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The colloidal stabilization of young red wine by Acacia
senegal gum: The involvement of the protein backbone from
the protein-rich arabinogalactan-proteins

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Abstract

The arabinogalactan-proteins (AGPs) ~~type biopolymers~~ from *Acacia senegal* gum are used as protective colloid in young red wines to prevent the precipitation of the coloring matter. Usually, *Acacia senegal* gum is chosen based on its efficiency to stabilize iron hexacyanoferrate salts in hydro-alcoholic solution. In this study, the protective activity of *Acacia senegal* gum and its ~~three~~ macromolecular fractions (HIC-F1, HIC-F2 and HIC-F3), separated according to their hydrophobicity, towards the precipitation of iron hexacyanoferrate salts and polyphenols in hydro-alcoholic solutions are reported. ~~The~~ AGPs prevent the colloidal instability of both iron hexacyanoferrate salts and polyphenols in “model” hydro-alcoholic solutions and young red wine with a good correlation between results obtained on both systems. This result ~~then~~ strengthens the use of iron hexacyanoferrate salts (hydro-alcoholic – mineral test) for the evaluation of the protective activity of *A. senegal* gum in young red wine. The precipitation of iron hexacyanoferrate salts is avoided by the electrostatic binding of Ca^{2+} , the driver of the instability, with ~~the~~ negatively charged carried by amino-acids from the protein backbone or both by amino acids and uronic acid monosaccharides localized close to the protein backbone of AGPs. The protective activity closely depends on the protein content of AGPs in both iron hexacyanoferrate salts and polyphenols hydro-alcoholic solutions: the more they are rich in proteins, the more their colloidal stabilizing efficiency is (HIC-F3>HIC-F2>HIC-F1). The differences observed in the protective activity between AGPs from the three HIC fractions are relied not only to their protein content but also to their related rate of glycosylation that modulates the protein accessibility to its environment, then their physicochemical properties as their hydrophobic behavior.

Keywords: Acacia gum, Arabinogalactan proteins, colloidal stability, young red wine, coloring matter

1. Introduction

Red wine is a mixture of water, alcohols, organic acid and of complex molecules at the origin of its organoleptic properties and in constant evolution during ageing. The color as well as the clarity of red wines are ones of the qualities required by the consumers. Red wines must present a colloidal stability not only at the time of bottling but also during ageing and storage until its consumption (Ribéreau-Gayon, Glories, Maujean & Dubourbieu, 2006). The phenolic compounds, responsible of the organoleptic perceptions, are also unstable molecules able to aggregate and precipitate both in young and aged red wines (Ribéreau-Gayon et al., 2006; Alcade-Eon, Garcia-Estévez, Puente, Rivas-Gonzalo & Escribano-Bailon, 2014). Among others, the phenolic compounds can react with ferric iron to form soluble complexes that may then flocculate and precipitate during ageing (Ribéreau-Gayon et al., 2006). The colloidal stability in young red wines may be ensured by several techniques as a cold treatment, fining or addition of protective colloids such as carboxymethyl cellulose, metatartaric acid, mannoproteins and Acacia gum (Waters, Pellerin & Brillouet, 1994; Waters, Pellerin & Brillouet, 1994a; Dupin, Stockdale, Williams, Jones, Markides & Waters, 2000; Escot, Feuillat, Dulau & Charpentier, 2001; Riou, Vernhet, Doco & Moutounet, 2002; Riberau-Gayon et al., 2006; Poncet-Legrand, Doco, Williams & Vernhet, 2007; Pérez-Lamela, García-Falcón, Simal-Gándara & Orriols-Fernández, 2007; Gerbaud, Gabas, Blouin & Crachereau, 2010; Teissedre, 2012; Gordillo, Cejudo-Bastante, Rodríguez-Pulido, Jara-Palacios, Ramírez-Pérez, González-Miret & Heredia, 2014). The cold and fining treatments cause the precipitation of existing particles responsible of the turbidity as well as those potentially formed during ageing. Therefore, both treatments have a clarifying and a stabilizing action (Riberau-Gayon et al., 2006). In young red wine, Acacia gum is used as a

protective colloid in order to prevent or limit the aggregation and precipitation of unstable colloids as the coloring matter (Ribereau-Gayon et al., 2006; Teissedre, 2012). In particular, Acacia gum macromolecules bind to polyphenols by the involvement of both hydrogen bonding and hydrophobic interactions that prevents their interaction and aggregation with proteins (de Freitas, Carvalho & Mateus, 2003; Mateus, Carvalho, Luis & de Freitas, 2004; Soares, Gonçalves, Fernandes, Mateus & de Freitas, 2009; Soares, Mateus & de Freitas, 2012; Chung, Rojanasasithara, Mutilangi & McClements, 2016). Hence, Acacia gum is mainly used in young red wine for its colloidal stabilizing properties. It stabilizes young red wine but does not clarify it.

Acacia gum is a natural exudate obtained from the trunk and branches of *Acacia senegal* and *Acacia seyal* trees (Williams & Phillips, 2000). Acacia gum macromolecules are highly glycosylated hydroxyproline-rich arabinogalactan-proteins (AGPs) belonging to the glycoprotein superfamily (Akiyama, Eda & Kato, 1984). AGPs from Acacia gum are mainly composed by sugars (D-galactose, L-arabinose, L-rhamnose, D-glucuronic acid, and 4-O-methyl-D-glucuronic acid) with a small fraction of proteins and minerals (Idris, Williams & Phillips, 1998; Lopez-Torrez, Nigen, Williams, Doco & Sanchez, 2015). The sugars are organized into hyperbranched polysaccharide blocks covalently linked to the polypeptide backbone in serine- and hydroxyproline-rich domains (Lopez-Torrez et al., 2015). The highly branched polysaccharide structure is formed by main chains of 1,3-linked β -D-galactopyranose substituted by side chains in O-6 position. Units of α -L-arabinofuranosyl and α -L-rhamnopyranosyl are distributed in the main and side chains while β -D-glucuronopyranosyl and 4-O-methyl- β -D-glucuronopyranosyl are mostly end-units (Anderson, Hirst & Stoddart, 1966; Anderson & Stobbart, 1966; Lopez-Torrez et al., 2015).

