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1 **Relationship of molecular characteristics of acacia gum to their functional**  
2 **properties: PLS analysis combined with VIP scores on SEC-MALLS**

3

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16

17

18 **Abstract:**

19 The study objective was to rank molecular parameters that impact rheological qualities of  
20 Acacia gum (*Acacia senegal*) using partial least squares regression (PLS-R). 47 samples were  
21 analyzed across 46 molecular descriptors, while viscosity, emulsifying property (EP) and  
22 emulsion stabilization (ES) were response variables. PLS model accounted for 92, 76 and 77%  
23 of the variability in viscosity, EP and ES, respectively. PLS-R and determination of very  
24 important parameters (VIP) scores have allowed identification and ranking of molecular  
25 parameters: 9, 29 and 27 variables contributed to, while 4, 13 and 15 were considered as the  
26 most critical factors for viscosity, EP and ES variability, respectively. Molar mass distribution  
27 (but not proportion) of arabinogalactan protein complex had the strongest contribution to all  
28 three response variables. This study highlighted that EP and ES were more complex properties  
29 and involved a larger number of molecular characteristics compared to viscosity, which might  
30 be easier to control.

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34 **Keywords:** Acacia gum, Emulsion, AGP, Molecular distribution, molecular variables, PLS-R,  
35 VIP.

36

37 **1. Introduction:**

38 Acacia gum, known as gum Arabic (GA), is a hydrocolloid complex, polysaccharide-based gum  
39 exudate. The gum is secreted by the trunk, branches or fruit of Acacia trees (i.e *Acacia senegal*  
40 and *Acacia seyal*) in response to a scission, injury or fungal infection. Acacia trees are natives  
41 to dry areas of the sub-saharian region, from Senegal to East Africa, of which Sudan, Chad, and  
42 Nigeria produce nearly 95% of GA exported to the world market.

43 The dried gummy exudate, consisting of soluble fibers of low viscosity, is an interesting edible  
44 ingredient for several industrial applications (Williams & Phillips, 2009). Thanks to its  
45 technological features such as good emulsification, encapsulation, stabilization and adhesion  
46 properties, the GA is extensively used in the food, pharmaceutical, printing, textile, and  
47 cosmetic industries (Verbeken, Dierckx, & Dewettinck, 2003). Added to liquid food systems,  
48 they can adjust and improve their rheological properties. GA generally function as stabilizer,  
49 emulsifier, film former, thickener, flocculant and surface-finishing agent. These intrinsic  
50 properties make GA a recognized food additive (E414). They are found in the production of  
51 beverages, confectionery, emulsions, flavor encapsulations, bakery products and brewing  
52 (Verbeken et al., 2003; Wickens, Nations, Din, Sita, & Nahal, 1995).

53 These exceptional functional properties are thought to be tightly related to the molecular  
54 structure characteristics of both GA and its components. The molecular structure of GA consists  
55 of a mixture of highly branched and acidic polysaccharides. The main linear chain is composed  
56 of a core of 1,3-linked  $\beta$ -D-galactopyranose monomers with branches linked through C-6 but  
57 also C-4 or C-2. The branched chains consist of galactose and arabinose terminated by  
58 rhamnose and glucuronic acid (Al-Assaf, Phillips, & Williams, 2005a, 2005b; Siddig, Osman,  
59 Al-Assaf, Phillips, & Williams, 2005).

60 Gel permeation and hydrophobic chromatography studies have confirmed that both *Acacia*  
61 *senegal* and *Acacia seyal* gums are composed of three distinguish components. The main

62 fraction (88-90%) consists of arabinogalactan-peptide (AG) displaying a molecular weight  
63 (Mw) about 300kDa. The second component accounts for 10%, corresponds to arabinogalactan  
64 protein complex (AGP) with a 9% protein content and Mw starting from 1400kDa. Finally, a  
65 much smaller fraction consists of glycoprotein (GP) and displays the highest protein content  
66 (24.6-50%). These different values may change depending on several factor such as origin, age,  
67 or storage conditions.

68 The biochemical composition and molecular characteristics of GA can be affected by internal  
69 factors such as Acacia specie and age of trees. They can also change depending of external  
70 factors such as tree location, weather conditions, site and tapping method. The post-harvesting  
71 processes can also impact the molecular features and, as a result, the technological properties  
72 of GA. Very little data exists on the importance of the complex and versatile molecular variables  
73 contributing to the establishment of the functional properties of GA. Therefore, the overall  
74 objective of this work was to identify molecular variables that have the strongest effect on GA  
75 quality using partial least squares regression (PLS-R). PLS analysis is a useful statistical method  
76 that is suitable when there are more independent variables than response variables and there is  
77 multicollinearity among the independent variables (Abdi, 2010).

78 One of the most popular variable selection methods in partial least squares (PLS) projection  
79 method is Variable Influence on Projection (VIP), also known as Variable Importance in  
80 Projection scores (Wold, Johansson, & cocchi, 1993). VIP scores are useful to give an  
81 estimate of the contribution of a given predictor to a PLS regression model.

82 In this work PLS-R analysis combined with VIP scores on SEC-MALLS data will be used to  
83 understand and gain more insight about the relationship between molecular variables of GA  
84 and their functional properties. The results from this investigation will help gain a better  
85 understanding of the qualitative and quantitative molecular mechanisms controlling the  
86 establishment of GA qualities.

