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1 **Fractionation of *Acacia seyal* gum by ion exchange chromatography**

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25 **ABSTRACT**

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

43

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

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49 **1. Introduction**

50 *Acacia* gum is a complex polysaccharide-based plant gum exudate, obtained from trees of
51 selected *Acacia* species (i.e. *Acacia senegal* and *Acacia seyal*), whom exude this substance to
52 protect trees against external attacks. Much work can be found in literature about several
53 aspects of *Acacia senegal* gum (*Asen*), such as its composition (Islam, Phillips, Sljivo,
54 Snowden, & Williams, 1997; Idris, Williams, & Phillips, 1998; Verbeken, Dierckx, &
55 Dewettink, 2003), polydispersity, structure (Fincher, Stone, & Clarke, 1983; Idris et al.,
56 1998; Renard, Lavenant-Gourgeon, Ralet, & Sanchez, 2006; Sanchez et al., 2008), physico-
57 chemical properties (Phillips, Takigami, & Takigami, 1996; Renard et al., 2006; Mejia
58 Tamayo et al., 2018; Buffo, Reineccius, & Oehlert, 2001; Al-Assaf, Phillips, Aoki, & Sasaki,
59 2007; Dickinson, 2008). *Asen* has been much more studied over years since it largely
60 dominated the *Acacia* gum commerce with a market share of about 70%. However *Acacia*
61 *seyal* gum (*Asey*) has been gaining interest and importance in recent years in the *Acacia* gum
62 trade (Rahim, van Ierland, & and Weikard, 2010). *Acacia* gums have been largely used for
63 different purposes in all human history (Sanchez et al., 2018). Currently, this natural
64 substance is employed in a variety of applications such as food, pharmaceutical and other
65 industrial applications (Verbeken et al., 2003). *Asey* is constituted by about 37-44% of
66 galactose, 33-48% of arabinose, 2-3% of rhamnose, 7-13% of glucuronic acid, 6% of 4-*O*
67 methyl glucuronic acid, 1% of protein, and 4% of minerals (Gashua, Williams, Yadav, &
68 Baldwin, 2015; Lopez-Torrez, Nigen, Williams, Doco, & Sanchez, 2015). The
69 arabinogalactan moiety of *Asey* is formed by a core of β -1,3 linked galactose units with
70 branches linked through the 6 position consisting of galactose and arabinose terminated by
71 rhamnose and glucuronic acids (Al-Assaf, Phillips, & William, 2005; Siddig, Osman, Al-
72 Assaf, Phillips, & Williams, 2005; Street & Anderson, 1983). The minor proteinaceous

73 material is covalently attached to this arabinogalactan moiety (Mahendran, Williams,
74 Phillips, Al-Assaf, & Baldwin, 2008; Siddig et al., 2005). Besides, *Asey* macromolecules
75 present a lower hydrodynamic volume than those from *Asen*, that can be interpreted as a
76 more compact conformation (Al-Assaf et al., 2005; Flindt, Al-Assaf, Phillips, & Williams,
77 2005; Elmanan, Al-Assaf, Phillips, & Williams, 2008; Lopez-Torrez et al., 2015).

78 *Asen* has been demonstrated as a continuum of macromolecules which shows varying
79 protein/sugar ratios, molar masses and charge density (Siddig et al., 2005; Renard et al.,
80 2006). Consequently, the fractionation of *Asen* was done by different methods such as the
81 anion-exchange chromatography (Osman et al., 1995), the size exclusion chromatography
82 (SEC) (Ray, Bird, Iacobucci, & Clark 1995) or the hydrophobic interaction chromatography
83 (HIC), which was the most used fractionation procedure (Randall, Phillips, & Williams,
84 1989; Osman, Menzies, Williams, Phillips, & Baldwin, 1993; Ray et al., 1995; Renard et al.,
85 2006). Using HIC, three main AGP fractions can be obtained from *Asen*: (i) a dominant
86 fraction in percentage with low protein content and low mean molar mass (HIC-F1), (ii) a
87 fraction presenting a high molar mass and rich in protein (HIC-F2) and, finally, (iii) a
88 fraction showing high molar mass and the highest protein content (HIC-F3) (Renard et al.,
89 2006). In a previous work, we presented the successful fractionation of *Asen* by Ionic
90 Exchange Chromatography (IEC) from which two fractions were obtained: one protein-poor
91 fraction with low molar mass and one protein-rich fraction with high-molar mass showing a
92 great tendency to aggregate (Apolinar-Valiente et al., 2019). In comparison with *Asen*,
93 fractionation of *Asey* has been notably less reported. Siddig et al. (2005) undertook both GPC
94 and HIC to compare gums from *Asey* and *Asen* varieties. Using HIC fractionation, they found
95 for *Asey* practically the same class of fractions than those found for *Asen*. On the other hand,
96 they found a fourth fraction with low molar mass and rich in protein when *Asey* was

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

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

111 *Asey* has been therefore fractionated by IEC through the use of DEAE Sephacel gel as
112 stationary phase. Two main fractions were recovered, one of which was rich in protein with
113 very high molar mass. These fractions were subsequently characterized in terms of
114 biochemical composition (amino acid and sugar residues), structure (weight-average molar
115 masses M_w , number-average molar mass M_n , polydispersity index M_w/M_n , intrinsic viscosity
116 $[\eta]$) and volumetric properties (partial specific volume v_s° and partial specific adiabatic
117 compressibility β_s° coefficients and hydrodynamic volume from intrinsic viscosity).