A. senegal gum can be defined as a continuum of AGPs differing by their sugar, amino acid and mineral content and composition, sugar to amino acid ratio, polarity, number of charges,

99 molar mass, size and shape (Randall, Phillips & Williams, 1989; Islam, Phillips, Sljivo,
 100 Snowden & Williams, 1997; Renard, Lavenant-Gourgeon, Ralet & Sanchez, 2006; Mejia
 101 Tamayo, Nigen, Apolinar-Valiente, Doco, Williams, Renard & Sanchez, 2018). These AGPs
 102 can be separated according to their polarity using hydrophobic interaction chromatography
 103 (HIC) with the recovery of three fractions (Randall et al., 1989; Renard et al., 2006). These
 104 three fractions were historically named arabinogalactan (AG), arabinogalactan-protein (AGP)
 105 and glycoprotein (GP) according to their protein content and elution order. ~~However, all the~~
 106 ability of these three fractions to bind the β -glucosyl Yariv's reagent suggests that they all
 107 belong to the AGP family ~~because of their reactivity to the Yariv's reagent~~ (Osman et al.
 108 1993). Hence, to avoid any confusion, we named them HIC-F1 (arabinogalactan, AG), HIC-
 109 F2 (arabinogalactan-protein, AGP) and HIC-F3 (glycoprotein, GP) in their order of elution,
 110 reflecting also an increasing hydrophobic index. HIC-F1 is the most abundant fraction (85-
 111 92% of the whole gum) as compared to HIC-F2 (6-16% of the whole gum) and HIC-F3 (1-3%
 112 of the whole gum). The sugar composition was similar between the three fractions, with
 113 however a larger content of arabinose in HIC-F2 and HIC-F3 and a larger content of charged
 114 sugars in HIC-F1. HIC-F3 was the richest fraction in proteins with values around 25-40%,
 115 while the amount of proteins was around 8-10% and 0.5-1% for HIC-F2 and HIC-F1,
 116 respectively. These three HIC fractions differed also by their mean molar mass (M_w) and high
 117 M_w AGPs content (AGPs with M_w upper than 10^6 g.mol⁻¹). HIC-F1 was mainly composed of
 118 low M_w AGPs, while HIC-F2 and HIC-F3 were richest in high M_w AGPs.

119 In young red wines, it is advised to use *Acacia senegal* gum at a maximal concentration of
 120 300 mg/L (Code International des Pratiques Œnologiques, Fiche Code OIV (12/72) – Edition
 121 01/2015 II.3.3-7) even if no legal limit exists in the EC 606-2009. Before its addition in young
 122 red wines, the efficiency of *A. senegal* gum towards wine colloidal instability is evaluated
 123 according to an “efficacy test” of the International Oenological Codex (International

Oenological Codex: COEI-1-GOMARA, 2000). This “efficacy test” consists in determining the quantity of *A. senegal* gum required to prevent the flocculation of a colloidal iron hexacyanoferrate solution in hydro-alcoholic medium by calcium salt. Hence, the *A. senegal* gum efficiency towards wine colloidal instability is usually evaluated using a hydro-alcoholic – mineral solution which seems in appearance far enough from wine matrices on their biochemical and physicochemical properties. The colloidal stabilization of two so different matrices by *A. senegal* gum raises some questions on the colloidal stability mechanism and especially on the AGPs at the origin of this colloidal stabilization.

The aim of this study was to investigate the colloidal stabilizing properties of AGPs from *A. senegal* gum in three hydro-alcoholic solutions: a hydro-alcoholic – mineral solution, “wine-like” solution and young red wine. The AGPs responsible of the colloidal stability in the three hydro-alcoholic solutions were identified. Furthermore, the colloidal stabilization mechanism involving AGPs from *A. senegal* gum in hydro-alcoholic – mineral solution was characterized.

2. Materials and Methods

2.1. Materials

Instant spray dried Acacia *senegal* gum was provided by ALLAND & ROBERT Company - Natural and organic gums (Port Mort, France). *A. senegal* gum was fractionated by hydrophobic interaction chromatography (HIC) according to Renard et al. (Renard et al., 2006). Three HIC fractions were isolated ~~that were~~ and named HIC-F1, HIC-F2 and HIC-F3 according to their elution order, and then their hydrophobic behaviour. The biochemical composition and structural properties (mean molar mass) of *A. senegal* gum and HIC fractions were described by Mejia-Tamayo (Mejia-Tamayo et al., 2018) and presented in supplementary data (Table S1).

The pronase was from *streptomyces griseus* (lot 10165921001, Roche). The grape marc powder rich in polyphenols (64%) was provided by Grap'Sud Company (Cruviers-Lascours, France). The unstable young red wine (Cabernet sauvignon/Merlot, 2015) was provided by BioLaffort Company (Bordeaux, France). All other reagents were of analytical grade from Sigma-Aldrich (USA).

2.2. Hydrolysis of *Acacia senegal* gum and HIC-F1 fraction by pronase

A. senegal gum and HIC-F1 fraction were dissolved at a concentration of 10 mg.mL⁻¹ in ultra pure deionised water (18.2 mΩ resistivity) containing 0.02% NaN₃ and stirred overnight at room temperature. The pH was adjusted at 6.5 using a small aliquot of NaOH solution before to add pronase at a final concentration of 0.14 mg.mL⁻¹. The enzymatic hydrolysis occurred during 48h at 35°C. The enzymatic reaction was stopped by removing the pronase from the samples by centrifugation at 3000 rpm using a centricon Vivaspin 20 (cut-off of 50 000 Da). The hydrolysis products were also removed by the centrifugation step. The samples were then washed four times with ultra pure deionised water (18.2 mΩ resistivity) by centrifugation at 3000 rpm using a centricon Vivaspin 20 (cut-off of 50 000 Da), before their freeze-drying.

2.3. Preparation of *Acacia senegal* gum and its HIC fractions

Stock solution of *A. senegal* gum and HIC fractions were dissolved in ~~ultra-pure~~ ultra-pure deionised water (18.2 mΩ resistivity) and stirred overnight at room temperature. The solutions were centrifuged at 12 500 rpm for 30 min at 20°C to remove insoluble materials and air bubbles.

2.4. Electrophoretic mobility measurements

The electrophoretic mobility of *A. senegal* gum prepared in hydro-alcoholic solutions (tartaric acid: 3 g.L⁻¹; potassium sulfate: 1 g.L⁻¹; ethanol: 12% v/v) at pH 3.1, 3.5 and 4 was determined using a Zetasizer 1000 (Malvern, United Kingdom). The measurements were performed at 25°C. The data are the average of three measurements.

2.5. Multi-detector high performance size exclusion chromatography

HPSEC experiments were performed using a Shimadzu HPLC system (Shimadzu, Kyoto, Japan) coupled to one Shodex OHpak SB-G pre-column followed by four Shodex OHpak SB columns in series (SB 806 HQ, SB 805 HQ, SB 804 HQ and SB 803 HQ). 50 µl of *A. senegal* gum and HIC fractions solutions (1 mg.ml⁻¹) were injected. The elution was performed with 0.1 mol.L⁻¹ LiNO₃ solution containing 0.02% NaN₃ at a flow rate of 1 ml.min⁻¹ and 30°C.