87

## 88 **2. Material and methods:**

### 89 **2.1 *Acacia gum samples***

90 GA samples (n=47) from *Acacia senegal* were provided by NEXIRA company (Rouen, France).  
91 Spray-dried industrial powder went through dissolution, filtration and pasteurization. Grade  
92 quality of samples were sorted by their stabilizing potency for oil in water emulsion (turbiscan  
93 method). Here we propose to characterize and quantify functional potency of GA in emulsion  
94 with technical variables such as viscosity, EP (ability to decrease size distribution of oil droplet)  
95 and ES (ability to maintain size distribution over different conditions and time).

### 96 **2.2 *Viscometry***

97 For each GA sample, dry matter was quantified (105°C, 5h). GA solutions were prepared with  
98 35% (w/w) dry matter and left to hydrate (3h, 20°C) prior to viscosity measures. Viscosity  
99 quantification was done using Brookfield LVF viscometer (spindle 2, 60rpm).

### 100 **2.3 *Emulsion***

101 For each GA sample, continuous phase was prepared with a 20% GA solution (w/w) in water  
102 containing 0.75% of citric acid, with a settling period of hydration (1h) after powder dispersion  
103 in liquid. Oil phase (containing a mix (50:50 w/w) of limonene (3x) and glycerol ester of wood  
104 rosin (Estergum 8BG) as weighting agent) was added during a shearing treatment performed  
105 using Ultra Turrax® T50 (maximum speed, 3min) in order to obtain a coarse pre-emulsion.  
106 After a 10min rest, solution went through 2 passes of homogenization including each 2  
107 contiguous stages, respectively at 4,060 and 580psi.

108        **2.4 Quantification of emulsifying property (EP) and emulsion stabilization (ES)**

109        The EP variable was defined as d(0.9), diameter of distribution for which 90% of oil droplets  
110        sizes are smaller. EP was quantified, for each GA sample, on fresh homogenized emulsion by  
111        laser light scattering using Malvern Mastersizer Hydro2000 (Fraunhofer SOP).

112        The ES variable was defined, for each GA sample, as the individual relative difference  
113        (expressed as percentage) of d(0.9) after a destabilizing stress applied on emulsion.  
114        Destabilizing stress corresponded to a heat treatment (09°C, 20min) followed by an accelerated  
115        stability study (50°C, 3months).

116        **2.5 Size exclusion chromatography (SEC)/MALLS system and procedure**

117        GA samples (60mg) were dispersed and incubated at room temperature for 48h with 30.0mL  
118        of 0.03M sodium chloride containing 0.02% (w/v) azide, used as preservative. Samples were  
119        filtered through 0.45µm regenerated cellulose filters (MACHEREY-NAGEL GmbH & Co. KG,  
120        France) before injection (100µL) into the SEC-HPLC/MALLS system.

121        SEC-HPLC fractionation was done using an Eclipse3 F System (Wyatt Technology, Santa  
122        Barbara, CA, USA) serially connected to a UV detector (Agilent 1200, Agilent Technologies,  
123        Germany), a MALLS detector (Dawn multi-angle Heleos TM, Wyatt Technology Corporation,  
124        Europe) and an interferometric refractometer (RI) set at 35°C (Optilab rEX, Wyatt Technology  
125        Corporation, Europe). Absorbance was measured at 214nm. The separation was performed  
126        using OHPAK SB 804HD and 806HD columns (Shodex, Tokyo, Japan) in series. An Agilent  
127        1200 Series Isocratic HPLC Pump (Agilent Technologies, Germany) with an in-line vacuum  
128        degasser delivered the carrier flow to the channel. A 0.1µm in-line regenerated cellulose filter  
129        (Gelman Sciences, France) was installed between the main pump and the Eclipse system.  
130        Sodium chloride (0.03M) containing 0.02% (w/v) azide, was used as the mobile phase and  
131        filtered through a 0.1mm membrane (Gelman Sciences, France). The flow rate of eluent was  
132        set at 0.5ml.min<sup>-1</sup>.

133 All necessary constants for molecular weight distribution calculation were determined using a  
134 value of 0.141ml.g<sup>-1</sup> as refractive index. A pullulan110 standard was used to normalize the  
135 diodes of the MALLS detector. The calculated MW of pullulan110, pullulan200 and  
136 pullulan800 standards were reasonably close to the nominal value. Calculations of the number-  
137 average ( $M_n$ ), weight-average ( $M_w$ ), z-average ( $M_z$ ), uncertainty-weighted average ( $M_{avg}$ )  
138 molar mass, polydispersity ( $M_w/M_n$ ), number-average ( $R_n$ ), weight-average ( $R_w$ ), z-average ( $R_z$ ),  
139 uncertainty-weighted average ( $R_{avg}$ ) mean square radius, RI mass fraction, relative proportion  
140 form UV and UV area/RI area ratio were performed using ASTRA 7.4 software (Wyatt Techno.,  
141 Santa Barbara, CA). Zimm first-order formalism was used for molecular calculations.

## 142 ***2.6 Partial least square regression***

143 PLS-R analyses were performed on the collected data using the PLS-Toolbox 7.5 (Ei-genvector  
144 Research, Inc., Manson, WA, USA) in Matlab software versionR2018a. All relevant parameters  
145 will be developed in the results section.