118

119 **2. Material and Methods**

120

121 *2.1. Material*

122

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

125

126 *2.2. Preparation of Acacia seyal gum (Asey) dispersions*

127

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

132

133 *2.3. Ion exchange chromatography (IEC)*

134

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

144

145 *2.4. Yariv detection*

146

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

154

155 *2.5. Amino acid analysis*

156

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

160

161 *2.6. Sugar composition as trimethylsilyl derivatives*

162

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

166

167 *2.7. Glycosyl-linkage compositions.*

168

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

172

173 *2.8. Molar mass distribution and intrinsic viscosity.*

174

175 Fractions IEC-F1 and IEC-F2 from *Asey* were analysed by size exclusion chromatography
176 (SEC) as described by Apolinar-Valiente et al. (2019). The SEC line was constituted,
177 depending on the analysis, by an OHPAK SB-G guard pre- column followed by four columns
178 (OHPAK SB 803, 804, 805 and 806 HQ, Shodex) for *Asey* and fraction IEC-F2 and by an
179 OHPAK SB-G guard pre- column followed by one column (OHPAK SB 805 HQ, Shodex)
180 for fraction IEC-F1. In the latter, only one Shodex column was used in order to decrease
181 anomalous elution of high M_w hyperbranched macromolecules. The AGPs were eluted with
182 filtered (0.1 μm filter, Millipore) solution (0.1 M LiNO_3 + 0.02% NaN_3) at a flow rate of 1
183 $\text{mL}\cdot\text{min}^{-1}$ and 30°C. The samples were dissolved in filtered Milli-Q water (1 $\text{mg}\cdot\text{mL}^{-1}$),
184 stirred gently (24 h) and centrifuged (10 000 rpm, 10 min). Subsequently, 75 μL of IEC-F2
185 fraction and *Asey* and 10 μL IEC-F1 fraction were injected. Intrinsic viscosity was
186 determined using an online viscometer (Viscostar II Wyatt, Santa Barbara, CA, USA) and a
187 differential refractometer (Optilab T-Rex, Wyatt, Santa Barbara, CA, USA).. The refractive
188 index increment values (dn/dc) were determined as 0.155, 0.158 and 0.155 $\text{mL}\cdot\text{g}^{-1}$ for *Asey*,
189 IEC-F1 and IEC-F2, respectively.

190

191 *2.9. Partial specific volume and partial specific adiabatic compressibility*

192

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

201

202 *2.10. FTIR spectral acquisition.*

203 Spectral acquisitions were performed on dry gum samples using a Vertex 70V Fourier-
204 transform mid-spectrometer equipped with an ATR Diamond cell (SPECAC, Smyrna,
205 GA, USA). Each recorded spectrum was the average of 128 repetitions from 900 to
206 1800 cm^{-1} with a 4 cm^{-1} spectral resolution. A micrometric screw applying constant
207 pressure ensured good contact between the sample and the crystal. The spectra were
208 acquired and analysed using OPUS Software version 7.5. A sample-less spectrum was
209 recorded between two polysaccharide fractions to monitor the stability of the
210 background. Each spectrum was subjected to linear standardisation at absorbance values
211 from 0 to 1 for the respective wave numbers of 1800 and 1025 cm^{-1} for proper spectra
212 exposure and interpretation.

213

214 **3. Results and discussion**

215

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

221

222 *3.1. Fractionation of Asey by IEC: yield of the recuperation*

223

224 The IEC fractionation procedure allowed recovering two fractions, IEC-F1 and IEC-F2,
225 which correspond respectively to 1.4% and 82.6% of the initial gum. The losses of material
226 can occur during the fractionation, concentration, diafiltration and spray-drying steps;
227 nevertheless, we consider that the total yield (84.0% of initial gum) was satisfactory.

228 Supplementary data (Fig. 1) shows the precipitation of both fractions IEC-F1 and IEC-F2 by
229 Yariv’s reagent, corroborating that they belong to AGP family. Fractions from *Acacia*
230 *senegal* gum obtained by different fractionation techniques, such as preparative GPC (Qi,
231 Fong, & Lamport, 1991), HIC (Osman et al., 1993) or IEC (Apolinar-Valiente et al., 2018),
232 were also found to interact with Yariv reagent.

