upon interaction with AGP surface residues. Changes in v_s° and β_s° are mainly attributed to changes in the cavities or hydration. The first one produces a positive effect on v_s° . On the other hand, hydration causes a negative effect on v_s° , which indicates the occurrence of a more compressible (more flexible) structure (Gekko & Noguchi, 1979; Chalikian & Breslauer, 1996). These two properties can be determined using ultrasound measurements as previously described by Mejia Tamayo et al. (2018).

Table 3B gives v_s° and β_s° of the fractions IEC-F1 and IEC-F2. Greater values of v_s° and β_s° are observed in IEC-F1 ($0.607 \text{ cm}^3 \cdot \text{g}^{-1}$ and $-7.4 \times 10^{-11} \text{ Pa}^{-1}$, respectively) compared to those of fraction IEC-F2 ($0.582 \text{ cm}^3 \cdot \text{g}^{-1}$ and $-12.4 \times 10^{-11} \text{ Pa}^{-1}$, respectively) and those of *Asey* ($0.577 \text{ cm}^3 \cdot \text{g}^{-1}$ and $-13.2 \times 10^{-11} \text{ cm}^3 \cdot \text{g}^{-1}$, respectively). Therefore, it can be concluded that the fraction IEC-F1 displays a more compressible and less hydrated structure than the IEC-F2 fraction. Moreover, fraction IEC-F1 from *Asey* presents less negative β_s° value than the similar fraction obtained from *Asen* (-7.4×10^{-11} vs $-9.4 \times 10^{-11} \text{ Pa}^{-1}$) (Apolinar-Valiente et al. (2019), indicating less hydration of the former. On the other hand, the same compressibility values were found for IEC-F2 fraction coming from the two gums. It can be noted that a link between protein interfacial properties and their molecular compressibility was clearly demonstrated with globular proteins (Gekko & Yamagami, 1991; Damodaran, 2008). It can then be assumed that fraction IEC-F1 from *Asey* would present better interfacial properties as compared to unfractionated *Asey*. All these results are in agreement with the data of IEC fractions from *Asen* previously reported by Apolinar-Valiente et al. (2019).

3.2.6. Basic molecular characteristics

Using several biochemical and structural properties, we have estimated the contribution of the polysaccharide and protein moiety of IEC fractions to basic molecular properties (Table 4). By means of the sugar (Table 1) and amino acid (Table 2) composition, we have calculated the average M_w of the polysaccharide and protein part. The content of free OH in the polysaccharide portion was estimated by taking into account the linkage of the neutral sugars (Table 1B) and the number of possible interacting sites of the protein portion. Observing the protein moiety, the fraction IEC-F1 shows a much greater number of potential charged and polar interacting sites (~1 200) as compared to fraction IEC-F2 (35). Their corresponding content of amino acid residues (~1 800 and 40, respectively) explains this behavior. Concerning the polysaccharide part, the number of charges contributed by fraction IEC-F1 also presents much higher values (~47 000) than those from fraction IEC-F2 (~12 000), which is explained by their corresponding content of sugar residues (~17 000 and ~5 000, respectively).

4. Conclusions

We have succeeded to recover a fraction rich in protein and with high molar mass from *Asey* gum by ion exchange chromatography. This fraction, called IEC-F1, has been deeply characterized, together with the other obtained fraction, named IEC-F2. Significant differences between the characteristic ratio arabinose/galactose, the glucuronic acid content and the ratio of terminal to branched glycosidic residues of IEC-F1 and IEC-F2 fractions were observed. Fraction IEC-F1 shows the lowest content of hydroxyproline and serine, as