The HPSEC system was fitted in series to a Dawn Heleos II multi-angle laser light scattering (Wyatt Technology Corp., Santa Barbara, Ca, USA) and an Optilab T-rEX refractometer (Wyatt Technology Corp., Santa Barbara, Ca, USA). The molar mass distribution and the weight-average molar mass (M_w) were calculated using ASTRA software 6.1.1.17 (Wyatt Technologies, Santa Barbara, CA). The data were analyzed using the Zimm's model (1st order) and a refractive index increment (dn/dc) of 0.155, 0.162, 0.160 and 0.145 ml.g⁻¹ for *A. senegal* gum, HIC-F1, HIC-F2 and HIC-F3, respectively, as determined experimentally.

2.6. Colloidal stability of hydro-alcoholic - mineral solution

The hydro-alcoholic – mineral solution was prepared with ~~ultra-pure~~ultra-pure deionised water (18.2 mΩ resistivity) according to the ~~International~~ Oenological International Codex (O.I.V.). This solution ~~It~~was composed of tartaric acid (3 g.L⁻¹), potassium sulfate (1 g.L⁻¹), ethanol (12% v/v), potassium ferrocyanide (60 mg.L⁻¹) and iron ~~solution~~–(5 mg.L⁻¹~~00~~). ~~When~~The addition of iron (Fe³⁺) ~~was added~~to the hydro-alcoholic – mineral solution

198 ~~containing potassium ferrocyanide ($K_4[Fe(CN)_6]$)~~, induced the formation of a soluble
 199 “Prussian blue” colloidal solution of $K[Fe^{III}Fe^{II}(CN)_6]$ ~~is obtained~~. The hydro-alcoholic –
 200 mineral solution was stirred during 2 h at room temperature before to adjust ~~the~~ its pH at 3.1
 201 using NaOH ~~solution~~ (1 mol.L⁻¹). The hydro-alcoholic – mineral solution was then filtered
 202 through a 0.45 µm regenerated cellulose membrane (GE Healthcare Life Sciences, Germany).
 203 For the studies according to pH, the hydro-alcoholic – mineral solution was prepared at pH
 204 3.1, 3.5 and 4.0.

205 The hydro-alcoholic – mineral solution was destabilized by the addition of CaCl₂ (22 g.L⁻¹)
 206 prepared in ultra-pure deionised water (18.2 mΩ resistivity). The added Ca²⁺ bound to
 207 $K[Fe^{III}Fe^{II}(CN)_6]$ inducing the formation of “Prussian blue” precipitates ($Ca[Fe^{III}Fe^{II}(CN)_6]_2$).
 208 Most of experiments were performed at a final CaCl₂ concentration of 0.67 g.L⁻¹. Some
 209 experiments were performed at a final concentration of 0.33, 0.5, 1 and 1.33 g.L⁻¹ to
 210 investigate the influence of calcium. Each measurement was triplicated.

211 The efficiency of *A. senegal* gum and its HIC fraction to prevent the colloidal mineral
 212 destabilization was studied by varying their concentration in the hydro-alcoholic – mineral
 213 solution. The final concentration of *A. senegal* gum or its HIC fractions in the hydro-alcoholic
 214 – mineral solution ranged between 0 and 1 g.L⁻¹. *A. senegal* gum or HIC fraction solutions
 215 were added to the hydro-alcoholic – mineral solution before the addition of CaCl₂ solution.
 216 Each measurement was triplicated.

217

218 2.7. Colloidal stability of hydro-alcoholic – grape marc solution and young red wine

219 The hydro-alcoholic – grape marc solution (wine-like medium) was prepared with ultra-pure
 220 deionised water (18.2 mΩ resistivity). It contained tartaric acid (2.7 g.L⁻¹), potassium sulfate
 221 (0.9 g.L⁻¹), grape marc powder (3 g.L⁻¹) and ethanol (12% v/v). The solution was gently
 222 stirred during 2 h at room temperature before to adjust the pH at 3.5 using NaOH solution (1

mol.L⁻¹). The solution was then filtered through a 0.45 µm regenerated cellulose membrane (GE Healthcare Life Sciences, Germany).

The colloidal destabilization of the hydro-alcoholic – grape marc solution and the young red wine was induced by cooling the solutions to 10°C. The efficiency of *A. senegal* gum and its HIC fraction to prevent the destabilization of the hydro-alcoholic – grape marc solution and the young red wine was studied at 10°C by varying their concentration between 0 and 2 g.L⁻¹. Each measurement was triplicated.

2.8. Characterization of the colloidal stability of hydro-alcoholic model solutions and young red wine

The colloidal stability of the hydro-alcoholic – mineral and hydro-alcoholic – grape marc solutions, and the young red wine was investigated using a Turbiscan Tower equipped with a pulsed near infrared light source ($\lambda = 880$ nm) and two synchronous detectors, a transmission (T) and a backscattering (BS) detectors (Formulaction, France). The samples were loaded into cylindrical glass tubes and scanned throughout its entire height. The transmittance (T, in %) was measured every 1 min and 50 sec during 24 h at 25°C for the hydro-alcoholic – mineral solutions and during 48 h at 10°C for the hydro-alcoholic – grape marc solutions and young red wine.

The transmittance (T) profiles were analyzed using the TowerSoft software, version 1.1.0.36 (Formulaction, France). ΔT (%) corresponded to the difference in transmittance between the scan_i and the first scan. The Turbiscan Stability Index (TSI) was also determined as the following:

$$TSI = \sum_i \frac{\sum h |scan_i(h) - scan_{i-1}(h)|}{H}$$

The TSI corresponded to the transmittance variation at all measured position (h) throughout the entire height (H) of the sample between the scan_i and the scan_{i-1} . The plot of TSI according to time corresponds to the kinetic behavior of the samples that takes into account all the physical phenomena (especially destabilization mechanism) occurring during the measurement.

The TSI kinetics were used to plot the TSI values at 24h in hydro alcoholic – mineral solution and 48h in hydro alcoholic – grape marc solution and young red wine according to AGPs concentration. This representation assimilated to a “stability curve” was used to experimentally determine the AGPs concentration, named critical concentration, necessary to obtain the colloidal stability of the solutions.