146

## 147 **3. Results and discussion:**

### 148 ***3.1 Acacia gum functional attributes***

149 The emulsifying activities of gum samples were determined by characterizing emulsions using  
150 particle size analysis with laser light scattering (Aoki, Decker, & McClements, 2005). This  
151 technique is more suitable for that kind of measurement than the turbidometric technique (Buffo,  
152 Reineccius, & Oehlert, 2001; Pearce & Kinsella, 1978). The EP expressed as d(0.9) ranged  
153 from 1.21 to 1.7 with an average value of 1.41 and coefficient of variation (CV) of 8.53% (Table  
154 1). CV is a dimensionless measure of the dispersion of our samples, indicating that our samples  
155 were moderately dispersed. The ES percentages oscillated between 3.49 and 69.9 with an  
156 average of 18.38. As judged by the higher CV, gum samples presented a larger range of



157 dispersion. The third studied parameter was viscosity. Samples exhibited an average viscosity  
158 of 847.89 with CV of 13.85% demonstrating medium to high dispersion.

159 **Table 1.** Viscosity, emulsifying activities and stabilities of Arabic gum samples.

<b>Parameters</b>	<b>Viscosity<sup>a</sup></b>	<b>Emulsifying property<sup>a</sup></b>	<b>Emulsification stabilisation<sup>a</sup></b>
<b>minimum</b>	666	1.21	3.49
<b>maximum</b>	1134	1.7	69.9
<b>Average</b>	847.89	1.41	18.37
<b>SD</b>	117.46	0.12	13.80
<b>CV %</b>	13.85	8.52	75.14

160 (a) Mean value of three experiments (n = 3).

161

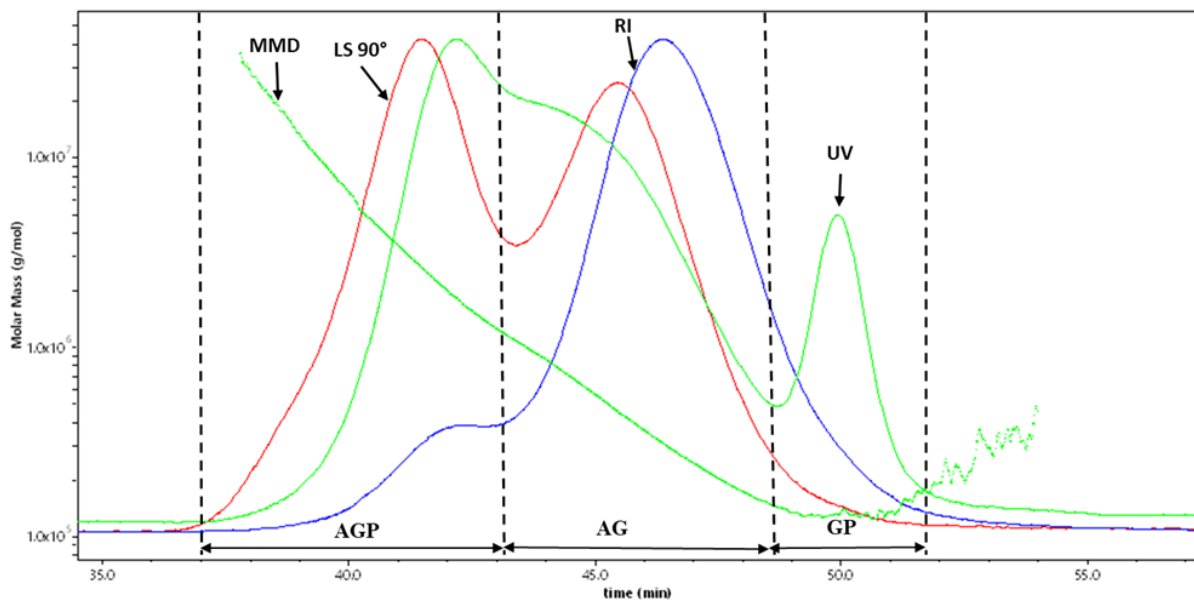
### 162 **3.2 Functional properties correlation**

163 Linear correlation coefficient ( $r^2$ ) was calculated between paired functionality properties. The  
164 negative correlation between emulsifying property and viscosity was very weak ( $r^2 = 0.16$ ).  
165 There was no correlation between emulsion stabilization and viscosity ( $r^2 = 0.01$ ). The negative  
166 correlation between emulsion stabilization and emulsifying property was very weak ( $r^2 = 0.18$ ).  
167 Those data confirm a high level of independency between the 3 functional properties and that  
168 we should consider and assess them independently.

169 The lack of correlation between functional properties is in agreement with industrial  
170 observations where the potency of EP is not systematically associated with ES efficacy over  
171 time. Stoke's law describes the velocity of emulsion sedimentation or creaming, based on a  
172 formula significantly and positively affected by viscosity (Chanamai & McClements, 2000).  
173 Creaming instability process of emulsion, however, is a different and later event than deviation  
174 of oil droplet (ES), the latter reflecting coalescence (merging of oil droplets). We should  
175 therefore assume that viscosity could help maintain and stabilize large oil droplets, but is not  
176 implicated in the preliminary coalescence process.

