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Abstract

In sparkling wine, foam characteristics are one of the major attributes. The foam quality depends on wine components. Bentonite is added to the base wine to facilitate the riddling process, but causes a loss of foamability. *Acacia* gum can be used as additive in wine. We have studied if the addition of *Acacia senegal* gum (*Asen*G), *Acacia seyal* gum (*Asey*G) and different *Asen*G fractions could improve the foamability of different base wines treated with bentonite. The foamability differs depending on the gum or the gum fraction treatment but also on the wine, being these differences linked to some aspects of their respective compositions and molecular parameters. *Asen*G and *Asey*G increase the foamability (by Mosalux - sparging procedure), respectively, in five and seven out of eight base wines treated with bentonite. Therefore, *Asen*G and *Asey*G are potential treatments increasing the foamability of these wines.

Keywords: Sparkling wine; bentonite; *Acacia* gum; foamability; polysaccharides; amino acids.

1. Introduction

Many sparkling wines are elaborated following the traditional or bottle-fermented method with a consequent second fermentation in closed bottles of base wines. The most famous sparkling wines include, among others, champagne from France, cava from Spain or prosecco from Italy. In sparkling wines, foam characteristics are major attributes observed by the consumer when serving and also when drinking them, being a key parameter of their quality (Martínez-Lapuente, Guadalupe, Ayestarán & Pérez Magariño, 2015). For this reason, winemakers are very interested in understanding the factors that affect the foamability of wine. Foam is a two-phase system of gas and bubbles, being separated by thin liquid layers. Foam characteristics depend on wine components that reduce surface tension and enhance the viscosity of the film between the bubbles (López-Barajas, Viu-Marco, López-Tamames, Buxaderas & de la Torre-Boronat, 1997). But wine is a very complex matrix which is composed mainly of water, alcohols, polyols, organic acids, nitrogenous compounds and polyphenolic compounds. Moreover, there are also complex carbohydrate molecules, including polysaccharides and oligosaccharides originating from grapes, yeasts and bacteria during the winemaking. Several authors (Abdallah, Aguié-Béghin, Abou-Saleh, Douillard & Bliard, 2010; Martínez-Lapuente et al., 2015) have investigated the impact of macromolecules on foam quality. Proteins seem to have a main role in foam stability, although several works designed and focused in various ways (Girbau-Sola, López-Tamames, Buján & Buxaderas, 2002; Vanrell, Canals, Esteruelas, Fort, Canals & Zamora, 2007; Coelho, Reis, Domingues, Rocha & Coimbra, 2011) show contradictory conclusions. They could be explained by environmental conditions variety, which could strongly influence the proteins of grapes (Ferreira, Piçarra-Pereira, Monteiro, Loureiro & Teixeira, 2002), as well as by the use of different matrices such as reconstituted wines, base wines or sparkling

73 wines. Polysaccharides have been also implicated in sparkling wine foam characteristics
74 (Girbau-Sola et al., 2002; Abdallah et al., 2010; Martínez-Lapuente et al., 2015).
75 Oligosaccharides are carbohydrates consisting of two to ten monosaccharide residues, and
76 their composition and content of base wines can be influenced by winemaking process, grape
77 variety and vintage (Jégou et al., 2017). However, there are very few works relating
78 oligosaccharides with the foaming properties of wine. The synergistic interaction of the
79 active foam compounds, such as peptides, proteins and complex carbohydrates, could modify
80 their surface-active properties and, hence, the foaming properties (Martínez-Lapuente et al.,
81 2015), but most of the studies present contradictory results (López-Barajas et al., 1997; Lao,
82 Santamaria, López-Tamames, Bujan, Buxaderas & de la Torre- Boronat, 1999; Girbau-Sola
83 et al., 2002).
84 Bentonite, a montmorillonite clay, is usually employed to prevent the protein haze in white
85 wine. Champagne and Cava winemakers often add bentonite to the wine in order to facilitate
86 the riddling process (Vanrell et al., 2007). Addition of bentonite carries a net negative charge
87 at the pH of wine, interacting electrostatically with the positively charged wine proteins, and,
88 therefore, causing their flocculation (Sauvage, Bach, Moutounet & Vernhet, 2010). Marchal,
89 Chaboche, Douillard and Jeandet (2002) and Dambrouck, Marchal, Cilindre, Parmentier and
90 Jeandet (2005) reported a loss of wine foamability after addition of bentonite, relating it to
91 the drastic reduction of protein amount. Therefore, it seems obvious that the wine industry
92 must search for new techniques to prevent or to reduce/compensate the undesirable effects of
93 bentonite treatment on the quality of the wine foam.
94 *Acacia* gum is a natural highly glycosylated hydroxyproline-rich arabinogalactan-peptide and
95 arabinogalactan-proteins exuded by *Acacia* trees species (i.e. *Acacia senegal* and *Acacia*
96 *seyal*). The composition and molecular characteristics of *Acacia* gum differ depending on

several aspects such as the *Acacia* specie (Lopez-Torrez, Nigen, Williams, Doco & Sanchez, 2015). *Acacia senegal* gum can be separated using the hydrophobic interaction chromatography (HIC) in three main fractions: a major fraction (HIC-F1), with low protein amount and low molar mass, a second fraction (HIC-F2) rich in protein and showing high molar mass and finally a minor fraction (HIC-F3) with the highest protein content and also with high molar mass (Renard, Lavenant-Gourgeon, Ralet & Sanchez, 2006; Sanchez et al., 2018). *Acacia* gum is employed in several industrial applications, such as pharmaceutical, cosmetic and textile uses, as well as a food additive (E414) (Sanchez et al., 2018). In wine production, *Acacia* gum is authorized as additive, being largely employed as a protective colloid to prevent the precipitation of the coloring matter in red wine (Pellerin & Cabanis, 1998). This substance also confers body to the wine (Sanchez et al., 2018). According to International Organisation of Vine and Wine (OIV, 2019), the dose used of *Acacia* gum shall not exceed 300 $mg \cdot L^{-1}$. On the other hand, one of the valuable features of *Acacia* gum is the possibility of forming complex with proteins, which may stabilize air/water interfaces in several foamed products (Dickinson, 2008). The adsorption of *Acacia* gum/protein complexes at the air bubble interfaces can improve the stability of the foam (Schmitt & Kolodwiejczyk, 2010). Therefore, these properties could be used to form and stabilize foams (Sanchez et al., 2018).

In this work, the main objective was to see if the addition of *Acacia senegal* gum (*Asen*G) and *Acacia seyal* gum (*Asey*G) could improve the foam characteristics of sparkling base wines. The secondary goal was to deepen in the knowledge about the possible link between foam properties and gum composition. For this reason, we have also included the use of HIC-separated fractions (HIC-F1, HIC-F2 and HIC-F3) from *Asen*G, due to their different composition (in protein content, amino acid composition and molecular weight distribution).

First of all, we have added *Acacia* gums and their fractions in a synthetic wine, trying to eliminate the potential matrix effects. Subsequently, the foam parameters were measured. Secondly, based on the results obtained in the synthetic wine, we have studied the foam features adding the same treatments in base wines from different grape cultivars (Moscatel, Macabeo, Chardonnay and Pinot noir) and several origins (Tarragona, Saragossa and Malaga, in Spain; Champagne region in France). Finally, we have related the foam features with some composition aspects and molecular parameters of base wines and also of *Acacia* gums and *AsenG* fractions. Eight base wines and two different gums, together with three HIC-separated fractions from *Asen*G have been used to fulfil these aims. In our knowledge, this is the first work which studies the effect of addition of *Acacia* gum and gum fractions on the foam properties of sparkling base wines.

2. Material and methods

2.1. Wine samples

A synthetic wine (SYWI) devoid of grape and yeast colloids was prepared containing 12% (v/v) ethanol and 3 $g \cdot L^{-1}$ of tartaric acid, and its pH was adjusted to 3.2 with 4M NaOH. Moreover, eight monovarietal base wines were elaborated by the traditional white winemaking method. Three wines were elaborated in three different Spanish regions: in Malaga (MA) from Moscatel grapes and in Saragossa (SA) and Tarragona (TA) from Macabeo grapes. The origin of the other five wines was the French region of Champagne (close to Reims). Two monovarietal base wines of Chardonnay were elaborated at the cooperative winery Nogent l'Abbesse (NO1 and NO2), whereas the rest of monovarietal base wines elaborated with Pinot noir (RU1) and Chardonnay (RU2 and RU3) were provided by

145 Reims University. The enological characteristics of the eight wines are within the classical
146 values for base wines (alcoholic degree: between 10 and 13% v/v; titratable acidity: between
147 3 and 7 $g \cdot L^{-1}$, expressed in sulfuric acid; pH: between 3.0 and 3.5). One part of all the eight
148 base wines were treated with bentonite (20 $g \cdot hL^{-1}$; Microcol Alpha®, Laffort), stirred gently
149 for a few hours, kept in cold storage (10 days, 4° C), racked and filtered (1 μm). The obtained
150 bentonite-treated wines were coded as CO (control wine) followed by its corresponding
151 origin, resulting in COMA (control wine from Malaga), COSA (control wine from
152 Saragossa), COTA (control wine from Tarragona), CONO1 and CONO2 (control wines from
153 the cooperative winery Nogent l'Abbesse) and CORU1, CORU2 and CORU3 (control wines
154 from the University of Reims) and forming the CO wines. A sample without bentonite was
155 performed in each wine, and these non-bentonite-treated wines were coded as ORI (original
156 wine) followed by its corresponding origin, resulting in ORIMA (original wine from
157 Malaga), ORISA (original wine from Saragossa), ORITA (original wine from Tarragona),
158 ORINO1 and ORINO2 (original wines from the cooperative winery Nogent l'Abbesse) and
159 ORIRU1, ORIRU2 and ORIRU3 (original wines from the University of Reims) and forming
160 the ORI wines.
161
162 *2.2. Isolation of polysaccharide and oligosaccharide fractions from base wines*
163 Following the methodology previously described (Jégou et al., 2017; Apolinar-Valiente,
164 Ruiz-García, Williams, Gil-Muñoz, Gómez-Plaza & Doco, 2018), 5 mL of wine were
165 partially depigmented onto a polyamide column, being eluted the not retained
166 polysaccharides and oligosaccharides. High-resolution size exclusion chromatography was
167 subsequently performed and polysaccharides and oligosaccharides were separately collected
168 according to their elution time. Elution was performed using a Superdex-30 HR column (60 x

169 1.6 cm, Pharmacia, Sweden) with a precolumn (0.6 x 4 cm) equilibrated at 1 mL·min^{-1} with
170 30 mM ammonium formiate (pH 5.6). The isolated fractions were freeze-dried, redissolved
171 in water, and freeze-dried again four times to remove completely the ammonium salt.
172
173 *2.3. Complex carbohydrate analysis of base wines*
174 Reported by Apolinar-Valiente et al. (2018), neutral monosaccharides were released after
175 hydrolysis of the wine polysaccharides by treatment with TFA (120° C, 75 min) and
176 quantified by gas chromatography (GC) analysis. The addition of all the neutral
177 monosaccharides was used to calculate the total polysaccharide content. We have calculated
178 the percentage of each polysaccharide family (mannoproteins (MPs), polysaccharides rich in
179 arabinose and galactose (PRAGs), and rhamnogalacturonans type II (RG-II)) based on the
180 neutral monosaccharide content, as previously reported (Apolinar-Valiente et al., 2018).
181 The total oligosaccharide content was calculated on the basis of the neutral and acidic sugar
182 composition of the wine oligosaccharide fraction. It was determined after solvolysis with
183 anhydrous MeOH containing 0.5 M HCl (80 ºC, 16 h), by GC of their per-O-
184 trimethylsilylated methyl glycoside derivatives (Doco, O'Neill & Pellerin, 2001).
185
186 *2.4. Amino acid composition of base wine proteins*
187 Following the method described by Lowry, Rosenbrough, Farr and Randall (1951), and
188 modified by Potty (1969), 25 mL of trichloroacetic acid (TCA) at 10% were added to 10 mL
189 of wine and were kept at 4ºC for 2 hours to precipitate the proteins of base wines. The tubes
190 were centrifuged (38 400 g, 20 min) and the supernatant liquid was removed. Four washes
191 were realized with 1 mL of MilliQ water each one, and after transference to hydrolysis tubes
192 the samples were freeze-dried.