233

234 *3.2. Fractions IEC-F1 and IEC-F2: composition and structure*

235

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

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

240

241 3.2.1. *Sugar composition*

242

243 Table 1A presents the neutral sugar and uronic acid composition of *Asey*. We found high
244 content of arabinose (48.5%) and galactose (34.2%), whereas rhamnose only represents
245 3.2%. Concerning the uronic acids, glucuronic acid and 4-O-methyl glucuronic acid show
246 values of 7.7% and 6.4%, respectively. This sugar composition corresponds with data largely
247 reported in literature (Biswas, DeVido, & Dorsey, 2003; Flindt et al., 2005; Siddig et al.,
248 2005; Andres-Brull et al., 2015; Lopez-Torrez et al., 2015; Gashua, Williams, & Baldwin,
249 2016). When characteristic ratio Ara/Gal of *Asey* was calculated, it presents a value of 1.4,
250 which is in general also coherent with values found in literature (Siddig et al., 2005; Andres-
251 Brull et al., 2015; Lopez-Torrez et al., 2015). It can be noted that this ratio could notably vary
252 depending on the origin of the *Asey* gum (Biswas et al., 2003; Flindt et al., 2005).

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

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

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

$$276 \quad TU = \frac{2.3.4.Rha}{0.69} + \frac{2.3.5.Ara}{0.60} + \frac{2.3.4.Ara}{0.61} + \frac{2.3.4.6.Gal}{0.70}$$

277 We have calculated the branched units (BU) using:

$$278 \quad BU = \frac{2.6.Gal}{0.79} + \frac{2.4.Gal}{0.80} + 2x \frac{2.Gal}{0.84} + \frac{3.Ara}{0.70}$$

279 The coefficients used in these two formulas were reported by Sweet, Albersheim and Shapiro
 280 (1975). The TU/BU ratio is higher in fraction IEC-F1 (0.57) compared fraction IEC-F2
 281 (0.47). The highest TU/BU ratio of fraction IEC-F1 could suggest a less branched structure
 282 of the carbohydrate moiety. The data given in Table 1B enables us to calculate the content of
 283 free OH in the polysaccharide portion, as shown below in section 3.2.6.

284

285 3.2.2. Amino acid composition

286

287 Table 2A shows the total amino acid content and amino acid composition of *Asey* and the
288 two IEC fractions. The total content of amino acids for *Asey* is $7 \text{ mg}\cdot\text{g}^{-1}$ of sample, which is
289 in agreement with those obtained by other authors (Elmanan et al., 2008; Lopez-Torrez et al.,
290 2015; Gashua et al., 2016). Regarding the amino acid composition of *Asey*, hydroxyproline is
291 by far the major amino acid, followed by serine, and in less amount leucine, aspartic acid,
292 proline and threonine, as previously presented in literature (Osman et al., 1993; Flindt et al.,
293 2005; Siddig et al., 2005; Lopez-Torrez et al., 2015). Minor amino acids were isoleucine,
294 lysine, arginine and tyrosine (Table 2A).

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

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

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

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

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

326 Regarding the total content of amino acids, fraction IEC-F1 shows clearly the highest value
327 (74 mg amino acid·g⁻¹ of sample), matching with our previous results about IEC-
328 fractionation of *Asen* (Apolinar-Valiente et al., 2019). Literature shows varying protein
329 contents between fractions when *Asey* gum was fractionated by HIC (Siddig et al., 2005) or
330 by GPC (Flindt et al., 2005). Our results are corroborated by the infrared spectra of fractions
331 IEC-F1 and IEC-F2 (Supplementary Figure 2). The absorbance is clearly higher at 1650 and
332 at 1545 cm⁻¹ for fraction IEC-F1, indicating the emergence of Amide I and II vibration bands

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

339

340 3.2.3. Size exclusion chromatograms and M_w distribution

341

342 *Asey* chromatogram (Figure 1A) shows one main peak between 26 and 33 min elution time
343 (RI signal). This profile, together with the obtained M_w distribution as a function of the
344 elution time (Figure 1A), is in coherence with those previously reported by several authors
345 (Gashua et al., 2015; Lopez-Torrez et al., 2015). Figure 1B exhibits the molar mass
346 distribution analysis of *Asey*. It was divided into three selected ranges in order to estimate the
347 relative percentage in small, medium and large molar masses: range 1 (below $5 \times 10^5 \text{ g}\cdot\text{mol}^{-1}$)
348 1), range 2 (between 5×10^5 and $1 \times 10^6 \text{ g}\cdot\text{mol}^{-1}$) and range 3 (above $1 \times 10^6 \text{ g}\cdot\text{mol}^{-1}$). Thus,
349 we have obtained higher values for range 1 (42%) and 2 (34%) in comparison with the range
350 3 (24%).

351 The chromatogram of fraction IEC-F1 presents one major peak with elution time values (RI
352 signal) ranging from 6.4 to 8.9 min (Figure 1A). This population presents only high M_w
353 AGPs (upper than $10^6 \text{ g}\cdot\text{mol}^{-1}$). A second lower but well-defined peak can be deduced
354 between 8.9 and 11 min, showing AGPs with low M_w (below than $10^6 \text{ g}\cdot\text{mol}^{-1}$).