3. Results

3.1. Colloidal stabilization mechanism of the hydro-alcoholic – mineral solution by *A. senegal* gum

The colloidal stability of the hydro-alcoholic – mineral solution (pH 3.1) containing *A. senegal* gum was followed by measuring at 25°C the transmittance (T) of the solutions during 24h using a light scattering equipment. The figure 1 showed the temporal change in transmittance (ΔT) of three hydro-alcoholic – mineral solutions without and with 0.09 and 0.14 g.L⁻¹ of *A. senegal* gum (Figure 1A, 1B and 1C). After 24h, the transmittance (T) of the hydro-alcoholic – mineral solutions without and with 0.09 g.L⁻¹ of *A. senegal* gum increased respectively by 60% and 35% in the middle of the sample (cell height: 2 – 36 mm) reflecting a clarification of the solution. This phenomenon was confirmed by the loss of the blue color of the solutions after 24h (Figure 1A and 1B). The clarification resulted from the formation of a salt precipitate ($\text{Ca}[\text{Fe}^{\text{III}}\text{Fe}^{\text{II}}(\text{CN})_6]_2$) between $\text{K}[\text{Fe}^{\text{III}}\text{Fe}^{\text{II}}(\text{CN})_6]$ and Ca^{2+} that pelleted, as evidenced by the decrease of T by 5% and 2% after 24h in the bottom of the cell (cell height:

1 – 1.5 mm) for the solutions without and with 0.09 g.L⁻¹ of *A. senegal* gum, respectively (Figure 1A and 1B). On the other hand, the transmittance of the hydro-alcoholic – mineral solution containing 0.14 g.L⁻¹ of *A. senegal* gum was constant during the 24h of measurement, reflecting the colloidal stability of the solution. The influence of *A. senegal* gum concentration (0 – 0.14 g.L⁻¹) on the colloidal stability kinetics of the hydro-alcoholic – mineral solution was analyzed by plotting the TSI (Turbiscan Stability Index) according to time (Figure 2A). The increase of *A. senegal* gum concentration reduced and delayed the instability mechanism occurring in the hydro-alcoholic – mineral solution. The colloidal instability was totally prevented for an *A. senegal* gum concentration above around 0.11 g.L⁻¹. The minimal *A. senegal* gum concentration ([AG]_{critical}) necessary to obtain the hydro-alcoholic – mineral colloidal stability was determined by plotting the TSI values after 24h according to *A. senegal* gum concentration (stability curve) (Figure 2B). Below an *A. senegal* gum concentration of 0.05 g.L⁻¹, the TSI values at 24h was constant and similar to the control sample without *A. senegal* gum. In these experimental conditions, the concentration of *A. senegal* gum was too low. Above 0.05 g.L⁻¹ of *A. senegal* gum, the TSI values decreased progressively before to reach a constant TSI value (≤ 2) similar to the control without Ca²⁺ for *A. senegal* gum concentration upper than 0.11 g.L⁻¹. Based on the stability curve, the [AG]_{critical} was 0.114 ± 0.002 g.L⁻¹ (Figure 2B).

The colloidal stabilization mechanism was further investigated by modifying the physicochemical condition as the pH of the solutions and the amount of Ca²⁺ added to induce the colloidal instability mechanism. The stability curves of hydro-alcoholic – mineral solutions prepared at pH 3.1, 3.5 and 4.0 are shown in Figure 3A. As the pH increased from 3.1 to 4.0, the stability curves were shifted towards lower *A. senegal* gum concentrations necessary to prevent the colloidal instability. The [AG]_{critical} were 0.114 ± 0.002 , 0.094 ± 0.003 and 0.064 ± 0.002 g.L⁻¹ at pH 3.1, 3.5 and 4.0, respectively. Hence, the colloidal

296 stabilizing properties of *A. senegal* gum were greatly influenced by pH and enhanced with its
 297 increase in this pH range. AGPs from *A. senegal* gum are weakly charged glycoproteins
 298 characterized by a global negative charge in aqueous solution for pH ranging from 2.0 to 10.0
 299 (Burgess & Carless, 1984). Moreover, the negative AGPs μ_E increased from pH 2.0 to ~ 5.0
 300 in aqueous solutions reflecting the increase of the global negative charge (Burgess & Carless,
 301 1984; Schmitt, Sanchez, Thomas & Hardy, 1999). Thus, the electrophoretic mobility (μ_E) of
 302 *A. senegal* gum in hydro-alcoholic solutions prepared at pH 3.1, 3.5 and 4.0 was measured
 303 and plotted according to $[AG]_{critical}$ (Figure 3B). A good correlation was evidenced between
 304 *A. senegal* gum μ_E and its concentration necessary to stabilize the hydro-alcoholic – mineral
 305 solutions. The more the negative *A. senegal* gum μ_E was, the less the *A. senegal* gum quantity
 306 was required to prevent the colloidal instability. Hence, negative charges carried by AGPs
 307 were certainly involved in this colloidal stabilization mechanism.

308 Since the colloidal instability of the hydro-alcoholic – mineral solution is induced by the
 309 addition of Ca^{2+} , it was hypothesized that electrostatic interactions between Ca^{2+} and negative
 310 charges of AGPs could play an important role in the stabilization mechanism. Therefore, we
 311 determined the $[AG]_{critical}$ in hydro-alcoholic – mineral solutions (pH 3.1) containing different
 312 final Ca^{2+} concentrations (0.33 to 1.33 g.L⁻¹). A close linear relationship between the
 313 $[AG]_{critical}$ and the amount of Ca^{2+} added in the hydro-alcoholic – mineral solutions was
 314 obtained (Figure 4). The increase of Ca^{2+} concentration induced also the increase of
 315 $[AG]_{critical}$. Based on its structural and physicochemical properties, hyperbranched and
 316 negatively charged, AGPs from *A. senegal* gum appear as a natural mineral carrier that
 317 contains $\sim 2\text{--}3.4$ wt% of ash for this Acacia gum sample, and especially Ca^{2+} (Anderson,
 318 Douglas, Morrison & Weiping, 1990; Debon & Tester, 2001; Mhinzi, 2003). Lamport and
 319 Varnai evidenced that Ca^{2+} bound to AGPs with a fairly strong binding constant of 6.5×10^{-6}
 320 mol.L⁻¹ (Lamport & Varnai, 2013). They also showed that the Ca^{2+} binding sites of AGPs

were not naturally fully saturated by Ca^{2+} . Hence, as expected, we can easily suggest that AGPs stabilized the hydro-alcoholic – mineral solutions by its electrostatic binding with Ca^{2+} , the driver of the instability mechanism.