193 Amino acid composition from freeze-dried samples was determined following the
194 methodology previously described by Lopez-Torrez et al. (2015). Samples were hydrolyzed
195 with 6 N HCl and heating at 110°C for 24h. The excess of acid was eliminated by washing
196 twice with water (0.5 mL) and once with absolute ethanol (0.5 mL), and hydrolyzed samples
197 were analyzed by liquid chromatography with a Biochrom 30 analyser (BIOCHROM 30,
198 Cambridge, UK) using an ion-exchange column (Ultra-pac-8 lithium form; Amersham
199 Pharmacia Biotech, Piscataway). Lithium citrate (0.2 M, pH 2.2) was used as eluent and
200 norleucine as internal standard. The total amino acids content (TAAs) was calculated by
201 adding the amount of all the amino acids from the hydrolysis of the wine proteins
202 precipitated with TCA.
203
204 *2.5. Acacia gum samples*
205 *Acacia* gums from *Acacia senegal* trees (*Asen*G) (Lot: OF152413) and from *Acacia seyal*
206 trees (*Asey*G) (Lot: OF110724) were provided by ALLAND & ROBERT Company – Natural
207 and organic gums (Port Mort, France).
208
209 *2.6. Fractionation of AsenG by Hydrophobic Interaction Chromatography (HIC)*
210 Following the classical fractionation method (Renard et al., 2006), macromolecular
211 fractions, HIC-F1, HIC-F2, and HIC-F3 were obtained from *Asen*G soluble powder by HIC
212 performed at room temperature on one Phenyl-Sepharose CL-4B (Sigma, St. Louis, Mo)
213 column (40 x 2.6 cm) equilibrated with degassed 4.2 M NaCl. *Asen*G was dissolved in water
214 (100 $g \cdot L^{-1}$), stirred overnight to allow the complete hydration, loaded and eluted successively
215 by 4.2 M NaCl (fraction HIC-F1), 2 M NaCl (fraction HIC-F2), and finally water (fraction
216 HIC-F3) at a flow rate of 1 $mL \cdot min^{-1}$. HIC-F1 and HIC-F2 dispersions were desalted by

diafiltration against deionized water through an AKTA FLUX 6 system (GE Healthcare, Upsala, Sweden) using a transmembrane pressure of 15 psi. The membrane used was a polysulfone hollow fiber (GE Healthcare) with a nominal molecular weight cut off of 30 kDa. The samples were consequently concentred and spray dried. The excessive material losses during this procedure explain the different methodology used to remove salt from HIC-F3 fraction. The HIC-F3 fraction was concentrated using a rotovapor, dialysed for 72 h and freeze dried. Mejia Tamayo et al. (2018) reported their neutral sugars and uronic acid composition, their protein content measured by the Kjeldhal method and their basic molecular parameters.

2.7. Composition and total content of amino acids of Acacia gum samples

Amino acid composition of *Asen*G, *Asey*G, HIC-F1, HIC-F2 and HIC-F3 samples were determined following the methodology previously described by Lopez-Torrez et al. (2015). This procedure is stated above in the section on the amino acid composition of base wine proteins.

2.8. Supplementation of SYWI and CO wines by Acacia gums and AsenG fractions

On the one hand, *Asen*G, *Asey*G, HIC-F1, HIC-F2 and HIC-F3 were separately added to synthetic wine (SYWI) at 600 $mg \cdot L^{-1}$. On the other hand, *Asen*G and *Asey*G were separately added at 300 $mg \cdot L^{-1}$ to the eight CO wines, whereas HIC-F1, HIC-F2 and HIC-F3 were also separately added (300 $mg \cdot L^{-1}$) to two selected CO wines (COMA and CONO2). These wines treated with gums or *Asen*G fractions formed the CO-supplemented wines. In all the cases, gum or gum fraction powder was gently stirred (20 °C, 24 h).

241 *2.9. Sparging procedure (Mosalux method) to measure foaming parameters*

242 We have compared the separate addition of *Asen*G and *Asey*G on SYWI (at 600 mg·L^{-1})
243 using a classical gas-sparging method (the so-called Mosalux) as described by Maujean,
244 Poinsaut, Dantan, Brissonnet and Cossiez (1990). We have also used this method to compare
245 CO wines supplemented with *Asen*G and *Asey*G (at 300 mg·L^{-1}) with ORI and CO wines.
246 Moreover, we have studied the potential impact of the addition of the different studied
247 fractions from *Asen*G (HIC-F1, HIC-F2, HIC-F3) in SYWI at 600 mg·L^{-1}. Two selected CO
248 wines (COMA and CONO2) supplemented with *Asen*G fractions (HIC-F1, HIC-F2, HIC-F3)
249 were also compared to ORI and CO wines. 100 mL of the sample was introduced in a glass
250 cylinder having a glass frit (pore size 16–40 µm) at the bottom. The carbon dioxide gas was
251 injected through the glass frit at a constant rate flow (7 L·h^{-1}) and a constant pressure (1 bar).
252 Foam height was surveyed during gas injection for 5 min. We have measured the foamability
253 corresponding to the maximum height (HM) expressed in mm reached by the foam column.
254 Besides, we have noted the foam stability height (HS) representing the height at which the
255 foam stabilizes during gas injection, expressed as mm. The beginning of the foam
256 stabilization time varied between second and third min depending on the wine. All the
257 experiments were done in triplicate in a room with controlled temperature (18 ± 1° C).
258
259 *2.10. Pictures*

260 Pictures of control SYWI and SYWI-treated samples were taken after four minutes of gas
261 injection during the analysis by Mosalux in control and treated samples, with the objective of
262 achieving a better observation and analysis of the foam quality.
263
264 *2.11. Statistical procedures*

265 Pearson correlations and multiple regression analysis were applied to results from
266 Mosalux procedure to examine the relationships between foam features and the chemical
267 composition of wines, gums and HIC-fractions from *Asen*G. Pearson results are considered
268 significant when degree of significance (*p*) is lower than 0.05. Concerning multiple
269 regression analysis, we have taken into account only the significant relations when R^2 is
270 higher than 75%. This percentage maybe does not allow us to make precise prediction
271 equations, but it enables us ensuring consistent trends. Besides, we have used a maximum of
272 two independent variables for robust statistics. Statgraphics Centurion XVI.I software
273 (StatPoint Technologies, Inc., USA) was used to apply Pearson and multiple regression
274 analysis.
275

276 **3. Results and discussion**

277 At first, the foaming parameters measured by Mosalux procedure in control SYWI and
278 SYWI-treated samples are shown. The pictures taken after 4 minutes of gas injection to these
279 samples get information about the foam aspect and the size of bubbles. Secondly, the foam
280 features in ORI and CO wines, as well as in CO-supplemented wines are given. Finally, we
281 explore the link between the sparkling base wine foamability and the complex carbohydrate
282 and amino acid content of wines and gum treatments, as well as concerning some basic
283 molecular and structural parameters of gum treatments.
284 SYWI was not the final aim of this work but it represented a good tool to obtain deeper
285 knowledge which allows us achieving our objectives. For this reason, we consider that it
286 could be interesting to test treatments on SYWI at a dose (600 $mg \cdot L^{-1}$) greater than the
287 permitted concentration (300 $mg \cdot L^{-1}$) (OIV, 2019). However, and considering that the

288 primary aim of our work is focused in sparkling base wines, we preferred testing the
289 maximum levels permitted in these samples.
290
291 *3.1. Foaming parameters on SYWI after gum and HIC-fractions addition at 600 mg·L^{-1}*
292 HM and HS of control synthetic wine (SYWI), as well as of SYWI formulated with
293 *Asen*G, *Asey*G, HIC-F1, HIC-F2 and HIC-F3 (600 mg·L^{-1}) are presented in Figure 1. The fact
294 that we have eliminated all the possible matrix effects allows us to focus only on the
295 characteristics and composition of gum or gum fractions treatments. Both HM and HS
296 increased considerably when *Asen*G and mainly *Asey*G were added to SYWI. On the other
297 hand, these two foam parameters also improved when HIC-F1 was used, but they increased
298 greatly when HIC-F2 or HIC-F3 was added to SYWI.
299 The varying hydrophobicity of studied HIC-fractions could affect their general influence on
300 the foam height of SYWI. Onishi and Proudlove (1994) reported that the absolute level of
301 hydrophobic polypeptide is important to enter into and stabilize foam, rather than the ratio of
302 hydrophobic to hydrophilic polypeptides. We have therefore estimated the hydrophobic score
303 (Table 1) through the amino acid composition data of gum and HIC-fractions (Table 1) and
304 the hydrophobicity scale proposed by Monera et al. (1995), using the non-polar hydrophobic
305 amino acids (alanine, isoleucine, leucine, phenylalanine, proline and valine). For every gum
306 or HIC-separated fraction from *AsenG*, the content of each hydrophobic amino acid is
307 divided by the total content of amino acids, and the result is multiplied by its corresponding
308 coefficient from the hydrophobicity scale (footnote in Table 1). The sum of the values of all
309 the hydrophobic amino acids corresponds to the hydrophobic score of gums or HIC-
310 separated fractions from *AsenG*. The order of increased hydrophobicity of these gum
311 fractions was HIC-F1<HIC-F2<HIC-F3 according to the principle of separation method

312 (Mejia-Tamayo et al., 2018) as well as to the calculated hydrophobicity score (Table 1),
313 which corresponded with the order of the foamability. Brissonnet and Maujean (1993)
314 suggested that hydrophobic proteins accessed easier into the foam than hydrophilic ones.
315 According to these authors, polypeptides with a greatly hydrophobic exterior showed a
316 higher trend to interact with other compounds of bubble wall (interface gas/liquid), whereas
317 hydrophilic proteins presented a higher affinity for water than for bubble surfaces. Onishi and
318 Proudlove (1994) observed a correlation between polypeptide molar mass, hydrophobicity
319 and foam stabilizing activity in beer. In the same line, Moreno-Arribas, Pueyo and Polo
320 (1996) reported that the hydrophobicity of the characterized peptides could account for the
321 foamability of sparkling wine. Indeed, it is important to remark that the differences in
322 hydrophobicity of gums and gum fractions were intimately linked to their total amino acid
323 content, which followed the order HIC-F1<*Asey*G<*Asen*G<HIC-F2<HIC-F3 (Table 1). It can
324 be hence deduced that the impact of hydrophobicity was strongly related with the amount of
325 amino acids, resulting both of them in a great impact on the foam features of the different
326 synthetic wines. As will be discussed below, the form and the aspect of the bubbles could be
327 a reason for the reverse behavior of both *Acacia* gums when their foam parameters values
328 (*Asen*G<*Asey*G) were compared to their hydrophobicity values (*Asey*G<*Asen*G).
329 The foam pictures of control SYWI as well as SYWI with separate addition (600 $mg \cdot L^{-1}$) of
330 *Asen*G, *Asey*G and HIC fractions from *Asen*G after 4 minutes of gas injection using a
331 sparging procedure are shown in Figure 2. The foam aspect presented two distinguishable
332 regions in *Asey*G (Fig. 2). The first region was placed between 0.5 and 1.5 mm in height and
333 presented similar foam aspect than the other treated samples, including *Asen*G. The second
334 region was less compact and presented larger bubbles (between 2 and 4 mm in diameter at
335 the top of the foam). In contrast, for the rest of treated samples, the foam appeared in a single

region as compact and showed small bubbles (between 0.5 and 2 mm diameter at the top of the foam). In that connection, it is important to recall that HM increased in a major way when *Asey*G was added in comparison with addition of *Asen*G, even although *Asey*G showed lower TAAs, protein content and hydrophobicity score (Table 1). The larger bubbles observed in the second region (at the top of the foam) after *Asey*G addition could explain this apparent discrepancy. In beverages and foods, the foam's texture appears determined by size and distribution of the bubbles (Blasco, Viñas & Villa, 2011). Small bubbles are uniformly distributed and would result in soft foam (Wilde & Clark, 1996). Focusing on sparkling wines, Liger-Belair (2005) mentioned that their quality is often linked to the size of bubbles. According to this author, small bubbles would rise slowly, being preferred to large bubbles. Flavor release and mouthfeel could also be influenced by bubble size (Liger-Belair, 2005).