well as the greatly highest value of total amino acid content. The molar mass distribution of fraction IEC-F1 shows difference compared to fraction IEC-F2 and also to *Asey* gum, giving great percentages (82%) in the range 3 (molar masses above $1 \times 10^6 \text{ g}\cdot\text{mol}^{-1}$). Fraction IEC-F1 also shows the largely highest values of M_w , M_n and intrinsic viscosity. The higher hydrodynamic coefficient from the Mark-Houwink-Sakurada analysis corresponds to fraction IEC-F1, which would implicate more anisotropic conformation compared to fraction IEC-F2. The anisotropy parameter (ρ) remains constant around 0.9-1.1 in fraction IEC-F1, whereas it enhances from 0.65 to 0.90 in fraction IEC-F2. Besides, from the partial specific volume (v_s°) and the partial specific adiabatic compressibility (β_s°) coefficients, a more flexible and less hydrated structure in the fraction IEC-F1 compared to *Asey* gum can be concluded. Finally, from a large part of the obtained data, we could conclude a steric hindrance of some groups to interact with the DEAE gel during IEC fractionation. Based on the results shown in this work, further studies should be carried out in order to improve the knowledge about the potential physico-chemical characteristics of obtained *Asey* fractions and, hence, to raise their potential applications.

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Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

Compliance with ethics requirements

This article does not contain any studies with human or animal subjects.

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687 **Table 1.** Neutral sugars and uronic acids composition (% molar) (1A) and glycosidic-
688 linkages composition (% molar) (1B) of *Acacia seyal* gum (Asey) and fractions IEC-F1 and
689 IEC-F2 obtained using Ion Exchange Chromatography (IEC).

		Asey	Fraction IEC-F1	Fraction IEC-F2	
1A	Glycosyl composition				
	Arabinose	48.5	53.7	49.9	
	Rhamnose	3.2	2.2	3.5	
	Galactose	34.2	29.4	37.7	
	Glucuronic Acid	7.7	10.2	4.5	
	4- <i>O</i> methyl Glucuronic acid	6.4	4.4	4.4	
	Ratio Arabinose/Galactose	1.42	1.83	1.32	
1B	Glycosyl residue Linkage				
	2.3.4.6-Galactose ^a	T-Galp ^b	0.6	1.3	0.9
	2.3.4-Galactose	1→6 Galp	2.0	1.9	2.1
	2.4.6-Galactose	1→3 Galp	3.0	3.9	3.3
	2.3.6-Galactose	1→4 Galp	6.2	4.6	6.7
	2.6-Galactose	1→3.4 Galp	1.9	1.9	1.5
	2.4-Galactose	1→3.6 Galp	32.0	29.2	34.2
	2-Galactose	1→3.4.6 Galp	2.2	1.7	1.9
	Total Galactose		47.8	44.3	50.4
	2.3.5-Arabinose	T-Araf	11.2	12.4	8.6
	2.3.4-Arabinose	T-Arap	1.8	1.7	1.7
	3,4-Arabinose	1→2 Arap	12.6	12.3	12.6
	2.5-Arabinose	1→3 Araf	15.5	16.6	16.1
	3.5-Arabinose	1→2 Araf	7.1	9.8	6.7
	2.3-Arabinose	1→5 Araf	1.3	1.7	1.2
	3-Arabinose	1→2.5 Araf	0.6	0.9	0.5
	Total Arabinose		49.9	55.2	47.3
	2.3.4-Rhamnose	T-Rhap	2.3	0.8	2.4
	Total Rhamnose		2.3	0.8	2.4
	Ratio Terminal Units (TU)/Branched Units (BU)		0.53	0.57	0.47

Table 2. Amino acid composition (% by mass of each amino acid relative to total mass of all amino acids), total amino acid content (mg amino acid·g⁻¹ of sample) (2A) and amino acid families (%) (2B) of *Acacia seyal* gum (Asey) and fractions IEC-F1 and IEC-F2 obtained using Ion Exchange Chromatography (IEC).

2A	Amino Acid (%)	Asey	Fraction IEC-F1	Fraction IEC-F2
	Alanine	3.0	4.0	1.9
	Arginine	1.8	2.8	1.4
	Aspartic acid ^a	6.7	15.3	4.8
	Glutamic Acid ^a	5.2	7.5	3.4
	Glycine	3.3	5.3	2.6
	Histidine	3.6	2.5	5.2
	Hydroxyproline	31.6	5.6	34.3
	Isoleucine	1.6	4.2	0.5
	Leucine	7.8	9.8	7.7
	Lysine	2.0	5.0	1.7
	Phenylalanine	2.5	8.5	2.4
	Proline	6.8	6.6	7.4
	Serine	13.2	7.6	16.3
	Threonine	5.0	4.7	5.3
	Tyrosine	1.8	1.4	1.2
	Valine	4.6	9.0	3.9
	Total amino acid content (mg amino acid·g ⁻¹ of sample)	7.3	74.5	6.2
2B	Amino Acid Families (%)			
	Negatively charged AAs (asp+glu) ^b	11.8	22.8	8.2
	Positively charged AAs (arg+his+lys) ^b	7.3	10.3	8.3
	Hydrophilic or polar AAs (ser+thr+tyr) ^b	20.0	13.7	22.8
	Hydrophobic or non-polar AAs (ala+gly+ile+leu+phe+pro+val) ^b	29.6	47.4	26.4