3.2. Identification AGPs from *A. senegal* gum involved in the colloidal stability mechanism of the hydro-alcoholic – mineral solution

As previously mentioned, *A. senegal* gum is a continuum of AGPs differing by their biochemical, structural and physicochemical properties (Randall et al., 1989; Renard et al., 2006). To better identify the macromolecules involved in this colloidal stabilization mechanism, *A. senegal* gum was fractionated using Hydrophobic Interaction Chromatography (HIC). The three fractions obtained were named HIC-F1, HIC-F2 and HIC-F3 according to their elution order and consequently to their increasing hydrophobic index. The biochemical composition and some structural properties of *A. senegal* gum and its HIC fractions were previously described (Mejia-Tamayo et al., 2018) and presented in supplementary data (table 1). The three HIC fractions are composed of the same sugars (D-galactose, L-arabinose, D-glucuronic acid and 4-O-methyl-D-glucuronic acid) with some differences in their proportions. The arabinose to galactose (Ara/Gal) molar ratio was 0.69, 1.03 and 1.29 for HIC-F1, HIC-F2 and HIC-F3, respectively. HIC-F1 was also richer in uronic acid (glucuronic and 4-O-methyl-glucuronic acids) than HIC-F2 and HIC-F3 fractions (21.7 wt% vs. 16.2 wt% and 14.4 wt%, respectively). Hence, the carbohydrate blocks of HIC-F1 were supposed to carry more negative charges than HIC-F2 and HIC-F3 ones. Biochemically, the main difference between HIC fractions was the protein content and consequently the protein/sugar ratio. HIC-F3 showed a higher amount of proteins (13.8 wt%) and then protein/sugar ratio (0.169) as compared to HIC-F2 (6.3 wt% and 0.069) and HIC-F1 (0.5 wt% and 0.005). Structurally, the three HIC fractions differed mainly by their mean molar mass (M_w) and high

346 molar mass (HM_w) macromolecules content ($M_w > 10^6 \text{ g.mol}^{-1}$). HIC-F2 and HIC-F3 were
 347 richer in protein-rich high molar mass (HM_w) AGPs than HIC-F1 (73, 56 and 3%,
 348 respectively) (supplementary table 1).

349 The stability curves of the hydro-alcoholic – mineral solution in the presence of the three HIC
 350 fractions are shown in the Figure 5. They can be classified from the most effective to the least
 351 in terms of stabilization as: $HIC-F3 > HIC-F2 > HIC-F1$. Thus, the critical concentrations of
 352 HIC-F1, HIC-F2 and HIC-F3 fractions were 0.570, 0.090 and 0.012 g.L^{-1} , respectively.
 353 Though HIC-F3 was the minor fraction in weight (around 1 wt% of whole *A. senegal* gum),
 354 its efficiency for the colloidal stabilization of the hydro-alcoholic – mineral solution was 48,
 355 10 and 7.5 times larger than those of HIC-F1, *A. senegal* gum and HIC-F2, respectively.
 356 Hence, the colloidal stability properties of *A. senegal* gum towards the hydro-alcoholic –
 357 mineral solution were mainly due to its two minor fractions in weight, HIC-F2 and HIC-F3
 358 (around 11 wt% of whole *A. senegal* gum), that were especially richer in protein-rich HM_w
 359 AGPs than HIC-F1 (Table 1).

360 HIC-F1 (89 wt% of whole *A. senegal* gum) that was characterized by poor colloidal
 361 stabilizing property contained also a low amount (3%) of protein-rich HM_w AGPs. To precise
 362 whether the macromolecular origin of the colloidal stabilizing properties is due to this
 363 fraction, its HM_w AGPs were removed by an enzymatic hydrolysis treatment using the
 364 pronase, a protease mixture. Several studies showed the specific hydrolysis activity of pronase
 365 for protein-rich HM_w AGPs from *A. senegal* gum without degrading and affecting the
 366 structural properties of low M_w (LM_w) AGPs (Connolly, Fenyo & Vandeveld, 1988; Randall
 367 et al., 1989; Flindt, Al-Assaf, Phillips & Williams, 2005; Mahendran, Williams, Phillips, Al-
 368 Assaf & Baldwin, 2008; Renard, Lavenant-Gourgeon, Lapp, Nigen & Sanchez, 2014). The
 369 refractive index (RI) signal and the M_w distribution of HIC-F1 before and after the enzymatic
 370 treatment are shown in Figure 6A. The pronase hydrolyzed the protein-rich AGPs decreasing

the HM_w AGPs content from 3 to 1.7%, without affecting the quantity and the structural properties of LM_w AGPs (Figure 6A). The colloidal stabilizing properties of the hydrolyzed HIC-F1 towards the hydro-alcoholic – mineral solution were characterized and compared to HIC-F1 (Figure 6C). The hydro-alcoholic – mineral solutions was totally unstable until 1.2 g.L⁻¹ of hydrolyzed HIC-F1, a concentration twice more than the minimal HIC-F1 concentration necessary to totally prevent the colloidal instability ($[HIC-F1]_{critical} = 0.570 \text{ g.L}^{-1}$). Hence, the removal of less than 1.5% of AGPs from HIC-F1, corresponding to HM_w AGPs, induced the loss of its colloidal stabilizing properties. The main contribution of protein-rich HM_w AGPs was also confirmed by studying the colloidal stabilizing properties of hydrolyzed *A. senegal* gum by pronase. After the enzymatic treatment, the content of protein-rich HM_w ($M_w > 10^6 \text{ g.mol}^{-1}$) AGPs decreased from 14 to 6% (Figure 6B). As observed for HIC-F1, *A. senegal* gum also lost its stabilizing properties after the pronase treatment: the hydro-alcoholic – mineral solutions containing 0.12 g.L⁻¹ of hydrolyzed *A. senegal* gum was totally unstable while $[AG]_{critical}$ was found to be 0.114 g.L⁻¹. These results highlighted the major role of the protein-rich AGPs in the colloidal stabilizing mechanism of the hydro-alcoholic – mineral solution. Hence, HIC-F2 and HIC-F3 fractions prevented the colloidal instability of the hydro-alcoholic – mineral solution (i.e. formation of salt precipitates) by their electrostatic binding with Ca^{2+} . The key role of the protein-rich AGPs on the functional properties of *A. senegal* gum was also evidenced by studying its interfacial properties (Elmaman, Al-Assaf, Phillips & Williams, 2008). In this study, the removal of the protein-rich AGPs by a pronase treatment resulted in the loss of the interfacial properties of *A. senegal* gum.

3.3. Colloidal stabilization of a hydro-alcoholic – grape marc solution and an unstable young red wine by AGPs from *A. senegal* gum

In order to correlate the results obtained on the hydro-alcoholic – mineral solution to the wine matrix, the stabilizing properties of *A. senegal* gum and its HIC fractions were characterized on an unstable synthetic hydro-alcoholic – grape marc solution (wine-like medium) and an unstable young red wine.