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

357 enables to obtain high M_w AGP from *Asey*, corresponding to the fraction IEC-F1. However,
358 IEC separation seems not as good for *Asey* as we previously found for *Asen* (Apolinar-
359 Valiente et al., 2019), where we reported 96% of fraction IEC-F1 in the range 3. One likely
360 explanation could be the higher starting percentage of macromolecules from range 3 ($>10^6$
361 $\text{g}\cdot\text{mol}^{-1}$) in the case of *Asey* (24%) in comparison to *Asen* (15%, from Apolinar-Valiente et
362 al., 2019). This high number of macromolecules would result in a steric hindrance which
363 should hinder that macromolecules from ranges 1 and 2 should be fixed to the gel (Osman et
364 al., 1995). In such a way, these macromolecules from ranges 1 and 2 would pass easily
365 through the column and would be eluted as an integral part of fraction IEC-F1. Therefore, the
366 hydrodynamic volume of the macromolecules contributes in the efficiency of IEC separation
367 method. Another explanation, not in conflict with the previous account, could be the higher
368 negative attractive potential interaction in *Asen* compared to *Asey* due to the charged residues
369 content (Lopez-Torrez et al., 2015). This behaviour would explain the lower separation for
370 *Asey* compared to our previous work (Apolinar-Valiente et al., 2019).

371

372 3.2.4. Static and dynamic molecular parameters

373

374 The static and dynamic parameters of *Asey* and fractions IEC-F1 and IEC-F2 are given in
375 Table 3A. *Asey* shows values of $7.8 \times 10^5 \text{ g}\cdot\text{mol}^{-1}$ for M_w and $4.4 \times 10^5 \text{ g}\cdot\text{mol}^{-1}$ for M_n . These
376 values are close to those found in literature (Flindt et al., 2005; Elmanan et al., 2008; Lopez-
377 Torrez et al., 2015; Gashua et al., 2016).

378 Fraction IEC-F2 displays weight-average molar mass (M_w) and number-average molar mass
379 (M_n) of $8.1 \times 10^5 \text{ g}\cdot\text{mol}^{-1}$ and $4.7 \times 10^5 \text{ g}\cdot\text{mol}^{-1}$, respectively. In contrast, fraction IEC-F1
380 presents much higher values for M_w ($3.1 \times 10^6 \text{ g}\cdot\text{mol}^{-1}$) and M_n ($1.3 \times 10^6 \text{ g}\cdot\text{mol}^{-1}$). Flindt et

381 al. (2005) and Siddig et al. (2005) also detected differences in M_n and M_w between fractions
382 when *Asey* was separated, respectively, by GPC and by HIC. However, IEC technique allows
383 to obtain a fraction whose M_w is largely greater than the starting *Asey* gum. As mentioned
384 earlier, Siddig et al. (2005) obtained two high protein-content fractions by HIC: the classical
385 fractions HIC-F2 and HIC-F3. These fractions display, respectively, M_w values smaller (–
386 3.8%) and larger (+17%) than the M_w value of starting *Asey* gum. On the other hand, we
387 obtain by IEC a fraction (IEC-F1) whose M_w represents an increase of 283% with regard to
388 the starting *Asey* gum M_w . Concerning the polydispersity data (M_w/M_n), *Asey* presents a
389 value of 1.8, which is also close to values reported by other authors (Lopez-Torrez et al.,
390 2015; Gashua et al., 2016). Fraction IEC-F2 presents similar polydispersity (1.7) than *Asey*
391 (1.8), whereas fraction IEC-F1 shows the highest value for this parameter (2.6).

392 *Asey* gum presents a larger intrinsic viscosity ($[\eta]$) value ($23 \text{ mL}\cdot\text{g}^{-1}$) than those reported
393 previously ($12\text{-}18 \text{ mL}\cdot\text{g}^{-1}$) (Flindt et al., 2005; Elmanan et al., 2008; Lopez-Torrez et al.,
394 2015). These variations may be due to differences in the age of trees (Idris et al., 1998)
395 and/or the origin of the gum (Gashua et al., 2015). Fraction IEC-F2 shows similar values (22
396 $\text{mL}\cdot\text{g}^{-1}$) to *Asey* gum, whereas, as expected, the highest intrinsic viscosity ($[\eta]$) is found for
397 fraction IEC-F1 ($36 \text{ mL}\cdot\text{g}^{-1}$). This parameter in *Acacia* gum has been suggested as strongly
398 linked to the molar mass as well as to the protein content (Chikamai, Banks, Anderson, &
399 Weiping, 1996). In our case, the $[\eta]$ value (Table 3A) and the amino acid total content (Table
400 2A) of fraction IEC-F1 are in perfect coherence with these two remarks, which can be
401 explained by the fact that protein-rich macromolecules in *Acacia* gum display generally high
402 M_w .