^aThe acid hydrolysis used in amino acid composition analysis converts asparagine to aspartic acid and glutamine to glutamic acid, or destroys some amino acids such as tryptophan, so these residues are not measured and not reported in the table.

^bAAs: amino acids; asp: aspartic acid; glu: glutamic acid; arg: arginine; his: histidine; lys: lysine; ser: serine; thr: threonine; tyr: tyrosine; ala: alanine; gly: glycine; ile: isoleucine; leu: leucine; phe: phenylalanine; pro: proline; val: valine.

Table 3. Molecular parameters determined by *on line* SEC-MALLS, differential refractometer and viscometer (3A) and thermodynamic parameters (partial specific volume (v_s°) and partial specific adiabatic compressibility (β_s°)) (3B) of *Acacia seyal* gum (*Asey*) and fractions IEC-F1 and IEC-F2 obtained using Ion Exchange Chromatography (IEC).

3A	Molecular parameters	<i>Asey</i>	Fraction IEC-F1	Fraction IEC-F2
				706 707
	M_w (g·mol ⁻¹)	7.8 x 10 ⁵	3.1 x 10 ⁶	8.1 x 10 ⁵
	M_n (g·mol ⁻¹)	4.4 x 10 ⁵	1.2 x 10 ⁶	4.7 x 10 ⁵
	Polydispersity	1.8	2.6	1.709
	Intrinsic viscosity (mL·g ⁻¹)	23.0	35.6	22.2
	R_H (nm)	13.6	27.4	13.4 ⁷¹⁰
	R_g (nm) ^a	16.1	31.6	16.4 ⁷¹¹
3B	Thermodynamic parameters			
				712
	v_s° (cm ³ ·g ⁻¹)	0.577 ^b	0.607	0.582 ⁷¹³
	β_s° (x 10 ¹¹ Pa ⁻¹)	-13.2 ^b	-7.4	-12.4 ⁷¹⁴

^aOnly R_g values above 10 nm were considered, corresponding to about 61% of whole AGPs for IEC-F1 and 53% of whole AGPs for IEC-F2 and *Asey*.

^bValues taken from Mejia Tamayo *et al.* (2018).

Table 4. Basic molecular characteristics of *Acacia seyal* gum (Asey), as well as fractions IEC-F1 and IEC-F2 obtained using Ion Exchange Chromatography (IEC).

	Asey	Fraction IEC-F1	Fraction IEC-F2
M _w (g·mol ⁻¹)	780 000	3 100 000	810 000
Sugars (%) ^a	97.84	91.18	96.79
Proteins (%)	0.73	7.44	0.62
Protein moiety M _w (g·mol ⁻¹)	5 694	230 640	5 022
Average amino acid residue M _w (g·mol ⁻¹)	127.6	129.3	126.4
Number of amino acid residues	45	1 784	40
Non-polar amino acids (%)	25.8	42.1	24.0
Number of interaction sites	0.83	0.64	0.87
Potential number of charged and polar interacting sites (protein moiety)	38	1 149	35
Potential number of non-polar interacting sites (protein moiety)	8	635	5
Polysaccharide moiety M _w (g·mol ⁻¹)	774 306	2 869 360	804 978
Average sugar residue M _w (g·mol ⁻¹)	167.8	166.0	166.3
Number of sugar residues	4 614	17 283	4 840
Average total free OH/residue	2.55	2.71	2.58
Potential number of charged and polar interacting sites (polysaccharide moiety)	11 767	46 885	12 475