The hydro-alcoholic – grape marc solution and the young red wine that were stable at 25°C (control sample) were destabilized upon cooling to 10°C that induced the precipitation of the unstable colloidal substances as the coloring matter (Ribereau-Gayon et al., 2006; Alcade-Eon et al., 2014). The colloidal stability kinetics of the hydro-alcoholic – grape marc solution and the young red wine supplemented with *A. senegal* gum and HIC fractions were followed by measuring the transmittance of the solution during 48 h at 10°C. The increase of *A. senegal* gum concentration from 0 to 0.5 g.L⁻¹ led to the colloidal stabilization of the hydro-alcoholic – grape marc solution (Figure 7A). The solution supplemented with 0.5 g.L⁻¹ of *A. senegal* gum presented a similar kinetic behavior as the control sample at 25°C suggesting that AGPs from *A. senegal* gum totally prevented the colloidal instability. This result was in agreement with the use of *A. senegal* gum, and more globally the AGPs, as protective colloids in wines and model beverages to prevent the aggregation and precipitation of polyphenols and proteins (Ribereau-Gayon et al., 2006; Waters et al., 1994; Riou et al., 2002; Mateus et al., 2004; Luck, Liao, Murray, Grimmer, Warminski, Williamson, Lilley & Haslam, 1994; Liang, Liu, Qi, Su, Yu, Wang & He, 2013; Soares et al., 2009; de Freitas et al., 2003), and the chemical modifications of anthocyanins (Chung et al., 2016). The efficiency of *A. senegal* gum and its HIC fractions towards the colloidal instability of this hydro-alcoholic – grape marc solution were characterized by plotting the stability curves after 48h of kinetics (Figure 7B). The critical concentrations of *A. senegal* gum, HIC-F1, HIC-F2 and HIC-F3 fractions were found to be 0.245 ± 0.010 , 0.996 ± 0.090 , 0.118 ± 0.014 and 0.027 ± 0.003 g.L⁻¹, respectively. The colloidal stabilizing properties of *A. senegal* gum, HIC-F2 and HIC-F3 fractions were also

confirmed on an unstable young red wine (Figure 7C). They totally prevented the young red wine colloidal instability at 10°C for concentrations of 0.086 ± 0.007 , 0.045 ± 0.006 and 0.015 ± 0.002 g.L⁻¹, respectively. Unlike the other two HIC fractions, HIC-F1 was not efficient for the colloidal stabilization of the young red wine even at a concentration of 2 g.L⁻¹ (data not shown).

4. Discussion

The colloidal stabilizing properties of *A. senegal* gum and its HIC fractions isolated from Hydrophobic Interaction Chromatography were investigated on three colloidal hydro-alcoholic matrices: a hydro-alcoholic – mineral matrix, a hydro-alcoholic – grape marc solution (“wine-like” matrix) and an unstable young red wine. Whatever the hydro-alcoholic matrix tested, the HIC fractions can be classified in the same following order according to their colloidal stabilizing efficiency: HIC-F1 << HIC-F2 < HIC-F3. *A. senegal* gum that is a mixture of the HIC fractions presented some intermediate properties between those of HIC-F1 and HIC-F2. Before its addition in young red wines, *A. senegal* gum added in young red wines is usually chosen based on its ability to stabilize a hydro-alcoholic – mineral matrix. When we plot the critical concentrations of *A. senegal* gum and its HIC fractions determined in hydro-alcoholic – mineral solution as a function of those determined in hydro-alcoholic – grape marc solution or young red wine (Figure 8), a good correlation between both systems is obtained. The more the AGPs from *A. senegal* gum are efficient to stabilize the hydro-alcoholic – mineral solution, the better they are to stabilize the polyphenols in young red wines. Hence, these results confirmed and strengthened the relevance and the use of the hydro-alcoholic – mineral test for the evaluation of *A. senegal* gum colloidal stabilizing properties before its addition in young red wine.

445 In the hydro-alcoholic – mineral solution, the colloidal stability was assessed by the
 446 electrostatic binding of Ca^{2+} , the “positively charged” driver of the colloidal instability, with
 447 the negatively charged AGPs. According to the monosaccharide and amino acid compositions
 448 of *Acacia senegal* gum (Mejia-Tamayo et al., 2018), the negative charges of AGPs are carried
 449 out both by the carbohydrate moiety through the carboxylic groups of glucuronic and 4-O-
 450 methyl glucuronic acid monosaccharides, and the polypeptidic backbone by the carboxylic
 451 groups of aspartic and glutamic acid amino acid side chains. The content and proportion of
 452 these negatively charged uronic acids and amino acids are fraction dependent with a large
 453 excess of uronic acids in the three fractions. HIC-F1 has a higher uronic acid content (20.9
 454 wt% of whole fraction) as compared to HIC-F2 and HIC-F3 (14.9 wt% and 11.7 wt%,
 455 respectively) (supplementary table 1). Recently, Grein-Iankovski et al. (2018) characterized
 456 an acid dissociation constant (pK_a) between 3 and 4 for the AGPs from *A. senegal* gum that
 457 was attributed to the carboxylic group of glucuronic acids. This chemical property suggests a
 458 partial ionization of the carboxylic groups in the range of the pH studied in the hydro-
 459 alcoholic solutions, and moreover the increase of the negative charge of AGPs with the
 460 increase of pH from 3.1 to 4.0. This result is in well accordance with the decrease of the
 461 critical concentration of *A. senegal* gum when the pH is increased from 3.1 to 4.0. Recently,
 462 ‡The binding of Ca^{2+} with the carboxyl group of two uronic acids of a conserved O-Hyp-
 463 linked arabinogalactan polysaccharide was demonstrated using molecular dynamic
 464 simulations (Lamport & Varnai, 2013). Hence, based on the excess of uronic acid in the three
 465 HIC fractions and the molecular dynamic simulations it could be expected as a first
 466 approximation that the mineral colloidal stability occurred mainly by the binding of Ca^{2+} to
 467 the uronic acids of carbohydrate blocks. However, our results showed that HIC-F1, the richest
 468 fraction in uronic acids, had the weakest colloidal stabilizing properties, while HIC-F3, the
 469 one with the least uronic acids content, was the most effective fraction for the colloidal