403 The average sphere-equivalent hydrodynamic radius (R_H) of *Asey* gum (13.6 nm , Table 3A)
404 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

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

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

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

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

439

440 3.2.5. Thermodynamic volumetric parameters

441

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

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

462 Table 3B gives v_s° and β_s° of the fractions IEC-F1 and IEC-F2. Greater values of v_s° and β_s°
463 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
464 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*
465 ($0.577 \text{ cm}^3 \cdot \text{g}^{-1}$ and $-13.2 \times 10^{-11} \text{ cm}^3 \cdot \text{g}^{-1}$, respectively). Therefore, it can be concluded that the
466 fraction IEC-F1 displays a more compressible and less hydrated structure than the IEC-F2
467 fraction. Moreover, fraction IEC-F1 from *Asey* presents less negative β_s° value than the
468 similar fraction obtained from *Asen* (-7.4×10^{-11} vs $-9.4 \times 10^{-11} \text{ Pa}^{-1}$) (Apolinar-Valiente et al.
469 (2019), indicating less hydration of the former. On the other hand, the same compressibility
470 values were found for IEC-F2 fraction coming from the two gums. It can be noted that a link
471 between protein interfacial properties and their molecular compressibility was clearly
472 demonstrated with globular proteins (Gekko & Yamagami, 1991; Damodaran, 2008). It can
473 then be assumed that fraction IEC-F1 from *Asey* would present better interfacial properties as
474 compared to unfractionated *Asey*. All these results are in agreement with the data of IEC
475 fractions from *Asen* previously reported by Apolinar-Valiente et al. (2019).

476

477 3.2.6. Basic molecular characteristics

478

479 Using several biochemical and structural properties, we have estimated the contribution of
480 the polysaccharide and protein moiety of IEC fractions to basic molecular properties (Table
481 4). By means of the sugar (Table 1) and amino acid (Table 2) composition, we have
482 calculated the average M_w of the polysaccharide and protein part. The content of free OH in
483 the polysaccharide portion was estimated by taking into account the linkage of the neutral
484 sugars (Table 1B) and the number of possible interacting sites of the protein portion
485 Observing the protein moiety, the fraction IEC-F1 shows a much greater number of potential
486 charged and polar interacting sites (~1 200) as compared to fraction IEC-F2 (35). Their
487 corresponding content of amino acid residues (~1 800 and 40, respectively) explains this
488 behavior. Concerning the polysaccharide part, the number of charges contributed by fraction
489 IEC-F1 also presents much higher values (~47 000) than those from fraction IEC-F2 (~12
490 000), which is explained by their corresponding content of sugar residues (~17 000 and ~5
491 000, respectively).

492

493 4. Conclusions

494

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

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

516

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523

524 **Compliance with ethical standards**

525 **Conflict of interest**

526 The authors declare that they have no conflict of interest.

527 **Compliance with ethics requirements**

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

529

530 **References**

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685 Verbeken, D., Dierckx, S., & Dewettinck, K. (2003). Exudate gums: Occurrence,
686 production, and applications. *Applied Microbiology and Biotechnology*, 63(1), 10–21.

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

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

2A	Amino Acid (%)	<i>Asey</i>	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

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

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

701

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

3A	Molecular parameters	<i>Asey</i>	Fraction IEC-F1	Fraction IEC-F2
	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	1.7
	Intrinsic viscosity (mL·g ⁻¹)	23.0	35.6	22.2
	R_H (nm)	13.6	27.4	13.4
	R_g (nm) ^a	16.1	31.6	16.4
3B	Thermodynamic parameters			
	v_s° (cm ³ ·g ⁻¹)	0.577 ^b	0.607	0.582
	β_s° (x 10 ¹¹ Pa ⁻¹)	-13.2 ^b	-7.4	-12.4

715

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

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

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

	<i>Asey</i>	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

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

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731

732 **Fig. 1.** Size exclusion chromatograms showing the elution profiles monitored by
733 refractometer: relative refractive index (thick line) and molar mass (M_w ; $\text{g}\cdot\text{mol}^{-1}$; thin line) of
734 *Acacia seyal* gum (*Asey*, —) as well as of fractions IEC-F1 (—) and IEC-F2 (—) (Fig. A).
735 Cumulative percentage ranges (%) for molar masses of *Acacia seyal* gum (*Asey*) and
736 fractions IEC-F1 and IEC-F2; range 1 (■): molar mass below $5 \times 10^5 \text{ g}\cdot\text{mol}^{-1}$, range 2 (■):
737 molar mass between 5×10^5 and $1 \times 10^6 \text{ g}\cdot\text{mol}^{-1}$ and range 3 (■): molar mass above 1×10^6
738 $\text{g}\cdot\text{mol}^{-1}$ (Fig. B).