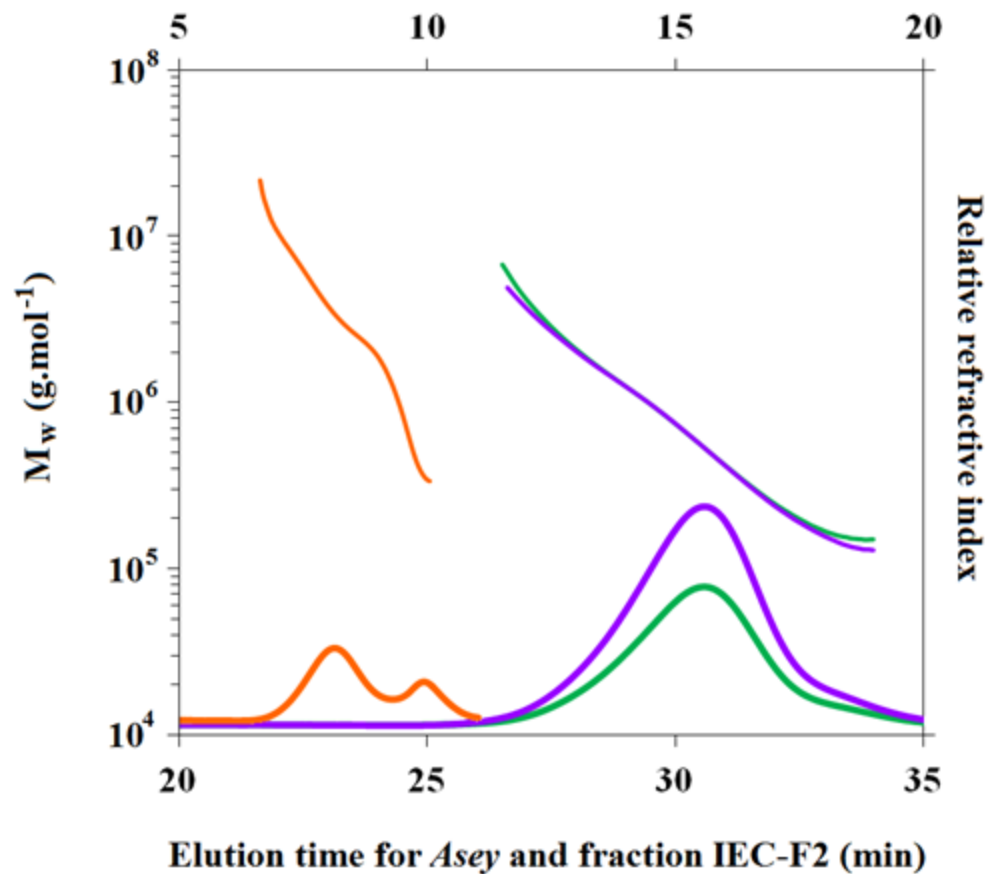
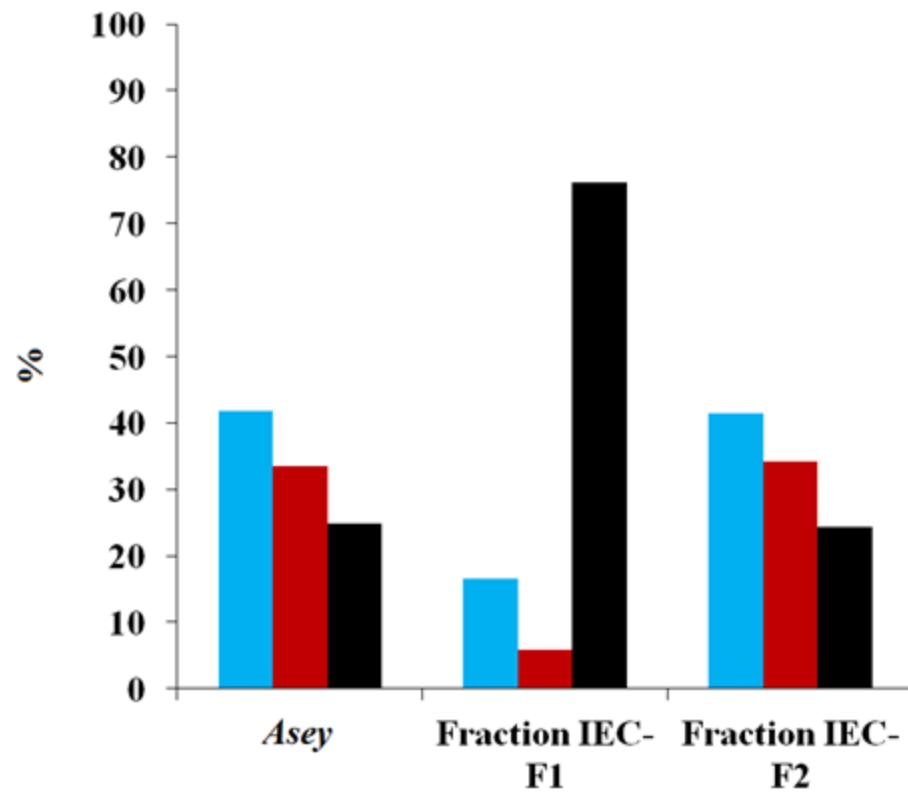
^aSugars percentage was determined by the difference of proteins and minerals from 100% of sample.