470 stabilization of the hydro-alcoholic – mineral solution (Figure 5). Hence, the stabilizing
 471 properties of AGPs could not be only explained by their uronic acids content. We also showed
 472 a significant decrease of the stabilizing properties of HIC-F1 and *A. senegal* gum after the
 473 hydrolysis of their protein-rich AGPs by a pronase treatment. The pronase is a cocktail of
 474 proteases hydrolyzing the accessible polypeptidic backbone of protein-rich AGPs without
 475 degrading the low molar mass (LM_w) AGPs poor in proteins (Renard et al. 2014). This
 476 enzymatic treatment gave rise to macromolecules presenting similar molar masses (M_w from
 477 1.8 to 6.0×10^5 g.mol⁻¹) and conformational properties as low molar mass (LM_w) AGPs
 478 naturally found in *A. senegal* gum (Connolly et al., 1988, Randall et al., 1989, Aoki, Al-
 479 Assaf, Katayama & Phillips, 2007, Renard et al., 2014). Since the conformational properties,
 480 and probably the sugar organization, of the released carbohydrate blocks of AGPs was
 481 seemingly not disturbed by the pronase treatment, this suggested that the uronic acids of the
 482 carbohydrate blocks were certainly not directly involved in the electrostatic Ca^{2+} binding in
 483 this colloidal stability test. Similar results were obtained on AGPs purified from a red wine by
 484 Waters et al. These authors showed that the greatest protein haze protective activity in
 485 Chardonnay wine was reached with AGPs presenting the highest protein content. Moreover,
 486 this protective activity was not affected by the sugar hydrolysis of AGPs using α -
 487 arabinofuranosidase and endo-(1 $\rightarrow$ 6)- β -D-galactanase, while it was totally lost when the
 488 protein moiety was altered with the loss of 75% of initial protein after smith degradation
 489 treatment (Waters et al., 1994). These results revealed the key role of the accessible
 490 polypeptidic backbone of AGPs in their colloidal stabilizing properties. In the hydro-alcoholic
 491 – mineral solution, it could be hypothesized that the electrostatic binding of Ca^{2+} occurred
 492 with only the negatively charged amino acids (aspartic and glutamic acids) or with the
 493 involvement of both negatively charged amino acids and uronic acid monosaccharides
 494 localized close to the protein backbone.

As observed in the hydro-alcoholic – mineral solution, the protein-rich AGPs from *A. senegal* gum, HIC-F2 and HIC-F3 fractions, were also found to be the most effective for the colloidal stabilization of the hydro-alcoholic – grape marc solution and the unstable young red wine. The surface and colloidal stabilizing properties of the protein-rich AGPs were previously demonstrated in other type of matrices. After *A. senegal* gum fractionation by size exclusion chromatography (SEC) and hydrophobic interaction chromatography (HIC), Ray et al. showed that the protein-rich AGPs made the best emulsions in model beverage (Ray, Bird, Iacobucci & Clark, 1995). Protein-rich AGPs were also found to be the most effective in decreasing the interfacial tension of n-hexadecane-water interface (Castellani, Al-Assaf, Axelos, Phillips & Anton, 2010), to preferentially adsorb at the interface of latex dispersions (Snowden, Phillips & Williams, 1987) and oil droplets (Randall, Phillips & Williams, 1988), and to stabilize carbon nanotube dispersions (Li, Zhang, Jin & Cai, 2018).

The stabilizing properties of the protein-rich AGPs towards such a chemical diversity of molecules (minerals, polyphenols, proteins, oils, latex, etc...) could be relied to their intrinsic properties. Structurally, the three HIC fractions seems not so different. AGPs from these fractions are hyperbranched glycoproteins adopting more or less extended ellipsoidal conformations (Sanchez, Schmitt, Kolodziejczyk, Lapp, Gaillard & Renard, 2008; Renard, Garnier, Lapp, Schmitt & Sanchez, 2012; Renard, Garnier, Lapp, Schmitt & Sanchez, 2013; Renard et al., 2014; Lopez-Torrez et al., 2015). AGPs from HIC-F2 and HIC-F3 fractions also appeared as more “flexible” than those of HIC-F1 (Mejia-Tamayo et al., 2018). Biochemically, HIC fractions presented some more marked differences, especially in their protein content and consequently in their protein to sugar ratio (supplementary table 1) that influenced their hydrophobic and hydration properties (Mejia-Tamayo et al., 2018). HIC-F1, HIC-F2 and HIC-F3 were eluted in that order according to their protein content and increasing hydrophobic behavior. From our results, the higher the HIC fraction is rich in

protein, the more effective it is for the colloidal stabilization of the three hydro-alcoholic solutions. In the hydro-alcoholic – grape marc solution and young red wine, these results were in accordance with the putative stabilization of polyphenols by their binding with AGPs via the establishment of hydrophobic interactions and hydrogen bonding (de Freitas et al., 2003; Mateus et al., 2004; Soares et al., 2009; Soares et al., 2012; Chung et al., 2016). Hence, the protein content and certainly the amino acids composition and distribution in the protein backbone of AGPs from *A. senegal* gum appeared as the main factor for the expression of their colloidal stabilizing properties in hydro-alcoholic matrices. This behavior agrees with the work performed by Dickinson et al. dealing with the surface properties of Acacia gums. Based on the surface activity of various Acacia gums with different protein content, these authors previously evidenced a good correlation between the Acacia gum protein content and its interfacial properties (Dickinson, Murray, Stainsby & Anderson, 1988; Dickinson, 2003). The protein content appeared also as essential for the colloidal stabilizing properties of mannoproteins, other glycoproteins considered as protective colloids in wine. The addition of mannoproteins containing a high proportion of proteins (10 to 30%) to red wine prevented the coloring matter precipitation and protein haze in white wine (Waters et al., 1994a; Escot et al., 2001; Charpentier, Escot, Gonzalez, Dulau & Feuillat, 2004; Poncet et al., 2007), while the mannoproteins with a low protein content were devoid of protective activity (Charpentier et al., 2004; Guadalupe, Palacios & Ayestaran, 2007; Poncet et al., 2007; Guadalupe & Ayestaran, 2008).

It is useful to remember that AGPs are constituted by a protein backbone covalently linked to hyperbranched carbohydrate blocks that partially hindered it from its environment. Therefore, if we consider the protein as the major component for the colloidal stabilizing properties of AGPs, it would seem appropriate to consider not only the protein content but also its accessibility to its environment that is closely link to the rate of glycosylation. HIC-F1,

presenting the weakest colloidal stabilizing properties, is highly glycosylated as compared to HIC-F2 and HIC-F3: their protein to sugar ratios (w/w) were 0.005, 0.069 and 0.169, respectively. The high glycosylation rate of HIC-F1 cause the protein less accessible to its environment as demonstrated by its resistance to the protein hydrolysis assay (Flindt et al., 2005; Renard et al., 2014). Moreover, the rate of glycosylation also influenced the structural and physicochemical properties of HIC fractions. The more the AGPs were glycosylated, then hydrated, the less their flexibility and hydrophobic behavior were marked (Mejia Tamayo et al., 2018). Therefore, we can advance that the surface properties of AGPs are mainly due to the physicochemical properties of their protein backbone which are subtly modulated and controlled by their rate of glycosylation.