3.2. Foaming parameters on ORI and CO wines

Figure 3 presents HM (Figure 3A) and HS (Figure 3B) of ORI wines (non-bentonite-treated wines) and CO wines (bentonite-treated wines). For the eight base wines studied, ORI wines presented the highest HM compared to the other three categories (Fig. 3A). This behavior was undoubtedly linked to the undesirable bentonite action causing a loss of foamability, as previously reported (Marchal et al., 2002; Dambrouck et al., 2005). The TAAs of the ORI and CO wines proteins is listed in Table 2, and was calculated by the sum of all the different amino acids from the hydrolysis of the wine proteins precipitated with TCA (Supplementary Table 1A and 1B). In agreement with previous works (Puig-Deu, Lopez-Tamames, Buxaderas & Torre-Boronat, 1999; Dambrouck et al., 2005), we observed that TAAs decreased largely (reduction ranged between 87% and 99%) after bentonite treatment in any wine. Therefore, the bentonite treatment successfully acted in all wines

studied. Furthermore, and knowing that *Acacia* gums present protein percentage of 2.15% (*Asen*G) and 0.77% (*Asey*G) (Table 2), their addition at 300 $mg \cdot L^{-1}$ would correspond, respectively, to ~6.5 and ~2.3 mg of protein per liter of base wine. This protein amount is lower than that removed by bentonite treatment, which ranged between ~15 and ~69 $mg \cdot L^{-1}$, Therefore, it would seem reasonable to assume that the addition of *Acacia* gums not would neutralize the positive effect of the bentonite treatment, which was to get rid of proteins.

Table 2 also shows that, between the ORI wines, ORIRU1 exhibited the highest TAAs, while ORIMA presented the lowest value. These different values were in agreement with the largely reported variability of the wine protein content because of the different cultivars, climatic and soils conditions and cultural and oenological practices (Ferreira et al., 2002).

On the other hand, and compared to CO wines, ORI wines presented the highest HS in every Spanish wine (ORIMA, ORISA and ORITA) and in one French wine (ORINO2), whereas the rest of ORI wines showed similar (ORINO1, ORIRU1, ORIRU2 and ORIRU3) values for this parameter (Fig. 3B). The different behaviors depending on the ORI wine could be explained by variations in their protein composition (Supplementary Table 1A). Some proteins have been described in the literature as poor foam formers but good stabilizers, whereas others are good foam formers but poor stabilizers (López-Barajas, López-Tamames, Buxaderas & de la Torre-Boronat, 1998; Lao et al., 1999). In this regard, correlations between protein concentration and foam stability have presented contradictory results (Girbau-Sola et al., 2002; Vanrell et al., 2007; Blasco et al., 2011; Coelho et al 2011).

3.3. Foaming parameters on CO wines after AsenG and AseyG separate addition at 300 $mg \cdot L^{-1}$

383 Figure 3 shows HM (Figure 3C) and HS (Figure 3D) of CO wines (bentonite-treated
384 wines), CO wines plus *Asen*G and CO wines plus *Asey*G. HM of all the Spanish CO wines
385 was significantly increased by the *Asen*G (COMA: +24%; COSA: +6%; COTA: +6%) and
386 also by the *Asey*G (COMA: +22%; COSA: +10%; COTA: +12%) treatments (Fig. 3C).
387 Concerning the French CO wines, HM of CONO1 and CONO2 was significantly enhanced
388 after *Asen*G (CONO1: +8%; CONO2: +11%) and *Asey*G (CONO1: +5%; CONO2: +7%)
389 additions. Moreover, CORU2 also showed a rising value (+6%) of HM when *Asey*G was
390 used (Fig. 3C). In short, *Asen*G addition increased HM in five out of eight CO wines,
391 whereas *Asey*G did likewise in six out of eight CO wines.
392 HS parameter could represent the wine's ability to produce stable foam or persistence of
393 foam collar in a glass (Martínez-Lapuente et al., 2015). COMA, COSA and CONO2
394 increased their HS when *Asen*G (+17%, +7% and +6%, respectively) or *Asey*G (+21%, +4%
395 and +8%, respectively) were used (Fig. 3D). In the case of COSA, the addition of *Asen*G
396 increased HS even over the HS value of ORISA. The addition of *Asey*G to CORU3 enhanced
397 its HS over the initial HS value (+9%). To resume, *Asen*G addition enhanced HS in three out
398 of eight CO wines, whereas *Asey*G did the same in four out of eight CO wines.
399
400 *3.4. Foaming parameters on two selected wines after separate addition of HIC-fractions at*
401 *300 mg·L^{-1}*
402 The impact of the addition of *Asen*G HIC-fractions to selected COMA and CONO2 wines
403 is presented in Figure 4. The first reason for the choice of these two wines was their different
404 country of origin. Within each origin, we selected the wine with a better improvement of
405 both foamability parameters (HM and HS) after separate *Acacia* gums additions, according to
406 Figures 3C and 3D. Compared to CO wines, no effect was observed on HM or HS

parameters after HIC-F1 addition. This behavior can be explained by the low TAAs value in this fraction (4%, Table 1), as well as by the low surface accessibility of amino acids to the high glycosylation of the polypeptide backbone. On the other hand, both HM and HS parameters were improved when HIC-F2 was added to CONO2 (+11% and +7%, respectively). This treatment only enhanced the HM in the case of COMA (+6%). An opposite effect on both foam parameters was observed when HIC-F3 was added: while COMA presented decreasing values with this treatment (HM: –18%; HS: –22%), CONO2 exhibited improving values (HM: +9%; HS: +7%). The differences in composition between COMA and CONO2 base wines (Table 2) could explain this reverse impact after HIC-F3 addition, as widely discussed in section 3.8.

In brief, all the results presented in the latter two sections shows that the foam behavior depended on the wines and/or the gum or gum fraction treatment. *Asen*G increased HM or HS in five out of eight CO wines (an efficiency of 63% of the cases), whereas *Asey*G did likewise in seven out of eight CO wines (an efficiency of 88% of the cases). Therefore, we can affirm that the separate addition of these two gums, and particularly of *Asey*G, is an interesting tool to achieve a certain foamability recovery after the bentonite treatment.

3.5. Complex carbohydrate composition of wines

Table 2 shows the wine polysaccharide families (%) and the total content of polysaccharides ($mg \cdot L^{-1}$), based on the glycosyl residue composition from the eight CO samples (Supplementary Table 1C). In general, Spanish CO wines exhibited lower percentages of MPs but higher percentages of RG-II than French CO wines, whereas the percentage of PRAG did not follow a trend depending on the origin. Table 2 presents the total oligosaccharides content from CO wines on the basis of their glycosyl composition

(Supplementary Table 1D), and significant differences were found between the Spanish CO wines (ranged between 134 and 148 $mg \cdot L^{-1}$) and French CO wines (ranged between 78 and 98 $mg \cdot L^{-1}$). Our results were in general in coherence with several authors working with base wines (Jégou et al., 2017; Martínez-Lapuente et al., 2018). Origin of grapes seemed to impact on the polysaccharide composition and the oligosaccharide content in base wines. Other aspects such as cultivar grape, the maturity or the enological treatments could also play a role (Apolinar-Valiente et al., 2014).

3.6. Effect of the complex carbohydrate composition of wines on their foamability after AsenG and AseyG separate additions

The total oligosaccharides content resulted in a positive Pearson correlation (+0.7902; p = 0.0196) with the variation percentage of HM when *Asey*G was added to the wines. In beer, Chen, Wang and Li (2015) reported that the maltooligosaccharides are of vital importance to the maintenance of foam quality.

We also performed multiple regression analysis trying to understand which among the independent variables (the complex carbohydrate composition and content of wines, using a maximum of two independent variables for a robust statistics) were related to the dependent variable (the variation percentages of HM and HS after *Asen*G and *Asey*G separate additions). We took into account the significant relations only when R^2 was higher than 75%. Only one significant correlation between the variation percentage of HM and the percentages of MPs and TPs was found (the variation percentage of HM = 49.6786 – 0.481138*MPs – 0.109002*TPs; R^2 = 76.0%; p = 0.0284) after *Asey*G addition. This seems in contradiction with the observations made by Blasco et al. (2011), who reported that yeast MPs of wine were the major foam promoters, favoring the formation of an adsorption layer to the foam

455 bubbles gas/liquid interface. Besides, Aguié-Béguin et al. (2009) observed that the less the
456 concentration of macromolecules of the wine, the lower rate of formation of the adsorbed
457 layer at the air/champagne liquid interfaced. However, Abou Saleh et al. (2007) concluded
458 that the structure of adsorption layer can change depending on unidentified factors. In our
459 case, the addition of *Asey*G could play the role of this unidentified factor. Further physico-
460 chemical studies should be hence realized in order to clear this point that seems at first sight
461 contradictory. The influence of the size, the molecular weight; and the composition of wine
462 polysaccharides on the wine foaming properties has been demonstrated (Coelho et al., 2011;
463 Martínez-Lapuente et al., 2015). Moreover, and according to Martínez-Lapuente et al.,
464 (2015), the synergistic interaction of the foam active compounds, such as peptides, proteins
465 and complex carbohydrates, could modify their surface-active properties and, hence, the
466 foaming properties.
467 From all the results shown in the present and the previous sections, it seems necessary to take
468 into consideration the composition and content of polysaccharides and oligosaccharides in
469 the wines depending on the gum treatment in order to achieve a significant foamability
470 improvement.
471
472 *3.7. Effect of the amino acid composition of the wine proteins precipitated with TCA on the*
473 *wine foamability after AsenG and AseyG separate additions*
474 Table 2 shows that, between the CO wines, CONO2 presented the highest TAAs, whereas
475 CORU3 showed the lowest value. However, we did not find any correlation between the
476 composition and the content of amino acids and the foamability of CO wines after *Asen*G and
477 *Asey*G separate additions. Maybe the very low protein content of wines after bentonite
478 treatment deactivated in some way their influence on the foaming features. Besides, when

addition of bentonite decreased the foamability, it was really hard to restore it even partially through the addition of exogenous protein. In this line, Marchal et al. (2002) found that addition of one exogenous protein, specifically lysozyme, to champagne base wines after bentonite treatment, did not restore their foaming properties. Starting from these two premises, it can be hence concluded that the proteins were important but not the only compounds affecting the foamability. This conclusion seems in accordance with results reported by Puff, Marchal, Aguié-Béghin and Douillard (2001). These authors observed discrepancies between the properties of the adsorption layer of purified invertase (one protein accounting between 30 to 50% or the champagne proteins) and those of macromolecules in champagne wine. From these results, they suggested that adsorption layer presents a complex composition. Aguié-Béghin et al. (2009) concluded that proteins alone cannot be used as a realistic model for the macromolecules forming the adsorption layer of champagne.