177 **3.3 SE-HPLC analysis of Acacia gum**

178



179 **Figure 1.** Example of size exclusion chromatogram of acacia senegal gum sample using  
180 UV-MALLS-RI detectors with molar mass distribution (MMD) overlay

180

181 Figure 1 shows a typical set of chromatograms representing UV detection (214nm), which is  
182 mostly sensitive to the peptide bond, refractive index (RI) response, light scattering (LS)  
183 response and molar mass distribution (MMD) versus elution time using NaCl ( $2\text{g.L}^{-1}$ ) as  
184 mobile phase (Kobayashi, Utsugi, & Matsuda, 1986; Kuipers & Gruppen, 2007).  
185 Chromatograms are comparable to previously published SEC-HPLC GA chromatograms  
186 (Atgié, Garrigues, Chennevière, Masbernat, & Roger, 2019; Idris, Williams, & Phillips, 1998;  
187 Padala, Williams, & Phillips, 2009; Denis Renard, Lavenant-Gourgeon, Ralet, & Sanchez,  
188 2006). However, our separation displays higher resolution in comparison with those obtained  
189 previously (Flindt, Al-Assaf, Phillips, & Williams, 2005).

190 Integration of RI peak areas enabled determination of the recovery of injected mass and  
191 calculation of the percentage of GA components. In this investigation, the elution recovery was  
192 100%. For all gum samples, the RI profile showed two distinct peaks. The first small peak  
193 (elution 37-43min, max 42min) was attributed to the arabinogalactan-protein (AGP) fraction as

194 reported previously (Randall, Phillips, & Williams, 1989). The second large peak (elution 43-  
 195 55min, max 47min) was attributed to the arabinogalactan (AG) fraction. The UV elution  
 196 profiles were significantly different from the RI profiles showing a huge peak with shoulder,  
 197 covering the first two fractions mentioned earlier, and a resolved peak (50min), proteinaceous  
 198 in nature and identified as the glycoprotein (GP) fraction (Randall et al., 1989). The LS and RI  
 199 profiles were not comparable and did not superimpose. The LS profiles clearly displayed two  
 200 response peaks (41 and 45min), allowing molar mass and size determination of the AGP and  
 201 AG fractions, respectively.

202 A Debye Plot (Zimm fit) of  $R\theta/K*c$  vs.  $\sin^2(\theta/2)$  for the GA components enabled a good  
 203 estimate of the molecular features. According to MMD (Figure 1), AGP and AG fraction  
 204 displayed a broad range of molar mass ranging from about  $1.4 \times 10^6$  to over  $10 \times 10^6 \text{g.mol}^{-1}$  and  
 205 from about  $1.8 \times 10^5$  to  $1.4 \times 10^6 \text{g.mol}^{-1}$ , respectively. In addition, the average mean square radius  
 206 values for AGP and AG ranged from 30 to over 100nm and from 20 to 30nm, respectively.

### 207 3.4 *Acacia gum molecular characteristics*

208 Molecular parameters were determined for each fraction (Figure 1): AGP fraction (36-43min),  
 209 AG fraction (43-48.8min), GP fraction (48.8-52min) and whole GA (36-52min). Most sample  
 210 molecular parameters displayed a  $CV > 5\%$  showing great dispersion in our sample population  
 211 (Table 2, 3 and 4).

212 **Table 2.** Molar mass and mass fraction of polymeric components of *Acacia senegal*  
 Arabic gum samples obtained from SE-HPLC-UV-MALLS-RI.

Parameters	Mass				
	Fraction <sup>a</sup> (%)	$M_w/M_n^a$	$M_n^a$ (g.mol <sup>-1</sup> )	$M_w^a$ (g.mol <sup>-1</sup> )	$M_{avg}^a$ (g.mol <sup>-1</sup> )
<b>AGP</b>					
<b>minimum</b>	8.2%	1.21	2.68E+06	3.32E+06	2.54E+06
<b>maximum</b>	15.6%	1.64	3.77E+06	6.01E+06	3.16E+06
<b>Average</b>	10.81%	1.39	3.09E+06	4.32E+06	2.86E+06
<b>SD</b>	1.86	0.11	2.79E+04	7.03E+05	1.53E+05
<b>CV %</b>	17.21	8.06	9.03	16.25	5.34

<b>AG</b>					
<b>minimum</b>	70.5%	1.173	4.78E+05	5.81E+05	4.28E+05
<b>maximum</b>	75.1%	1.298	5.93E+05	7.11E+05	5.46E+05
<b>Average</b>	73.20%	1.21	5.26E+05	6.41E+05	4.87E+05
<b>SD</b>	1.07	0.03	2.59E+04	2.87E+04	3.15E+04
<b>CV %</b>	1.46	3.25	4.93	4.48	6.48

<b>GP</b>					
<b>minimum</b>	12.1%	1.009	1.97E+05	2.01E+05	1.87E+05
<b>maximum</b>	18.9%	1.059	3.45E+05	3.59E+05	3.32E+05
<b>Average</b>	15.98	1.02	2.66E+05	2.72E+05	2.64E+05
<b>SD</b>	1.74	0.01	3.83E+04	4.00E+04	3.65E+04
<b>CV %</b>	10.89	1.06	14.39	14.72	13.81

**Whole Gum**

<b>minimum</b>		1.645	4.08E+05	7.72E+05	2.34E+05
<b>maximum</b>		2.669	6.08E+05	1.44E+06	5.05E+05
<b>Average</b>		2.00	4.93E+05	9.90E+05	3.86E+05
<b>SD</b>		0.26	4.60E+04	1.72E+05	7.78E+04
<b>CV %</b>		13.08	9.32	17.36	20.12

213 (a) Mean value of three experiments (n = 3).