5. Conclusion

In this research, the colloidal stabilizing properties of arabinogalactan-proteins from *A. senegal* gum in hydro-alcoholic – mineral and hydro-alcoholic – polyphenols solutions were investigated. The AGPs prevented the colloidal instability of both calcium iron hexacyanoferrate salts in “model” hydro-alcoholic solution and polyphenols in young red wine. A good relationship was evidenced between the stabilizing properties of AGPs determined in these two hydro-alcoholic solutions. The protein moiety of the AGPs appeared to be essential for these functional properties whatever the hydro-alcoholic solutions. The more the AGPs were rich in proteins, the more their colloidal stabilizing efficiency were. In the hydro-alcoholic – mineral solution, the AGPs avoided the precipitation of potassium ferrocyanide salts by their electrostatic binding with Ca^{2+} , the driver of the instability.

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Figure 1. Transmittance changes of hydro-alcoholic – mineral solutions without (A) and with 0.09 (B) and 0.14 g.L⁻¹ (C) of *A. senegal* gum. The transmittance was registered at 25°C during 24h. Pictures of the solutions before and after 24h of kinetic are presented on the right.

Figure 2. (A) Colloid stability kinetics of hydro-alcoholic – mineral solutions containing various *A. senegal* gum concentrations: 0 (●), 0.05 (●), 0.08 (●), 0.09 (●), 0.10 (●), 0.11 (●) and 0.14 (●) g.L⁻¹. (B) Colloidal stability curve of hydro-alcoholic – mineral solution in presence of *A. senegal* gum after 24h of kinetic. The experiments were performed at 25°C. The line (figure 2B) is shown to guide the eyes. The experiments were triplicated.

Figure 3. (A) Colloidal stability curves of hydro-alcoholic – mineral solutions in presence of *A. senegal* gum (AG) at pH 3.1 (○), 3.5 (○) and 4.0 (○) after 24h of kinetic. (B) Relationship between the electrophoretic mobility of *A. senegal* gum (AG) and its critical concentration in hydro-alcoholic – mineral solutions. The lines (figure 3A) are shown to guide the eyes. The experiments were triplicated.

Figure 4. Relationship between the concentration of Ca^{2+} in the hydro-alcoholic – mineral solution and the critical stabilizing concentration in *A. senegal* gum ($[\text{AG}]_{\text{crit}}$). The experiments were triplicated.

774 Figure 5. Colloidal stability curves of hydro-alcoholic – mineral solution in presence of A.
 775 *senegal* gum (○), HIC-F1 (○), HIC-F2 (○) and HIC-F3 (○) fractions after 24h of kinetic. The
 776 experiments were performed at 25°C. The lines are shown to guide the eyes. The inset figure
 777 is a zoom of the left part. The experiments were triplicated.
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782 Figure 6. Molar mass distribution (thick line) and normalized refractive index signal (thin
783 line) of HIC-F1 (A) and *A. senegal* gum (B) before (black) and after (red) pronase treatment.
784 Colloidal stability curves of hydro-alcoholic – mineral solution in presence of HIC-F1 (C) and
785 *A. senegal* gum (D) before ($\circ$) and after ($\bullet$) pronase treatment after 24h of kinetic. The lines
786 (figures 6C and 6D) are shown to guide the eyes. The colloidal stability experiments were
787 triplicated.

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792 Figure 7. (A) Colloid stability kinetic of hydro-alcoholic – grape marc solutions according to
 793 *A. senegal* gum (AG) concentration at 10°C. The concentrations of *A. senegal* gum were 0
 794 (●), 0.01 (●), 0.025 (●), 0.05 (●), 0.1 (●) and 0.5 (●) g.L⁻¹. The colloidal stability kinetic of
 795 the hydro-alcoholic – grape marc solution at 25°C without *A. senegal* gum is shown as a
 796 control (●) (B) Colloidal stability curves of hydro-alcoholic – grape marc solution in presence
 797 of *A. senegal* gum (○), HIC-F1 (○), HIC-F2 (○) and HIC-F3 (○) fractions after 48h of kinetic
 798 at 10°C. (C) Colloidal stability curves of a young red wine in presence of *A. senegal* gum (○),
 799 HIC-F2 (○) and HIC-F3 (○) fractions after 48h of kinetic at 10°C. The lines (figures 7B and
 800 7C) are shown to guide the eyes. The inset figure is a zoom of the left part. The experiments
 801 were triplicated.

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Figure 8. Relationship between the critical concentrations of *A. senegal* gum (AG) and its HIC-fractions determined in hydro-alcoholic – mineral solution and those determined in hydro-alcoholic grape marc solution (A) and young red wine (B). The experiments were triplicated.

Supplementary table 1. Biochemical and structural properties of *Acacia senegal* gum (AG), and its HIC fractions.

	AG	HIC-F1	HIC-F2	HIC-F3
Total dry matter (mg.g ⁻¹)	893.4	921.6	926.2	921.9
Sugars (mg.g ⁻¹)	944.4	961.3	918.3	813.0
Galactose	363.6	374.9	315.9	270.7
Arabinose	281.4	257.6	326.9	311.4
Rhamnose	120.9	120.2	125.8	113.0
Glucuronic acid	168.1	195.1	143.3	111.4
4-O-Me-Glucuronic acid	9.4	13.5	5.5	5.7
Proteins (mg.g ⁻¹)	21.5	4.9	63.1	137.7
Ash (mg.g ⁻¹)	34.1	30.5	19.3	49.3
M _w (g.mol ⁻¹)	6.8 × 10 ⁵	3.5 × 10 ⁵	1.5 × 10 ⁶	1.6 × 10 ⁶
High M _w content (%) ^a	14	3	73	56

^a The high M_w corresponded to macromolecules with a M_w upper than 10⁶ g.mol⁻¹.

Highlights.

- AGPs from *Acacia senegal* gum prevent the coloring matter precipitation
- The more AGPs are rich in proteins, the more their stabilizing efficiency are
- Stabilizing properties of AGPs are correlated in “synthetic” solutions and red wine
- In “synthetic” mineral solution, AGPs avoid the precipitation by Ca^{2+} binding

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