3.8. Influence of the composition and properties of gum or AsenG HIC-fraction on the foamability of two selected CO wines

We performed multiple regression analysis trying to correlate two independent variables (variation percentage of HM or HS of selected COMA and CONO2 after different treatments) with one dependent variable [the amino acid data (in Table 1), the protein content measured by Kjeldhal method (in Table 1, from Mejia Tamayo et al., 2018), the carbohydrate composition data (from Mejia Tamayo et al., 2018), the average molar masses (M_w; in Table 1, from Mejia Tamayo et al., 2018) and the hydrophobicity score (in Table 1) of the gums and the gum fractions].

A significant correlation was observed between the variation percentage of HM and the content of histidine (R^2 = 95.5%; p = 0.0451), hydroxyproline (R^2 = 99.0%; p = 0.0102),

503 serine (R^2 = 96.5%; p = 0.0352) and threonine (R^2 = 97.5%; p = 0.0255). Similarly, the
504 variation percentage of HS was significantly correlated with the content of serine (R2 =
505 95.4%; p = 0.0463) and threonine (R^2 = 95.4%; p = 0.0456). However, it is not easy explain
506 the foamability of a wine considering an amino acid alone, even if a correlation existed.
507 Otherwise, proteins are more consistent to biochemically explain a correlation with the foam.
508 In this connection, the variation percentage of HM was significantly correlated with the
509 content of protein of gums and gum fractions (R^2 = 96.7%; p = 0.0327). On the other hand,
510 the variation percentage of HS was significantly correlated with M_w (R^2 = 95.0%; p =
511 0.0498) and the content of protein (R^2 = 96.6%; p = 0.0345). These two correlations would
512 explain the no effect previously mentioned on HM or HS after the HIC-F1 addition to
513 COMA and CONO2, keeping in mind that this sample presents the lowest protein content
514 (4.9 $mg \cdot g^{-1}$ of sample) and M_w values (3.5 x 10^5 $g \cdot mol^{-1}$) (Table 1). Moreover, they also can
515 explain the positive increase of HS in CONO2 and also of HM in both selected base wines
516 when HIC-F2 was added to them. This fraction shows high protein content (63.1 $mg \cdot g^{-1}$ of
517 sample) and M_w values (1.5 x 10^6 $g \cdot mol^{-1}$) (Table 1). Several authors observed a close
518 relationship between protein concentration and foam features in base (Maujean et al., 1990;
519 Brissonnet & Maujean, 1993; Marchal et al., 2002) or sparkling (Vanrell et al., 2007;
520 Martínez-Lapuente et al., 2015) wines. However, Puig-Deu et al. (1999) observed that lower
521 levels of proteins may favor a higher stability time of foam.
522 The behavior of foamability after addition of HIC-F3 appears as much more complex and
523 more difficult to understand and explain from this point of view. As stated above, the
524 foamability parameters increased when it was added to CONO2, whereas they decreased
525 after addition to COMA. According to the obtained correlations as well as the highest protein
526 content and M_w values of HIC-F3 (Table 1), the consistent and predictable behavior of

foamability after its addition would correspond to CONO2. It may therefore be hypothesized that the different wine composition influenced distinctly on the impact of the gum or the gum fractions on the wine foamability. The influence of the polysaccharide families on the wine foaming properties has been widely demonstrated (Girbau-Sola et al., 2002; Abdallah et al., 2010; Martínez-Lapuente et al., 2015). The large varying composition of both wines concerning MPs (COMA: 37 $mg \cdot L^{-1}$; CONO2: 62 $mg \cdot L^{-1}$) and PRAGs (COMA: 41 $mg \cdot L^{-1}$; CONO2: 20 $mg \cdot L^{-1}$) (Table 1) could induce some contrasting type of interaction after the addition of gum fractions in some cases. MPs and PRAGs of wines show a protein moiety with hydrophobic and hydrophilic parts and a sugar hydrophilic portion. The protein moiety of both polysaccharide families would interact with protein moiety of added gums by, among others, hydrophobic forces. Different protein content, and hence, different hydrophobicity of the applied *Acacia* gums and gum fractions could imply unequal interactions depending on the wine composition. In certain cases, these interactions could lead to the formation of a viscoelastic film highly resistant to tension (Blasco et al., 2011), but in other cases they could maybe difficult this process. For example, the particular protein content of HIC-F3 might cause an unlike effect on the foamability depending on the MPs and PRAGs content of wine. This unequal occurrence results more obvious when HIC-F3 was added, but it can be also observed adding HIC-F2. As previously mentioned, this treatment increased positively both HM and HS foaming parameters in COMA wine, but only HS in CONO2. So, although less evident, this behavior after HIC-F2 addition also goes on to suggest that distinct protein content might cause different interactions. In view of these observations, it seems reasonable to think that the composition of wines influenced on the effect of the gums or the gum fractions on wine foamability parameters. Abdallah et al. (2010) remarked the complexity of the wine matrix, reporting that the adsorption layers were formed with various molecular

551 ranges, being the result of complex poly-macromolecular associations leading to a network at
552 the gas/liquid interface rather than the result of a single component. Further experiments
553 should be hence realized in order to deepen our understanding of this essential aspect.
554 A positive Pearson correlation (+0.9372; p = 0.0106) was also observed between the
555 variation percentage of HS and the percentage of non-polar amino acids of gums and gum
556 fractions. Non-polar amino acids were calculated subtracting from 100 the addition of the
557 percentages of polar and charged amino acids (arginine, aspartic acid, cysteine, glutamic
558 acid, glycine, histidine, hydroxyproline, lysine, serine, threonine and tyrosine). Martínez-
559 Lapuente et al. (2015) noted that amino acids with non-polar side chains presented greater
560 coefficients of correlation with foam parameters than amino acids with polar side chains.
561 Taking all these into account, the amino acid content and composition, the M_w and the
562 content of non-polar amino acids of the gums and the gum fractions showed therefore a main
563 role on the foamability features after their addition.
564 On the other hand, the differences between glycosyl composition of gums or *Asen*G fractions
565 added to the wines showed no effect on foam parameters. Maybe their high percentages of
566 carbohydrates (between 81.3% and 97.8%, from Mejia Tamayo et al., 2018) disabled the
567 potential influence of the variations of these compounds between gum samples.
568

569 **4. Conclusions**

570 In the light of all our results, we can say clearly that the addition of *Asen*G and *Asey*G, which
571 are already authorized additives in enology, appears as a precious tool to recover/partially
572 restore wine foaming parameters after bentonite treatments. Foam parameters were increased
573 with efficiencies of 63% and 88% of the cases when, respectively, *Asen*G and *Asey*G were
574 added. For this purpose, *Asey*G was more effective gum than *Asen*G. The foamability of

575 wines treated first with bentonite (CO wines) and subsequently with gum or gum fractions
576 differed greatly depending not only on the gum or the gum fraction treatment, but also on the
577 wine composition. Further research about the impact of enological practices on the increasing
578 foamability after gum addition, as well as about other type of wines, gums and gums
579 fractions, must be done to deepen our knowledge concerning the improvement of foam
580 behaviour in base wines.
581
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589
590 **Compliance with ethical standards**
591 **Conflict of interest**
592 The authors declare that they have no conflict of interest.
593 **Compliance with ethics requirements**
594 This article does not contain any studies with human or animal subjects.
595
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Table 1. Amino acid composition, total amino acid content (mg amino acid·g^{-1} of sample), protein content measured by the Kjeldhal method (mg·g^{-1} of sample), the average molar mass (M_w, g·mol^{-1}) and the hydrophobicity score of *Asen*G, *Asey*G and *Asen*G HIC-separated fractions.

Gum and gum fractions	Ala[a]	Arg[a]	Asp[a]	Cys[a]	Glu[a]	Gly[a]	His[a]	Hyp[a]	Ile[a]	Leu[a]	Lys[a]	Phe[a]	Pro[a]	Ser[a]	Thr[a]	Tyr[a]	Val[a]	TAAs[a]	Protein content[b]	M_w[b]	Hydrophobicity score[c]
*Asen*G	0.5	0.3	1.2	0.0	0.9	0.8	1.4	6.3	0.3	1.8	0.6	0.8	1.6	2.5	1.4	0.3	0.7	21.5	21.5	6.8×10^5	1.323
*Asey*G	0.2	0.1	0.5	0.0	0.3	0.3	0.3	2.1	0.1	0.6	0.1	0.2	0.6	1.0	0.3	0.1	0.3	7.1	7.7	7.1×10^5	1.289
HIC-F1	0.0	0.1	0.1	0.1	0.1	0.1	0.3	1.5	0.0	0.3	0.1	0.1	0.3	0.6	0.3	0.0	0.1	4.0	4.9	3.5×10^5	0.823
HIC-F2	0.8	0.5	2.3	0.2	2.2	1.5	3.1	13.3	0.5	4.2	1.0	2.1	3.4	5.7	3.5	0.3	1.6	46.1	63.1	1.5×10^6	1.442
HIC-F3	3.1	2.6	9.4	0.9	6.6	4.4	7.4	22.7	2.6	10.9	5.3	6.6	8.2	12.1	7.2	1.7	6.2	117.8	137.7	1.6×10^6	1.864

[a]Ala: alanine; Arg: arginine; Asp: aspartic acid; Cys: Cysteine; Glu: glutamic acid; Gly: glycine; His: histidine; Hyd: hydroxyproline; Ile: isoleucine; Leu: leucine; Lys: lysine; Phe: phenylalanine; Pro: proline; Ser: serine; Thr: threonine; Tyr: tyrosine; Val: valine; TAAs: total amino acids content.

[b]From Mejia Tamayo et al. (2018)

[c]From the hydrophobicity scale proposed by Monera et al. (1995), whose values for hydrophobic amino acids are: alanine: 4.1; isoleucine: 9.9; leucine: 9.7; phenylalanine: 10.0; proline: -4.6; valine: 7.7.

732 **Table 2.** Composition of studied wines: families of polysaccharides (%), total content of polysaccharides, (mg·L^{-1}) and total content of
733 oligosaccharides (mg·L^{-1}) from CO wines, as well as total amino acids content from the hydrolysis of the wine proteins precipitated with
734 TCA (mg·L^{-1}) of ORI and CO wines.