214

215 The average  $M_w$  for the AGP fraction of samples ranged from  $3.33 \times 10^6$  to  $6.02 \times 10^6 \text{g.mol}^{-1}$  with  
 216 an average value of  $4.33 \times 10^6 \text{g.mol}^{-1}$  and CV of 16.26% while that for the AG fraction oscillated  
 217 between  $5.8 \times 10^5$  to  $7.12 \times 10^5 \text{g.mol}^{-1}$  with an average value of  $6.41 \times 10^5 \text{g.mol}^{-1}$  and CV of 4.48%  
 218 (Table 2). In addition, the average  $M_w$  ranged from  $4.08 \times 10^5$  to  $6.09 \times 10^5 \text{g.mol}^{-1}$  for whole GA.  
 219 Comparing CV, the average  $M_w$  variability was more important for AGP fraction. The values  
 220 obtained agree reasonably with those cited in the literature. However, values of  $M_w$  calculated  
 221 for our samples were higher than those determined previously (Flindt et al., 2005; Gashua,  
 222 Williams, Yadav, & Baldwin, 2015; Idris et al., 1998; D. Renard, Garnier, Lapp, Schmitt, &  
 223 Sanchez, 2012; Siddig et al., 2005). This could be due to differences in column, solubilization,  
 224 elution conditions and or sample nature.

225

226

227

228

229

**Table 3.** Size distribution of polymeric fractions of *Acacia senegal* Arabic gum samples obtained from SE-HPLC-UV-MALLS-RI.

Parameters	$R_w^a$ (nm)	$R_z^a$ (nm)	$R_{avg}^a$ (nm)
<b><u>AGP</u></b>			
<b>minimum</b>	34.6	46.3	35.7
<b>maximum</b>	48.1	72.3	59.1
<b>Average</b>	39.72	56.62	44.50
<b>SD</b>	3.35	6.21	8.38
<b>CV %</b>	8.44	10.98	18.84
<b><u>AG</u></b>			
<b>minimum</b>	12.3	14.2	14.3
<b>maximum</b>	24.9	25.8	25.8
<b>Average</b>	16.46	18.05	18.09
<b>SD</b>	4.59	4.20	4.18
<b>CV %</b>	27.89	23.31	23.15
<b><u>GP</u></b>			
<b>minimum</b>	15.3	15.4	12.5
<b>maximum</b>	36.0	40.1	49.6
<b>Average</b>	22.44	23.44	22.27
<b>SD</b>	5.59	6.25	10.53
<b>CV %</b>	24.92	26.66	47.27
<b><u>Whole Gum</u></b>			
<b>minimum</b>	16.2	30.8	22.1
<b>maximum</b>	30.2	57.6	44.8
<b>Average</b>	21.28	41.34	29.10
<b>SD</b>	4.37	6.95	7.64
<b>CV %</b>	20.53	16.82	26.25

230

(a) Mean value of three experiments (n = 3).

231

232 The mean of AGP dispersity ( $M_w/M_n$ ) was 1.39 suggesting that this fraction is constituted of

233 heterogeneous polymers. The associated CV was about 8.06%, suggesting variation among

234 samples. Lower dispersity and CV were noted for AG and GP fractions (Table 2). The

235 measured average size parameters showed a strong variation. The  $R_w$  of AGP showed the lowest

236 CV while  $R_{avg}$  of GP had the highest CV (47.27%). The mean  $z$ -average size ( $R_z$ ) of AGP,

237 AG and GP were 56.62, 18.05 and 23.44nm, respectively. Recently, these fractions were found

238 to have diameters of 20 and 10nm, for AG and GP, respectively (Gashua et al., 2015) while  
 239 consensus was reached for AGP with maximum dimension of 64nm (Gashua et al., 2015; D.  
 240 Renard et al., 2012).

241

242 **Table 4.** Protein fraction proportions and proteins to polysaccharides ratio of *Acacia senegal* Arabic gum samples obtained from SE-HPLC-UV-RI.

Parameters	AGP		AG		GP		Whole gum
	UV/RI <sup>a</sup>	UV (%) <sup>a</sup>	UV/RI <sup>a</sup>	UV (%) <sup>a</sup>	UV/RI <sup>a</sup>	UV (%) <sup>a</sup>	UV/RI <sup>a</sup>
<b>minimum</b>	264.00	24.19	64.53	48.70	39.42	7.17	80.82
<b>maximum</b>	381.86	55.58	80.28	63.73	89.35	13.32	114.34
<b>Average</b>	309.42	35.68	69.91	54.32	63.44	10.65	94.82
<b>SD</b>	26.73	7.92	3.15	3.20	8.83	1.12	8.45
<b>CV %</b>	8.64	22.21	4.51	5.90	13.93	10.56	8.91

243 (a) Mean value of three experiments (n = 3).

244

### 245 3.5 PLS-R VIP

246 Collinearity tests were carried out. These tests showed that our independent parameters all  
 247 without exception showed high Variance Inflation Factor (VIF) values indicating the  
 248 presence of multicollinearity. Thus, PLS-R analysis was used.