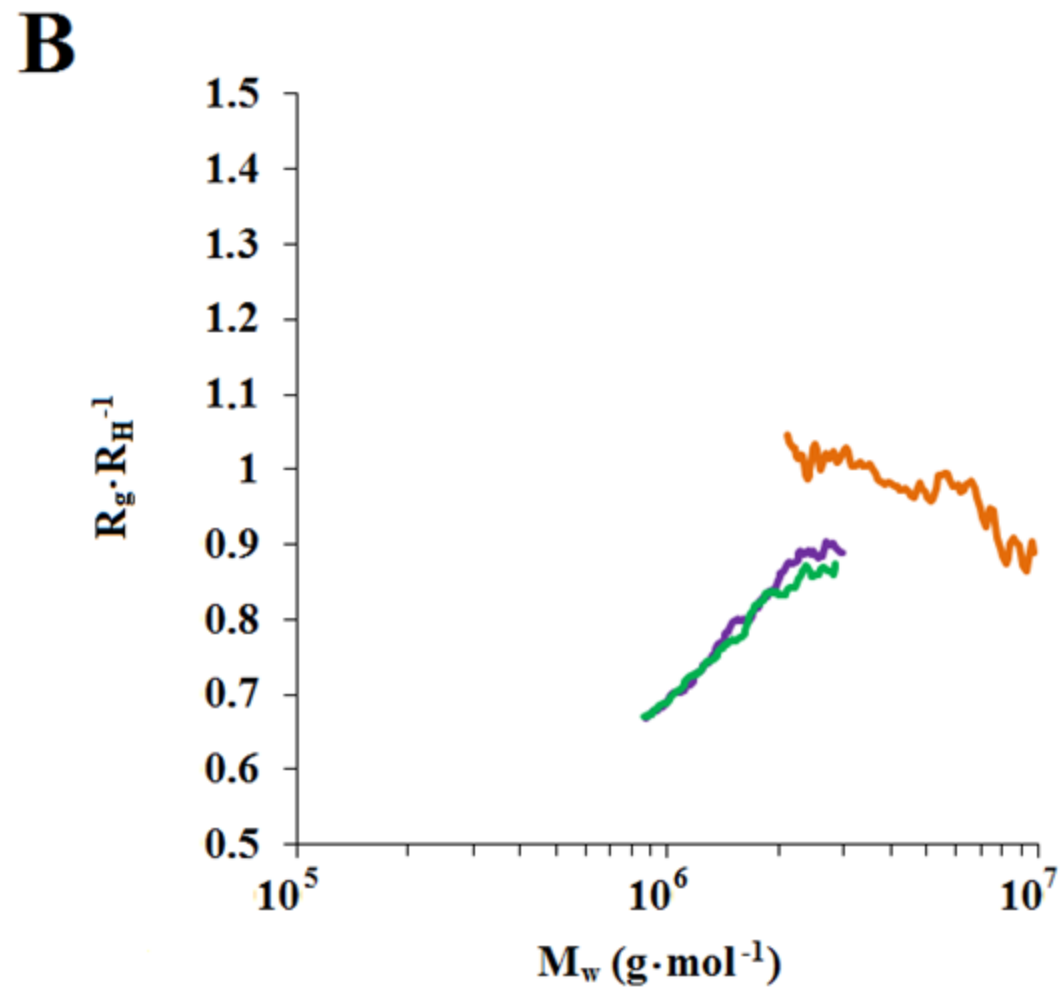
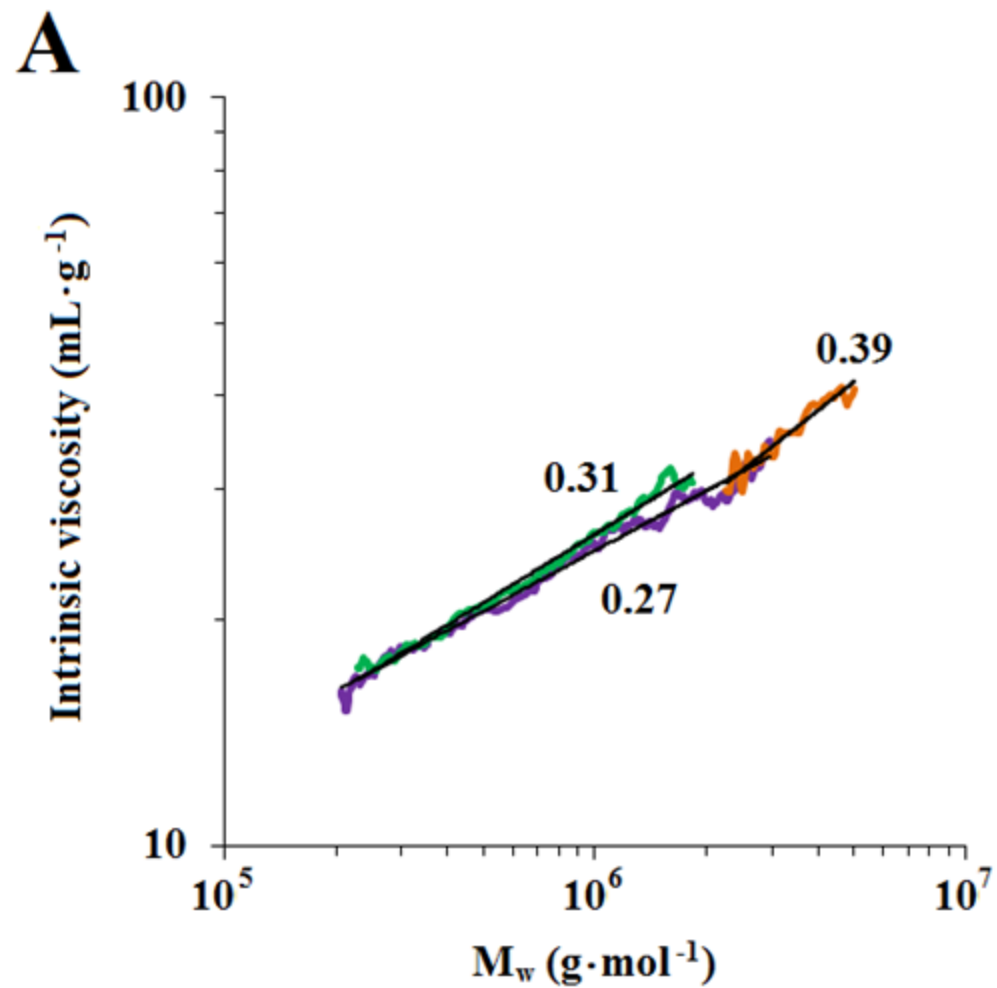
Fig. 1. Size exclusion chromatograms showing the elution profiles monitored by refractometer: relative refractive index (thick line) and molar mass (M_w ; $\text{g}\cdot\text{mol}^{-1}$; thin line) of *Acacia seyal* gum (Asey, —) as well as of fractions IEC-F1 (—) and IEC-F2 (—) (Fig. A). Cumulative percentage ranges (%) for molar masses of *Acacia seyal* gum (Asey) and fractions IEC-F1 and IEC-F2; range 1 (■): molar mass below $5 \times 10^5 \text{ g}\cdot\text{mol}^{-1}$, range 2 (■): molar mass between 5×10^5 and $1 \times 10^6 \text{ g}\cdot\text{mol}^{-1}$ and range 3 (■): molar mass above $1 \times 10^6 \text{ g}\cdot\text{mol}^{-1}$ (Fig. B).