Compound	Wine/Origin	Malaga (MA)	Saragossa (SA)	Tarragone (TA)	Champagne NO1	Champagne NO2	Champagne RU1	Champagne RU2	Champagne RU3
MPs[a]	CO wines	37	37	34	58	62	49	62	48
RGII[a]	CO wines	21	36	30	17	19	16	18	19
PRAGs[a]	CO wines	41	27	36	26	20	34	20	33
TPs[a]	CO wines	145	174	168	133	140	221	114	219
TOs[a]	CO wines	144	148	134	78	84	80	85	98
TAAs[a]	ORI wines	16.2	35.9	16.8	45.0	30.4	70.3	57.5	46.9
TAAs[a]	CO wines	1.2	1.6	1.6	2.5	3.9	1.0	1.1	0.7

735
736 [a] MPs: mannoproteins; RG-II: rhamnogalacturonans type II; PRAGs: polysaccharides rich in arabinose and galactose; TPs: total polysaccharide content; TOs: total
737 oligosaccharide content ; TAAs: total amino acids content.
738 The analyses were done in duplication.
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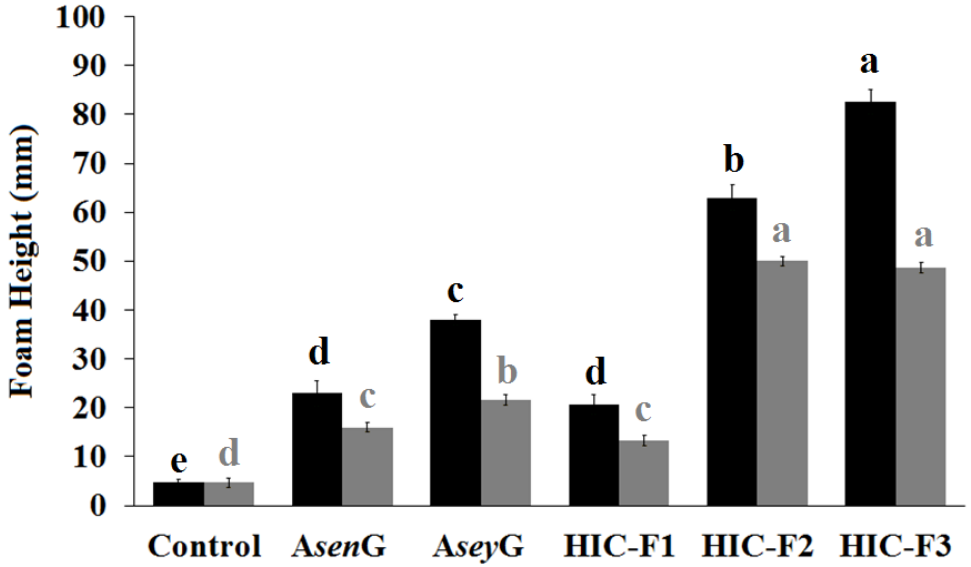

740

741 **Figure 1:** Maximum Foam Height (HM, ■; mm) and Foam Stability (HS, ■; mm) of control SYWI and SYWI with separate additions of *Asen*G,

742 *Asey*G and HIC-fractions from *Asen* (600 mg·L^{-1}).

743 Different letters in the same colour column represent significant differences according to an LSD test ($p < 0.05$).
744 Each bar represents the average value of three samples.
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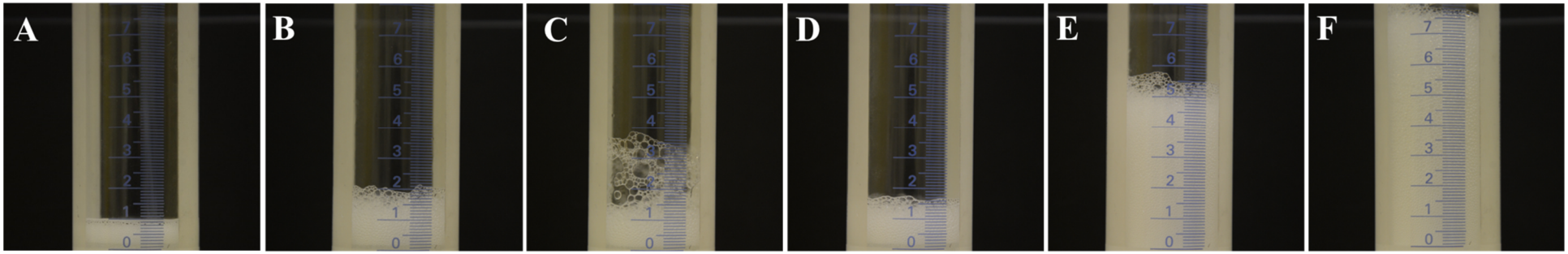
747 

748 **Figure 2.** Foam pictures of control SYWI (A) and SYWI with separate addition (600 mg·L^{-1}) of *Asen*G (B), *Asey*G (C) and HIC-F1 (D), HIC-F2

749 (E) and HIC-F3 (F) fractions from *Asen*G after 4 minutes of gas injection.

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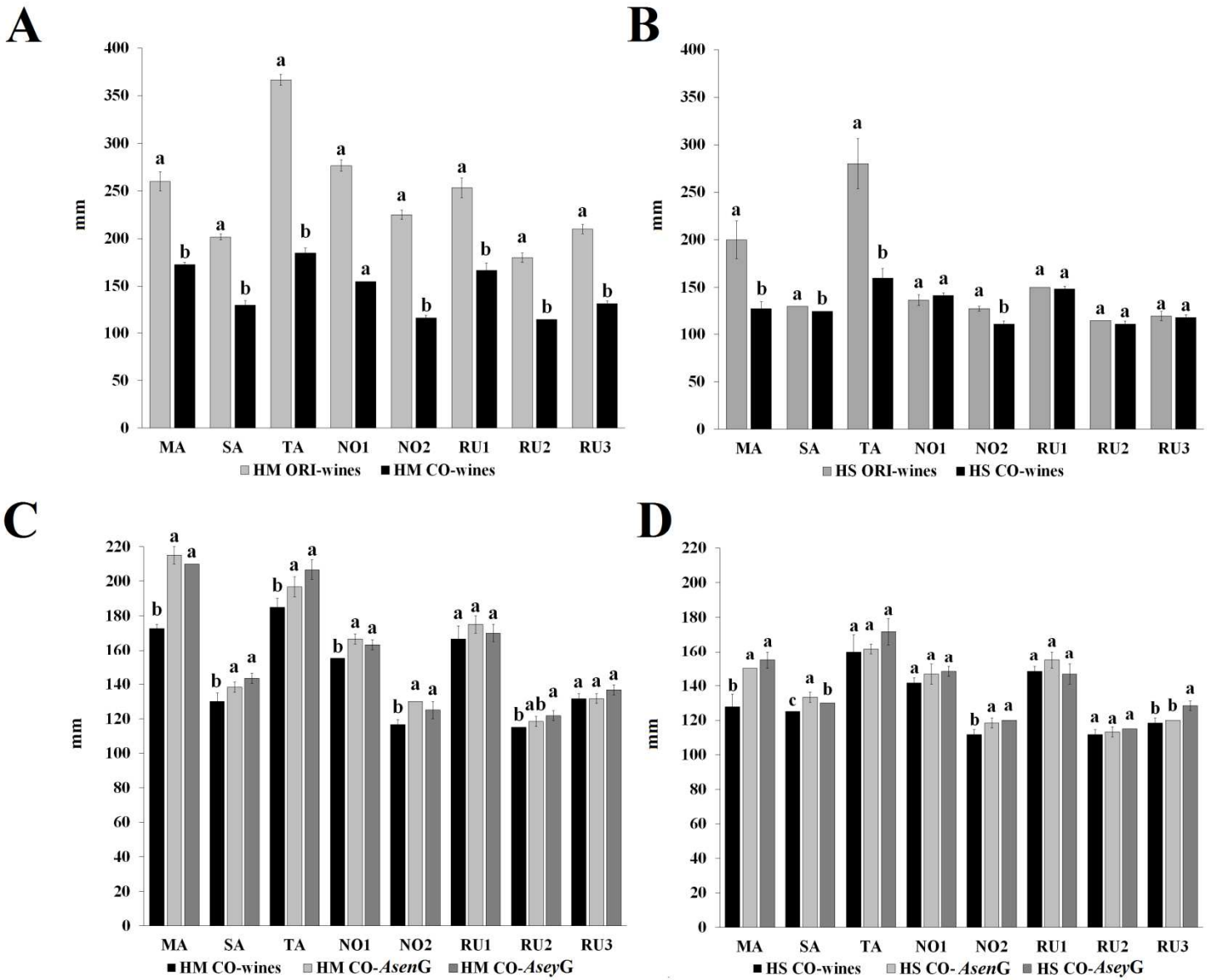
A
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HM CO-wines
HS ORI-wines
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HM CO-wines
HM CO-AsenG
HM CO-AseyG
HS CO-wines
HS CO-AsenG
HS CO-AseyG

752

753 **Figure 3.** A: Maximum Foam Height (HM; mm) of the ORI wines and CO wines. B: Foam Stability (HS; mm) of the ORI wines and CO
754 wines. C: Maximum Foam Height (HM; mm) of the CO wines and CO wines with separate addition of *Asen*G and *Asey*G at 300 $mg \cdot L^{-1}$. D:
755 Foam Stability (HS; mm) of the CO wines and CO wines with separate addition of *Asen*G and *Asey*G at 300 $mg \cdot L^{-1}$.

756 Different letters for each wine (MA: Malaga, SA: Saragossa, TA: Tarragone, NO1: Nogeant 1, NO2: Nogeant 2, RU1: Reims University 1, RU2: Reims University 2 and
757 RU3: Reims University 3) represent significant differences according to an LSD test ($p < 0.05$).
758 Each bar represents the average value of three samples.

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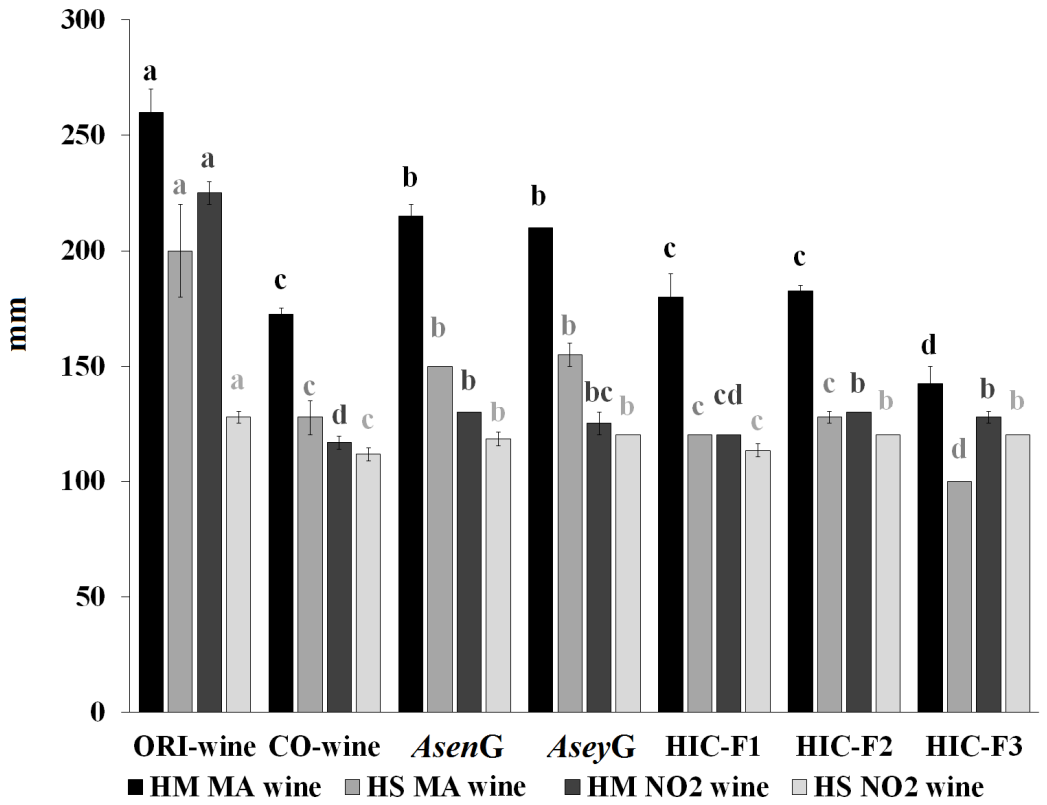


767

768 **Figure 4.** Maximum Foam Height (HM; mm) and Foam Stability (HS; mm) of ORIMA and ORINO2 wines, COMA and CONO2 wines and

769 COMA and CONO2 wines with separate addition of *Asen*G, *Asey*G, HIC-F1, HIC-F2 and HIC-F3 fractions at 300 mg·L^{-1}.