739

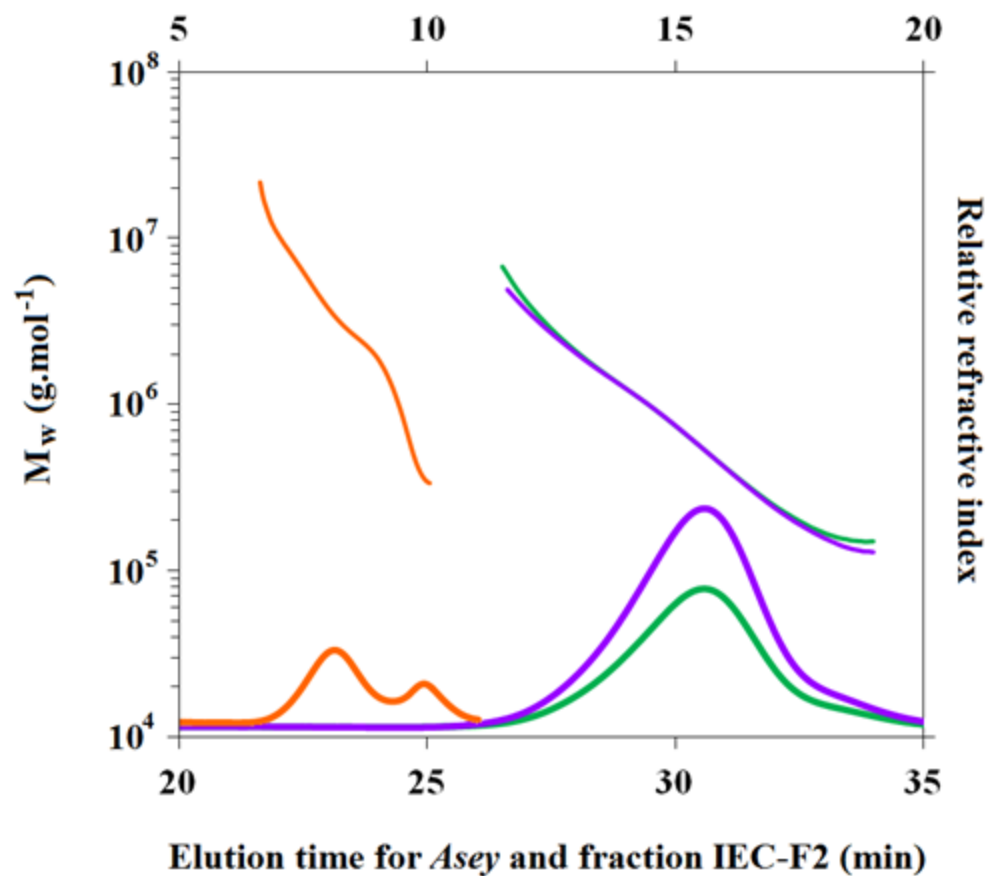
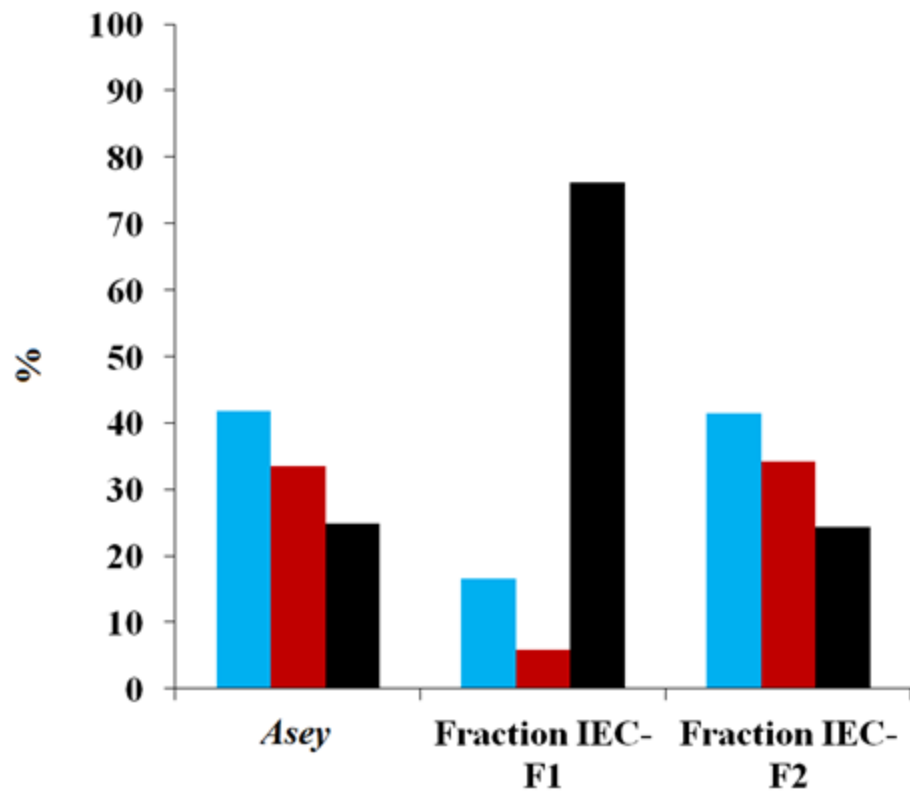
740 **Fig. 2. A:** Mark-Houwink-Sakurada plot showing the intrinsic viscosity ($[\eta]$; $\text{mL}\cdot\text{g}^{-1}$) as a
741 function of molar mass (M_w ; $\text{g}\cdot\text{mol}^{-1}$) for *Acacia seyal* gum (*Asey*, —) as well as of fractions
742 IEC-F1 (—) and IEC-F2 (—). Only intrinsic viscosity values corresponding to about 87% of
743 macromolecules from *Asey*, 52% of macromolecules from IEC-F1 and 87% of macromolecules from
744 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
745 *Acacia seyal* gum (*Asey*, —) and fractions IEC-F1 (—) and IEC-F2 (—). Only R_g values
746 above 10 nm were considered to calculate $R_g\cdot R_H^{-1}$, corresponding to about 53% of
747 macromolecules from *Asey*, 61% of macromolecules from IEC-F1 and 53% of
748 macromolecules from IEC-F2.