249 Using PLS-R, variable importance of projection was useful in understanding and selecting  
 250 the most influential and important explicative variables for each technological property. In  
 251 literature, VIP scores provide a useful measure to select variables that best explain variance  
 252 (Ceglar, Toreti, Lecerf, Van der Velde, & Dentener, 2016; Farrés, Piña, & Tauler, 2015;  
 253 Mkhabela, Bullock, & Sapirstein, 2018). VIP scores cut-offs are typically set at values  $\geq 0.8$   
 254 to determine the relative contribution of a given predictor to a PLS regression model.  
 255 However VIP scores  $\geq 1$  are required to improve the variable selection performance of the  
 256 PLS-VIP method (Chong & Jun, 2005). In this study, PLS analyses showed that there were  
 257 many independent variables with a VIP score  $\geq 0.8$  and most of them exhibited a VIP score

258  $\geq 1$  (Table 5). Therefore explicative variables with VIP scores between 0.8 and 1.2 were  
 259 considered as the very important variables while those having VIP scores  $\geq 1.2$  were  
 260 designated as the most critical factors (Mkhabela et al., 2018).

261 **Table 5.** Variable importance for projection (VIP) for viscosity, emulsifying activity and  
 262 emulsification stability.

Variables	VIP		
	Viscosity	Emulsifying property	Emulsification stabilisation
<b><u>AGP</u></b>			
$M_n$	<b><u>2.54</u></b>	<b><u>2.16</u></b>	<b><u>1.75</u></b>
$M_w$	<b><u>2.92</u></b>	<b><u>1.82</u></b>	<b><u>1.30</u></b>
$M_z$	<b><u>3.71</u></b>	<b><u>1.25</u></b>	<b><u>1.23</u></b>
$M_{avg}$	<b><u>1.05</u></b>	<b><u>2.13</u></b>	<b><u>1.22</u></b>
$M_w/M_n$	0.017	0.01	0.012
$R_n$	0.23	0.46	0.41
$R_w$	0.25	0.87	0.56
$R_z$	0.60	0.74	<b><u>1.01</u></b>
$R_{avr}$	<b><u>0.95</u></b>	<b><u>1.09</u></b>	<b><u>1.35</u></b>
Mass fraction RI	0.29	0.32	0.33
UV Area/RI Area	0.69	<b><u>1.15</u></b>	<b><u>1.13</u></b>
UV Area %	0.49	<b><u>1.10</u></b>	<b><u>1.32</u></b>
<b><u>AG</u></b>			
$M_n$	0.70	0.93	0.93
$M_w$	<b><u>0.91</u></b>	0.77	0.81
$M_z$	0.63	<b><u>1.01</u></b>	<b><u>1.09</u></b>
$M_{avg}$	0.55	<b><u>1.21</u></b>	<b><u>1.33</u></b>
$M_w/M_n$	0.002	0.006	0.002
$R_n$	0.28	0.77	0.78
$R_w$	0.32	0.38	0.77
$R_z$	0.29	0.35	0.72
$R_{avr}$	0.23	0.58	<b><u>0.82</u></b>
Mass fraction RI	0.34	<b><u>0.83</u></b>	0.84
UV Area/RI Area	0.72	0.78	0.69
UV Area %	0.59	<b><u>0.87</u></b>	0.69
<b><u>GP</u></b>			
$M_n$	0.62	<b><u>0.86</u></b>	<b><u>2.25</u></b>
$M_w$	0.52	<b><u>0.85</u></b>	<b><u>1.37</u></b>
$M_z$	0.53	<b><u>1.40</u></b>	<b><u>2.27</u></b>
$M_{avg}$	0.60	<b><u>1.91</u></b>	<b><u>2.23</u></b>
$M_w/M_n$	0.000	0.003	0.004

$R_n$	0.36	<b>1.01</b>	0.43
$R_w$	0.33	<b>0.98</b>	0.42
$R_z$	0.37	<b>1.12</b>	0.59
$R_{avr}$	<b>0.81</b>	<b><u>1.64</u></b>	<b>1.04</b>
Mass fraction RI	0.30	0.53	0.67
UV Area/RI Area	0.87	<b><u>1.35</u></b>	<b><u>1.28</u></b>
UV Area %	0.35	<b>0.84</b>	0.71

#### **Whole gum**

$M_n$	0.56	<b><u>1.59</u></b>	<b>1.03</b>
$M_w$	<b>1.13</b>	<b><u>1.36</u></b>	<b><u>1.37</u></b>
$M_z$	<b><u>3.64</u></b>	<b><u>1.64</u></b>	<b><u>1.43</u></b>
$M_{avg}$	0.71	<b><u>1.75</u></b>	<b><u>1.48</u></b>
$M_w/M_n$	0.05	0.02	0.04
$R_n$	0.16	0.42	0.54
$R_w$	0.16	0.37	0.48
$R_z$	0.39	0.45	<b>0.98</b>
$R_{avr}$	0.51	<b>1.07</b>	<b>0.94</b>
UV Area/RI Area	0.53	0.65	<b>0.92</b>

Bold VIP values are  $\geq 0.8$ ; bold underlined VIP values are  $\geq 1.2$ .