770 Different letters in the same colour column represent significant differences according to an LSD test ($p < 0.05$).
771 Each bar represents the average value of three samples.

Supplementary Table 1. Composition of studied wines. A: Amino acid composition (values given in %) from ORI wines; B: Amino acid composition (values given in %) from CO wines; C: Glycosyl composition (%), total content ($mg \cdot L^{-1}$) and families of polysaccharides ($mg \cdot L^{-1}$) from CO wines; D: Glycosyl composition (%) and total content ($mg \cdot L^{-1}$) of oligosaccharides from CO wines.

A	Wine/Amino acid	Cys ac[a]	Ala[a]	Arg[a]	Asp[a]	Cys[a]	Glu ac[a]	Gly[a]	His[a]	Hyd[a]	Ile[a]	Leu[a]	Lys[a]	Met[a]	Met sulf[a]	Phe[a]	Pro[a]	Ser[a]	Thr[a]	Tyr[a]	Val[a]
	ORIMA	2.4	5.4	3.4	10.1	0.1	11.6	5.6	2.0	0.5	3.6	5.8	5.7	0.0	0.6	3.9	23.5	4.6	4.8	1.7	4.7
	ORISA	1.7	5.5	3.3	11.1	0.1	12.0	5.6	1.5	0.6	3.2	4.7	3.9	0.1	0.0	4.4	26.6	4.8	5.4	1.8	3.9
	ORITA	2.6	6.1	3.6	11.8	0.1	13.6	6.4	2.1	0.6	3.8	5.9	5.2	0.1	0.3	4.2	15.0	5.5	5.8	2.4	4.9
	ORINO1	2.4	5.1	2.3	11.4	0.1	10.0	5.5	1.4	0.4	3.9	5.8	4.8	0.2	0.2	5.0	23.5	4.8	6.5	2.0	4.6
	ORINO2	2.1	5.3	2.3	10.3	0.1	10.6	5.1	1.6	0.3	3.8	5.8	5.2	0.2	0.2	4.5	25.2	4.9	5.7	2.1	4.7
	ORIRU1	2.6	6.1	5.6	13.1	0.6	10.3	6.1	1.7	0.3	3.9	5.9	5.1	0.2	0.0	5.9	12.8	5.5	7.4	2.0	4.9
	ORIRU2	1.1	7.6	4.7	8.8	0.1	14.4	4.4	1.4	0.5	3.1	4.5	4.3	0.1	0.1	3.1	27.3	4.6	4.7	1.3	4.1
	ORIRU3	0.7	8.2	3.2	10.3	0.6	15.8	4.8	1.3	0.3	3.5	5.1	4.4	0.3	0.0	4.0	20.4	4.6	5.7	2.3	4.4

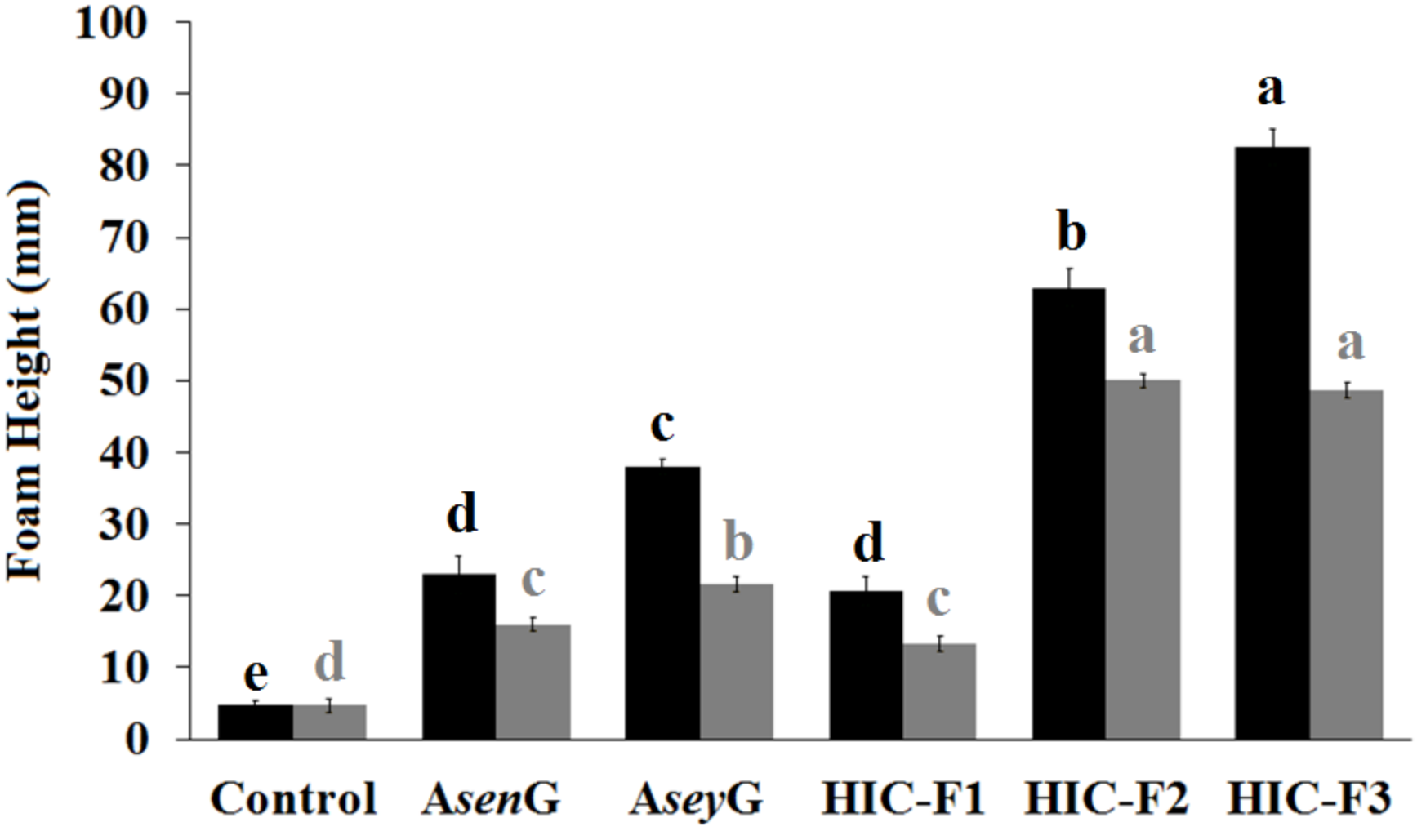
B	Wine/Amino acid	Cys ac[a]	Ala[a]	Arg[a]	Asp[a]	Cys[a]	Glu ac[a]	Gly[a]	His[a]	Hyd[a]	Ile[a]	Leu[a]	Lys[a]	Met[a]	Met sulf[a]	Phe[a]	Pro[a]	Ser[a]	Thr[a]	Tyr[a]	Val[a]
	COMA	5.7	7.5	4.3	9.9	0.1	13.3	8.7	2.0	0.0	3.3	8.3	4.8	0.0	0.5	3.9	6.4	9.4	5.4	1.6	5.0
	COSA	2.3	6.1	4.6	10.4	0.7	14.2	8.9	1.8	0.0	3.4	7.8	5.3	0.3	0.2	4.2	9.6	8.3	5.1	2.5	4.3
	COTA	3.0	6.7	5.3	11.0	0.5	12.9	7.3	2.0	0.0	4.1	8.4	6.6	0.0	0.7	4.9	5.8	7.5	5.7	2.2	5.4
	CONO1	4.5	6.1	5.0	10.5	0.5	12.0	6.4	2.0	0.0	4.6	8.9	7.7	0.2	0.3	4.8	6.1	7.1	5.3	2.7	5.5
	CONO2	1.5	5.5	3.6	11.0	0.2	11.1	6.4	2.8	5.0	4.2	8.6	7.3	0.0	0.4	4.3	5.4	9.1	5.8	0.8	6.7
	CORU1	4.2	7.1	4.7	12.2	1.1	10.9	7.1	1.5	0.0	3.6	8.7	6.0	0.0	0.7	6.1	5.5	7.1	6.7	1.6	5.0
	CORU2	10.0	6.3	5.7	8.5	1.0	13.3	7.5	1.5	0.0	2.7	8.9	4.7	0.0	0.6	3.9	5.8	8.9	5.0	1.0	4.6
	CORU3	3.3	8.5	4.8	9.6	1.6	13.6	7.0	2.2	0.0	2.0	10.1	4.8	0.0	0.0	4.5	10.8	8.1	4.4	1.8	3.7

C	Wine/Monosaccharide and Polysaccharide Family	Ara[b]	Rha[b]	Fuc[b]	Gal[b]	Glc[b]	Man[b]	Xyl[b]	2-OMeFuc[b]	2-OMeXyl[b]	Api[b]	MPs[b]	RGII[b]	PRAGs[b]
	COMA	11.3	5.3	0.5	37.4	1.9	41.4	0.4	0.5	0.5	0.9	53	31	59
	COSA	10.2	5.7	0.8	30.0	2.5	47.2	0.0	1.1	0.9	1.8	64	63	47
	COTA	9.1	4.9	0.4	37.2	4.8	40.4	0.4	0.8	0.7	1.3	57	51	60
	CONO1	6.4	4.0	0.5	21.5	1.8	64.2	0.2	0.4	0.3	0.6	77	22	34
	CONO2	5.8	3.5	0.3	18.0	2.0	68.4	0.3	0.5	0.3	0.8	87	26	28
	CORU1	10.1	3.1	0.2	28.9	2.2	54.1	0.1	0.4	0.4	0.5	109	36	75
	CORU2	5.5	3.8	0.5	18.1	3.4	67.1	0.2	0.5	0.3	0.6	71	20	23
	CORU3	8.8	4.5	0.3	29.7	1.7	53.2	0.2	0.4	0.4	0.7	105	41	73

D	Wine/Monosaccharide	Ara[b]	Rha[b]	Fuc[b]	Gal[b]	Glc[b]	Man[b]	Xyl[b]	4-O-MeGlc ac[b]	Gal ac[b]	Glc ac[b]	Xylitol[b]
	COMA	5.3	3.1	1.1	6.7	29.8	11.4	13.6	3.3	23.1	1.1	1.4
	COSA	11.6	11.9	1.6	14.1	15.4	15.7	9.7	4.1	12.2	2.4	1.6
	COTA	7.8	7.2	1.8	10.5	20.4	15.0	18.3	3.8	10.5	1.2	3.0
	CONO1	9.7	5.6	1.0	6.7	33.3	23.1	6.7	1.6	8.7	1.5	1.0
	CONO2	7.6	4.3	0.9	8.5	37.0	19.0	8.1	1.9	7.6	1.4	2.4
	CORU1	9.5	5.0	1.0	8.5	34.0	22.5	7.0	1.1	8.0	1.0	1.0
	CORU2	5.6	5.6	0.9	10.3	30.5	23.9	9.9	1.6	7.0	1.4	2.3
	CORU3	9.4	6.9	1.2	11.4	26.9	23.7	8.6	1.1	7.3	0.8	2.0

[a]Cys ac: cysteic acid; Ala: alanine; Arg: arginine; Asp: aspartic acid; Cys: Cysteine; Glu ac: glutamic acid; Gly: glycine; His: histidine; Hyd: hydroxyproline; Ile: isoleucine; Leu: leucine; Lys: lysine; Met: methyonine; Met sulf: methyonine sulfone; Phe: phenylalanine; Pro: proline; Ser: serine; Thr: threonine; Tyr: tyrosine; Val: valine.