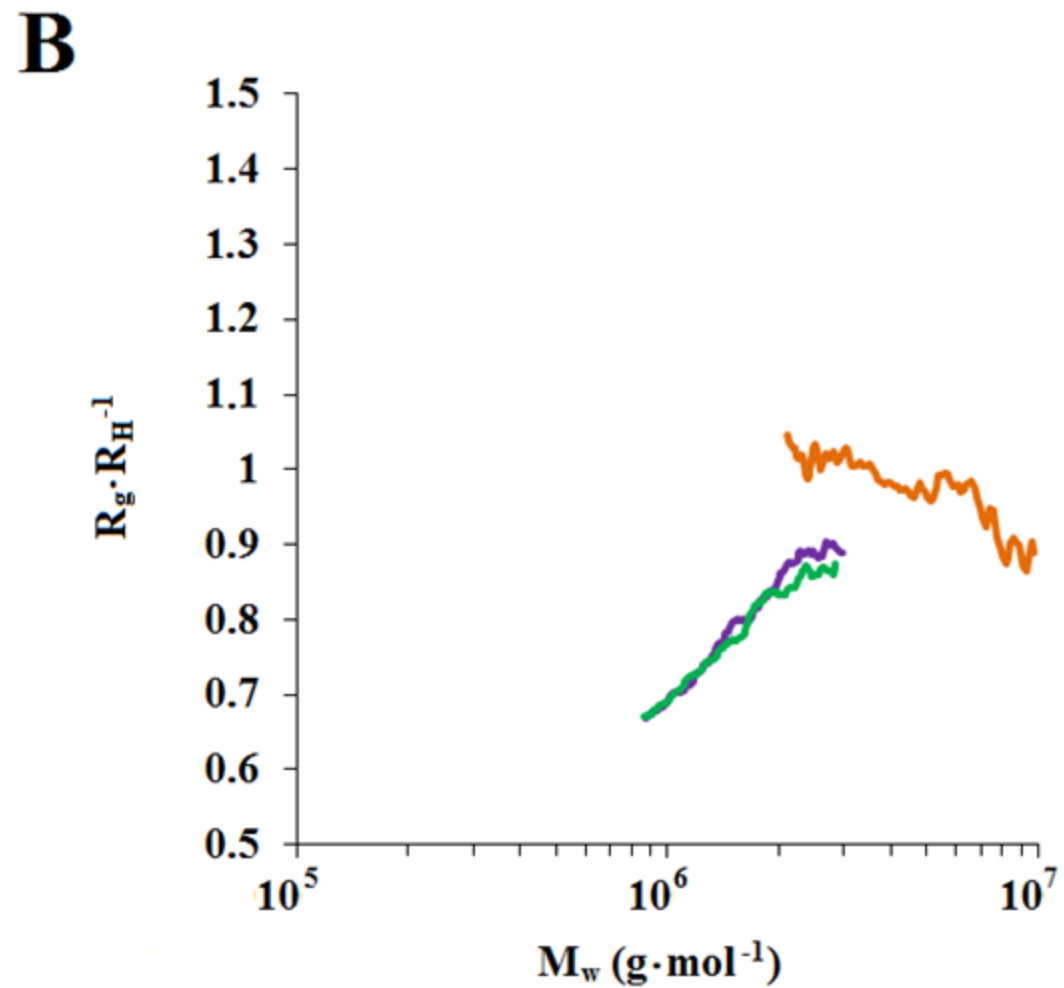
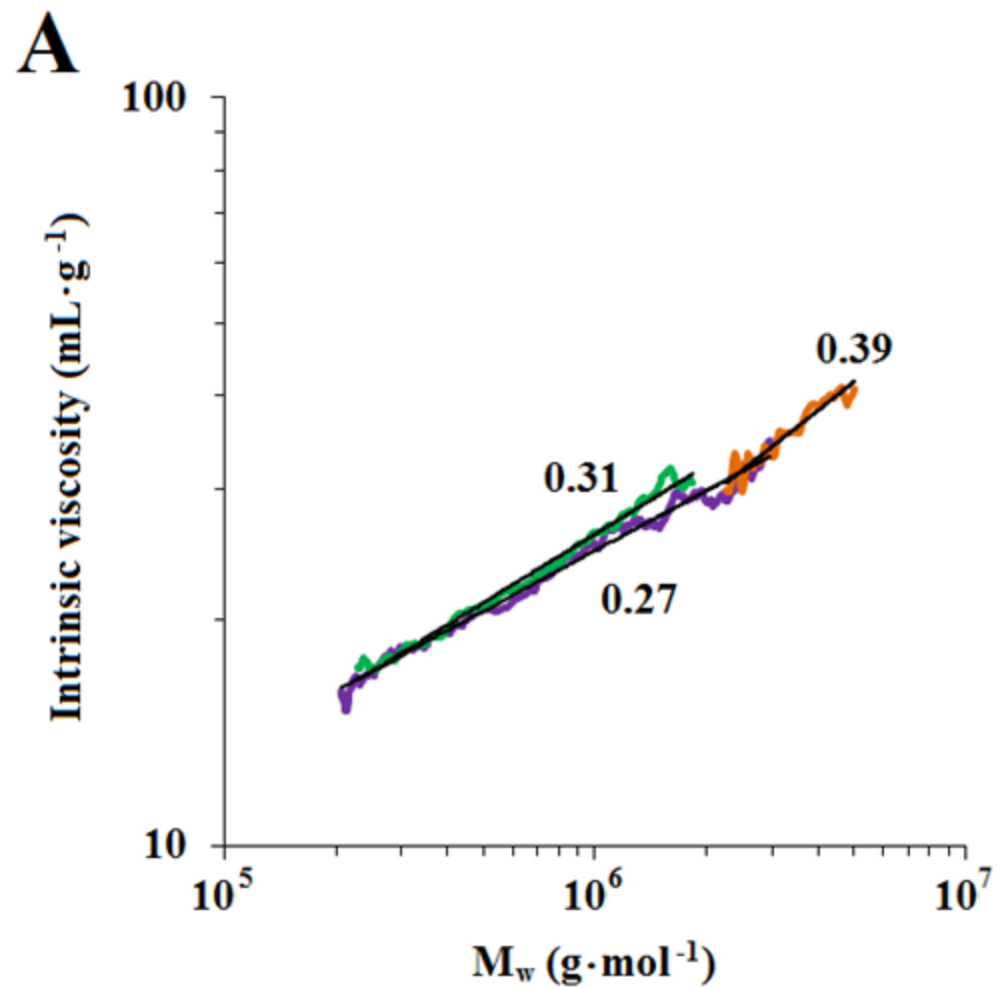
749