Fig. 2. A: Mark-Houwink-Sakurada plot showing the intrinsic viscosity ($[\eta]$; $\text{mL}\cdot\text{g}^{-1}$) as a function of molar mass (M_w ; $\text{g}\cdot\text{mol}^{-1}$) for *Acacia seyal* gum (Asey, —) as well as of fractions IEC-F1 (—) and IEC-F2 (—). Only intrinsic viscosity values corresponding to about 87% of macromolecules from Asey, 52% of macromolecules from IEC-F1 and 87% of macromolecules from IEC-F2 were considered. **B:** $R_g\cdot R_H^{-1}$ (ρ) ratio as function of molar mass (M_w ; $\text{g}\cdot\text{mol}^{-1}$) of *Acacia seyal* gum (Asey, —) and fractions IEC-F1 (—) and IEC-F2 (—). Only R_g values above 10 nm were considered to calculate $R_g\cdot R_H^{-1}$, corresponding to about 53% of macromolecules from Asey, 61% of macromolecules from IEC-F1 and 53% of macromolecules from IEC-F2.

A

Elution time for fraction IEC-F1 (min)

**B**





**Fraction
IEC-F1**

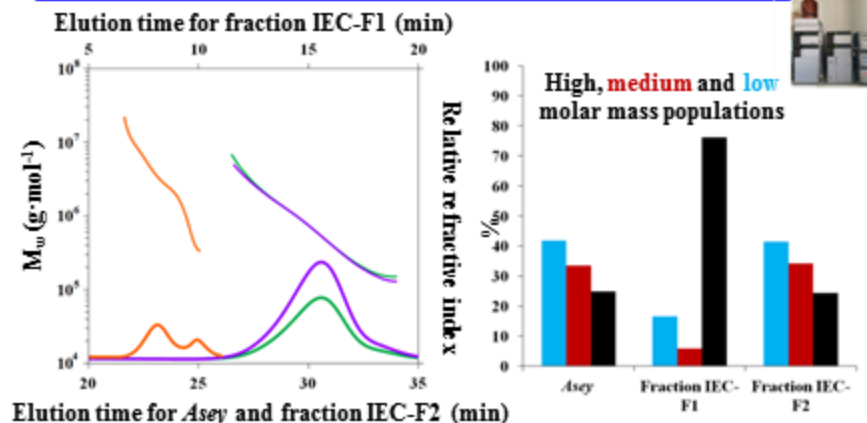


**Fraction
IEC-F2**

Characterization

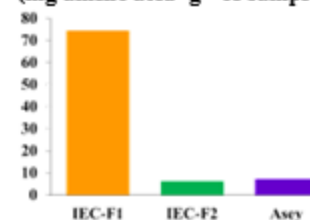
**Ionic
Exchange
Chromatography**

SEC MALLS CHROMATOGRAMS AND M_w DISTRIBUTION

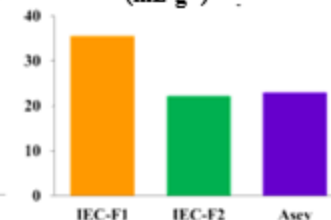


TOTAL AMINO ACID CONTENT AND INTRINSIC VISCOSITY

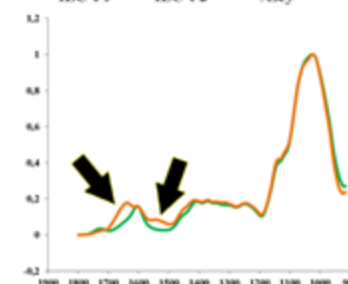
**Total amino acid content
(mg amino acid · g⁻¹ of sample)**



**Intrinsic viscosity
(mL·g⁻¹)**



FTIR



YARIV DETECTION