[b]Ara, arabinose; Rha, rhamnose; Fuc, fucose; Gal, galactose; Glc, glucose; Man, mannose; Xyl, xylose; 2-OMeFuc,2-O-CH_3-fucose; 2-OMeXyl, 2-O-CH_3-xylose; Api, apiose; MPs: mannoproteins; RGII: rhamnogalacturonans type II; PRAGs: polysaccharides rich in arabinose and galactose; 4-OMeGlc ac: 4-O-methyl Glucuronic acid; Gal ac: galacturonic acid; Glc ac: glucuronic acid.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80
70
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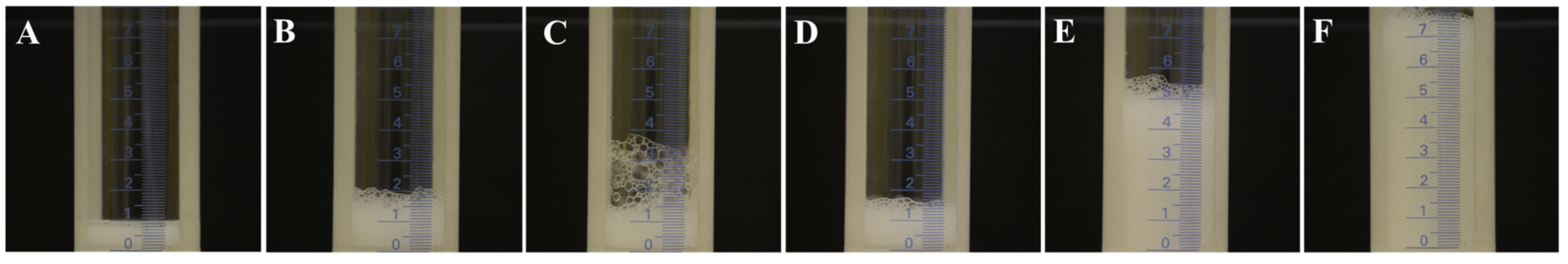
Control
AsenG
AseyG
HIC-F1
HIC-F2
HIC-F3


A
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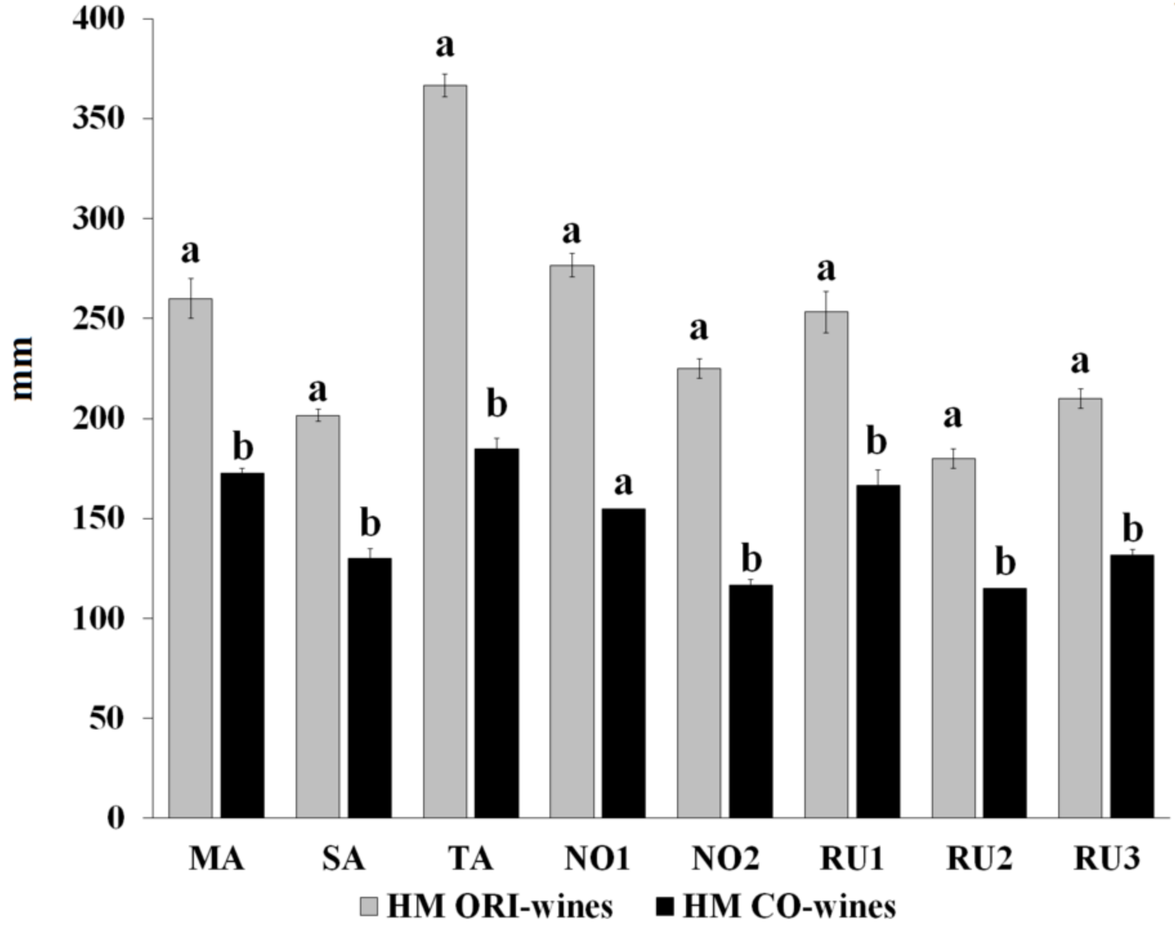
A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HM CO-wines