750

A

Elution time for fraction IEC-F1 (min)

**B**





Acacia seyal gum (Asey)

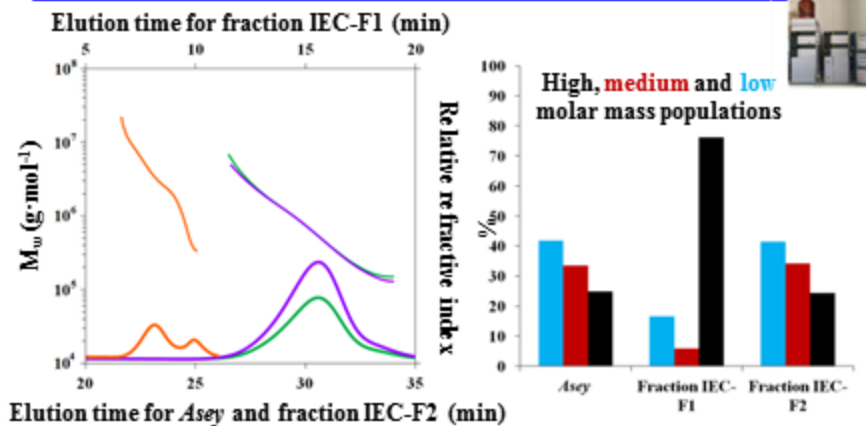


Ionic Exchange Chromatography

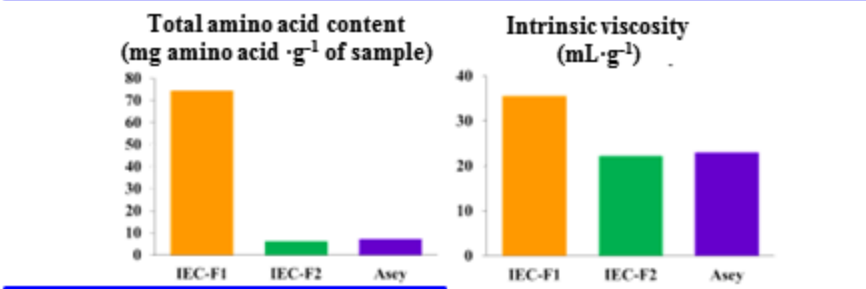
Fraction IEC-F1

Fraction IEC-F2

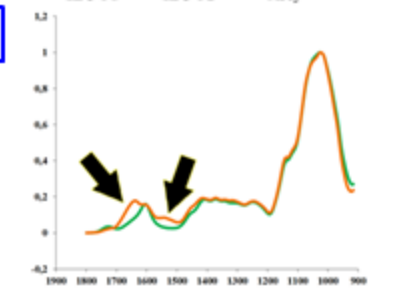
Characterization



TOTAL AMINO ACID CONTENT AND INTRINSIC VISCOSITY



FTIR



YARIV DETECTION