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### 265 **3.6 PLS-R VIP and Viscosity**

266 The best PLS model accounted for 92% of the variability in viscosity. This confirms that  
267 all main molecular variables implicated in viscosity have successfully been taken into  
268 account. Nine variables contributed significantly to the variability of viscosity, as shown  
269 by the VIP score  $\geq 0.8$ , while four variables were identified as the most critical factors (VIP  
270 score  $\geq 1.2$ ) that contributed to viscosity variability (Table 5). Based on VIP scores, the  
271 most critical factor was  $M_z$  of AGP, followed by  $M_z$  of whole GA polymers,  $M_w$  and  $M_n$  of  
272 AGP with VIP score of 3.72, 3.65, 2.92 and 2.54, respectively. Results suggested that the  
273 most influential parameters were tightly related to the molar mass of GA polymers but also  
274 in high extent to AGP. Furthermore, the average gyration radius ( $R_{avg}$ ) of AGP fraction  
275 exhibiting a VIP score of 0.95 contributed to viscosity variability. Although, mass fraction  
276 percentage of AGP is considered to impact the steady viscosity and moduli of whole gum



277 dispersion (Al-Assaf, Phillips, Aoki, & Sasaki, 2007), our data (VIP of 0.29) did not show  
278 importance of the relative percentage of AGP fraction on viscosity variability.  
279 This is the first time where a clear relationship have been established between AGP molar  
280 mass distribution and viscosity. Our findings are coherent with previous studies where the  
281 intrinsic viscosity was found to be tightly related to the gum molar mass and the  
282 hydrodynamic volume that control the hydration status and conformation of  
283 macromolecules (Apolinar-Valiente et al., 2019; Mudgil, Barak, & Khatkar, 2012; Robinson,  
284 Ross-Murphy, & Morris, 1982). Furthermore, authors reported an increase of 20-fold in  
285 viscosity with a rise of molar mass from  $4.24 \times 10^5$  to  $14.5 \times 10^5 \text{g.mol}^{-1}$  during maturation of  
286 GA (Al-Assaf et al., 2007). The role of the AGP molar mass could be explained by the fact  
287 that polymers have a tendency to aggregate in aqueous solution via intermolecular  
288 hydrophobic interactions between the polypeptide component and/or hydrogen bonds  
289 between the polysaccharide residues (Mahendran, Williams, Phillips, Al-Assaf, & Baldwin,  
290 2008; D. Renard et al., 2012). Hence, AGP constitute an elementary molecular background  
291 for aggregate establishment that affect the viscosity which is in accordance with previous  
292 reports (X. Li et al., 2009). They found that the viscosity diminishes as function of  
293 molecules disaggregation.

### 294 ***3.7 PLS-R VIP and Emulsifying property***

295 The best PLS model explained 76% of the variability in EP. This indicates that a majority  
296 of molecular variables have been taken into account for EP assessment. There are still other  
297 molecular parameters implicated, not studied here, which could complete this relationship.  
298 29 variables from the 46 studied contributed significantly to the variability of the EP, as  
299 shown by the VIP score  $\geq 0.8$ , while 13 variables were classified as the most critical factors  
300 (VIP score  $\geq 1.2$ ) that contributed to the EP variability (Table 5). In comparison, the number  
301 of variables contributing to this rheological parameter was much higher than that of

302 variables controlling the viscosity. The implication of molecular structure of GA in EP is  
303 more complex than for viscosity and involved a wide range of variables. Almost all the  
304 most critical factors were related to molar mass variables including molar mass of AGP,  
305 whole gum and GP. With VIP score of 2.16 and 2.14,  $M_n$  and  $M_{avg}$  of AGP were first and  
306 second among the most critical factors. It was clear that AGP molar mass were the most  
307 contributing factors in the establishment of the emulsifying activity. Gyration radius  
308 parameters with VIP score  $\geq 0.8$  were also crucial for this qualitative property mainly  $R_{avg}$   
309 of GP which was determined as one of the most critical factors with VIP value of 1.64.  $R_n$ ,  
310  $R_w$  and  $R_z$  of GP exhibiting VIP scores  $\geq 0.8$  outlined that not only molar mass but also the  
311 size of GP were essential determinants of EP.  $R_{avg}$  of AGP and whole gum contributed to  
312 the EP variability as they showed VIP scores  $\geq 0.8$ .

313 Once more, AGP% did not have a high VIP score meaning that relative percentage of AGP  
314 did not influence the formation of emulsion while mass fraction percentage of AG, with  
315 VIP value of 0.83, was shown to be essential for this qualitative parameter.

316 Interestingly, variables related to protein content (UV signal) were shown to be partially  
317 involved in the variability of EP. In addition, among these variables only UV area/RI area  
318 of GP fraction were identified as the most critical factors with VIP score of 1.35. UV%  
319 of GP displaying a VIP value of 0.84, showed a relevant role of this fraction in the  
320 emulsifying property variability. VIP values of AGP UV/RI ratio and AGP UV% and UV%  
321 of AG revealed that these factors which are related to peptides contributed to emulsification  
322 behavior.

### 323 **3.8 PLS-R VIP and Emulsion stabilization:**

324 The best PLS model justified 77% of the variability in the ES percentages. Similarly to EP,  
325 this indicates that a majority of molecular variables have been taken into account for ES  
326 assessment and that still other molecular parameters could be partially implicated. 27

327 variables were found significantly responsible for the variability of the ES parameter, as  
328 displayed by the VIP score  $\geq 0.8$ , while 15 variables were determined as the most critical  
329 factors (VIP score  $\geq 1.2$ ) that contributed to the ES variability (Table 5). In comparison with  
330 the viscosity, the number of the very important variables explaining the variability of the  
331 ES were three time larger, showing a much more complex relationship. Despite the absence of  
332 correlation between emulsification properties (EP, ES) and viscosity described earlier, similar  
333 results to viscosity were noted. All molar mass parameters of different GA fractions were shown  
334 to contribute to ES. More interesting, Molar mass of GP was among the most critical factors,  
335 and exhibited the highest values of VIP. The impact and role of fraction size was quite similar  
336 in variable number comparing to EP. However for ES, only  $R_{avg}$  of AGP was determined as the  
337 most critical factor while  $R_{avg}$  of GP, AG and whole gum as well as  $R_z$  of AGP and whole gum  
338 were considered as very important variables. As for EP, mass fraction percentage of AG was  
339 revealed to influence and contribute to ES as its VIP score was 0.84.

340 In addition, it was determined that the peptides represented by UV% of AGP, UV/RI ratio of  
341 AGP and UV/RI ratio of GP were involved in ES. UV% of AGP were one of the most critical  
342 factors while UV/RI ratio of AGP and UV/RI of AG were among the most important factor.

343 22 molecular parameters were common for the explanation of EP and ES. Without exception,  
344 all molar mass variables of all GA fractions were shown to be implied in emulsification  
345 variables and most of them were so as the most critical factors. The molar mass of AGP was  
346 closely involved in EP variability while molar mass of GP contributed strongly to ES variability.

347 EP was also shown to be dependent of gyration radius of GP and relative protein content. Thus,  
348 our study suggested that the molar mass of GA polymers depended on their heterogeneous  
349 composition of proteins and polysaccharides. In addition, it was obvious that the molar mass  
350 controlled gyration radius and surface active of polymers. It suggested that the quality of GA  
351 which is a well-established natural emulsifier agent, depends on polymer molar mass and

352 that importance resides in their ability of self-interaction due to their composition of  
353 amphiphilic proteins and hydrophilic polysaccharides. Therefore, our study complements  
354 previous reports showing the impact of some intrinsic parameters on GA properties. It has been  
355 reported that the relative proportion of the three main fractions governs the emulsification  
356 properties (Dickinson, Galazka, & Anderson, 1991; Dickinson, Murray, Stainsby, & Anderson,  
357 1988). Further, thanks to polypeptide chain hydrophobicity and carbohydrate  
358 hydrophilicity, AGP fraction showed to be the main responsible of the emulsifying  
359 behavior and interfacial properties of the GA (Anderson & Stoddart, 1966; Dickinson et al.,  
360 1988; Fauconnier et al., 2000; Randall et al., 1989). Our results which highlighted the weight  
361 of molar mass, relative proportion of proteins and protein to polysaccharide ratio in the  
362 establishment of GA properties, could explain previous statements describing the polymer  
363 aggregation (Al-Assaf, Sakata, McKenna, Aoki, & Phillips, 2009; Apolinar-Valiente et al.,  
364 2019; G.-Z. Li & Zeng, 2009; D. Renard et al., 2012, 2014). Taking into account these reports  
365 and our data, it is obvious that polymer molar mass governed by polysaccharides and  
366 polypeptides composition explains GA rheological behaviors that would be linked to the  
367 capacity of polymers to self-aggregate. The aggregation is due to molecular association via  
368 proteinaceous hydrophobicity interactions between aminoacid side chains in the polypeptide  
369 component and/or intermolecular hydrogen bonds between the polysaccharide residues.  
370 Then, the amount and the characteristics of the protein fractions could be considered crucial for  
371 intermolecular association. Further completion of PLS model for variability explanation could  
372 provide more information. In this aim, we should consider studies performing similar statistic  
373 tests and including other molecular variables related to protein composition as aromatic  
374 aminoacids, known for their higher hydrophobic behaviors (ie. UV 280/214 ratio; (Bérépion,  
375 2011)) or markers related charge as Zeta potential for each emulsion performed with  
376 fractionated AGP, AG and GP (Jayme, Dunstan, & Gee, 1999).

377 **4. Conclusions:**

378 This study confirmed that the 3 functional properties, viscosity, emulsifying property and  
379 emulsion Stabilization were rather independent and confirmed the relevance to evaluate them  
380 independently. Molecular variables from SE-HPLC-MALS considered in this study were  
381 relevant to assess relationship with viscosity, EP and ES as reflected by PLS model implication  
382 in their variability (92, 76 and 77% respectively). EP and ES have a much more complex  
383 molecular structure implication than viscosity. None of AGP, AG or GP quantity proportions  
384 were implicated in any functional properties. Molar mass of AGP and GP however played a  
385 critical role in all functional properties but with a higher implication in viscosity compared to  
386 EP and ES (while ES and EP were also impacted by molar mass of AG). Some other specifics  
387 between functional properties have been showed, as radius gyration of GP was more implicated  
388 in EP compared to radius gyration of AGP which was more implicated in ES. We should also  
389 highlight the weight of protein content (mainly from AGP and GP) for both EP and ES, while  
390 it is not implicated in viscosity. These data have shown the importance of understanding the  
391 role of component that have abilities to self-association via different kinds of surface  
392 interactions as has been reported in literature.

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