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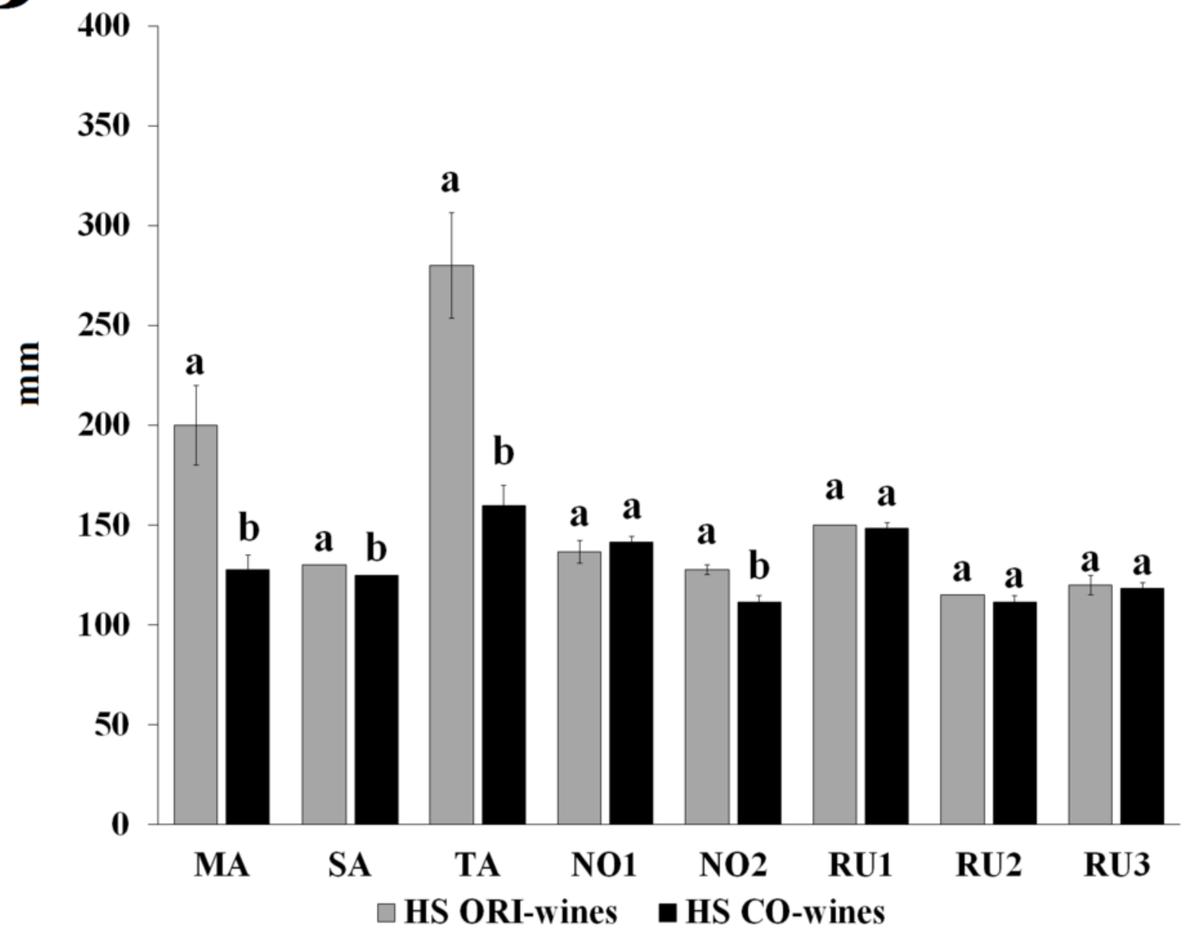
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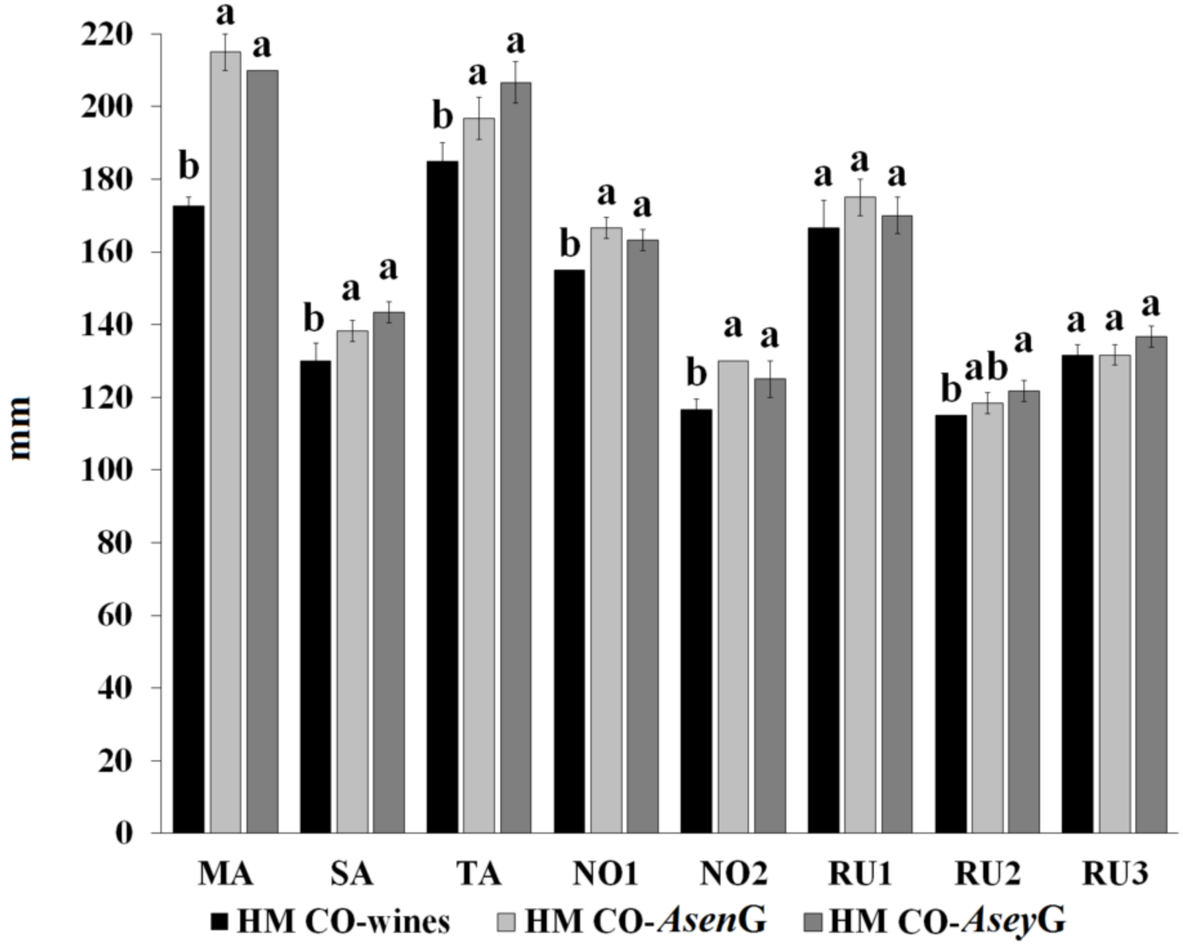
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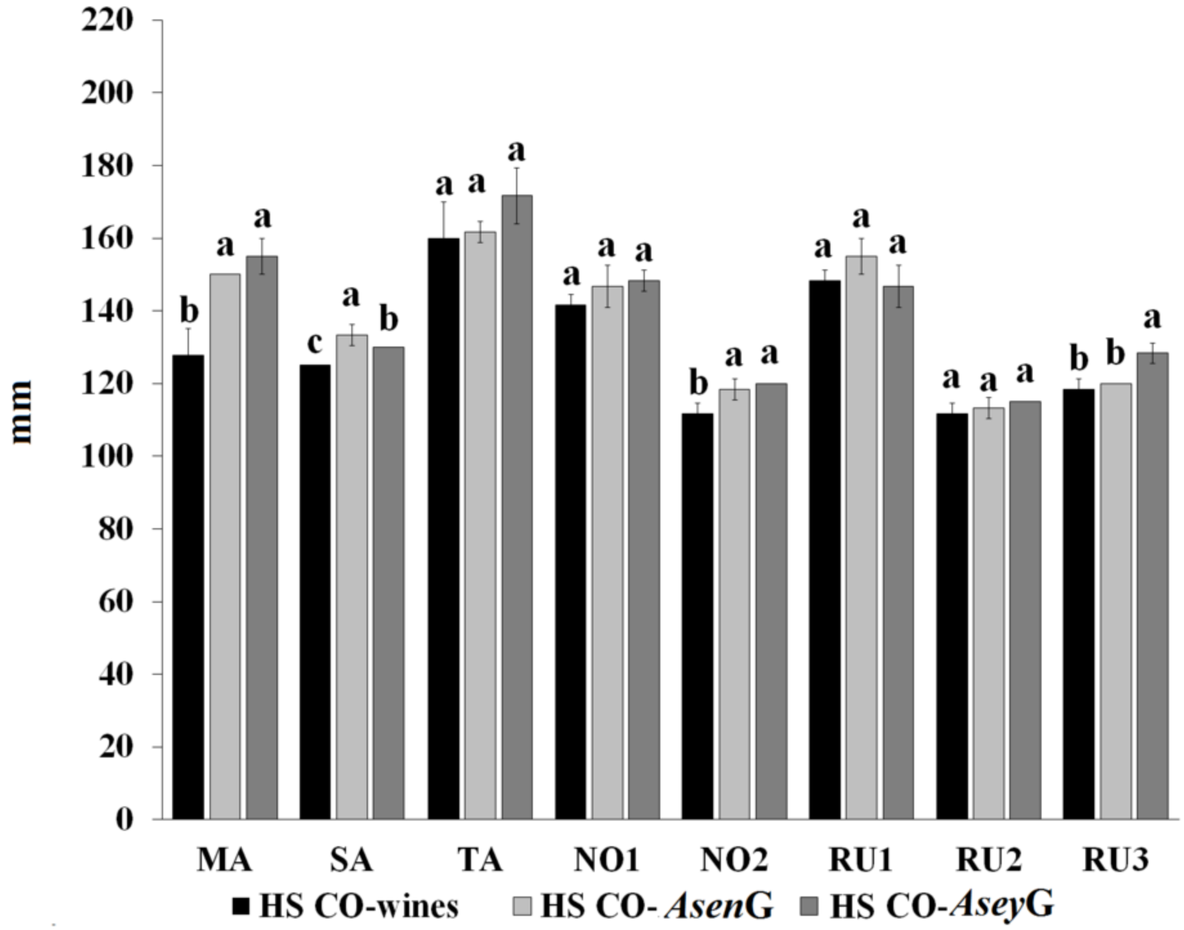
D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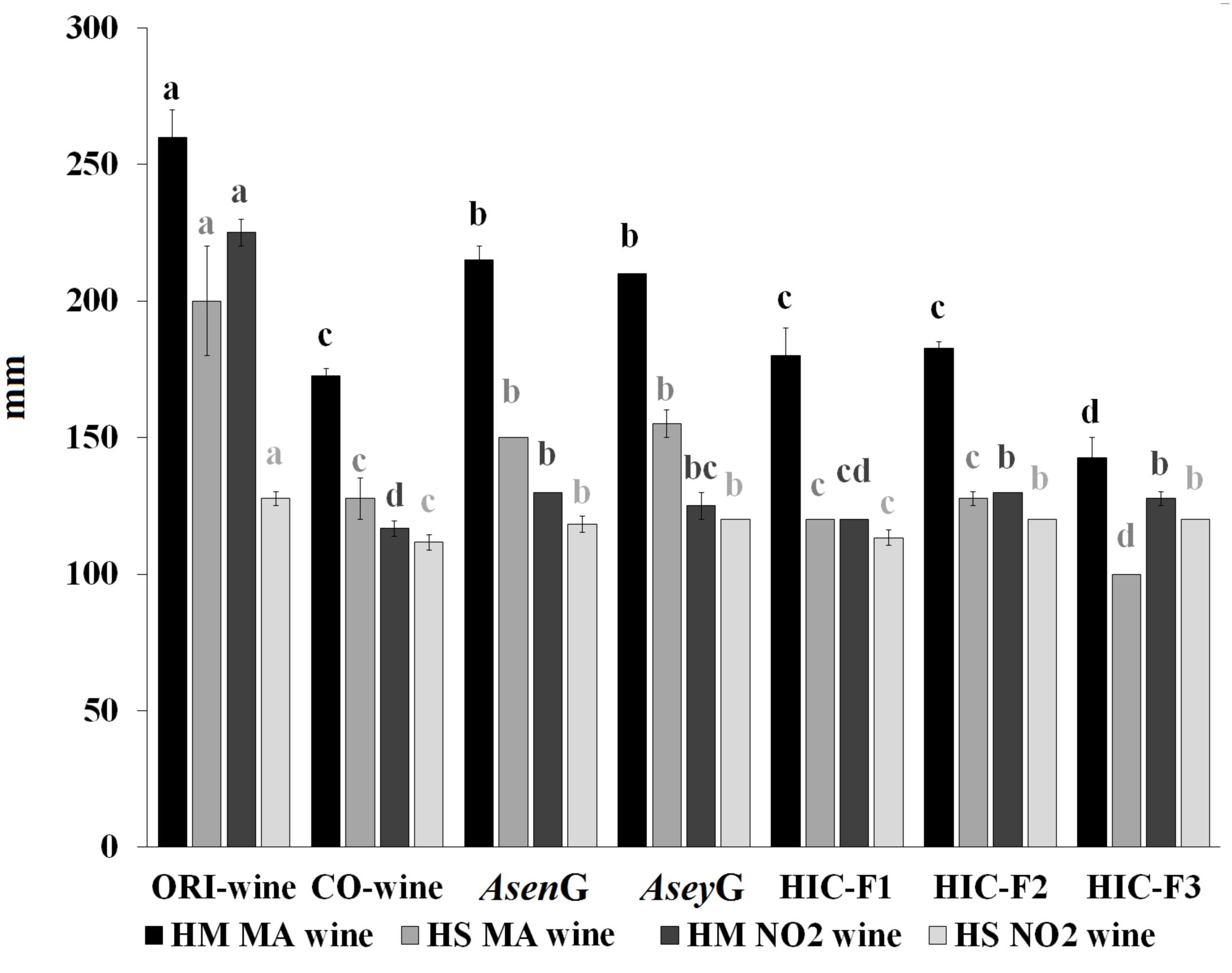
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ORI-wine
CO-wine
AsenG
AseyG
HIC-F1
HIC-F2
HIC-F3
HM MA wine
HS MA wine
HM NO2 wine
HS NO2 wine

Gum and gum fraction	Ala[a]	Arg[a]	Asp[a]	Cys[a]	Glu[a]	Gly[a]	His[a]	Hyp[a]	Ile[a]	Leu[a]	Lys[a]	Phe[a]	Pro[a]	Ser[a]	Thr[a]	Tyr[a]	Val[a]	TAAs[a]	Protein content[b]	M_w[b]	Hydrophobicity score[c]
Asen G	0.5	0.3	1.2	0.0	0.9	0.8	1.4	6.3	0.3	1.8	0.6	0.8	1.6	2.5	1.4	0.3	0.7	21.5	21.5	6.8×10^5	1,323
Asey G	0.2	0.1	0.5	0.0	0.3	0.3	0.3	2.1	0.1	0.6	0.1	0.2	0.6	1.0	0.3	0.1	0.3	7.1	7.7	7.1×10^5	1,289
HIC-F1	0.0	0.1	0.1	0.1	0.1	0.1	0.3	1.5	0.0	0.3	0.1	0.1	0.3	0.6	0.3	0.0	0.1	4.0	4.9	3.5×10^5	0.823
HIC-F2	0.8	0.5	2.3	0.2	2.2	1.5	3.1	13.3	0.5	4.2	1.0	2.1	3.4	5.7	3.5	0.3	1.6	46.1	63.1	1.5×10^6	1,442
HIC-F3	3.1	2.6	9.4	0.9	6.6	4.4	7.4	22.7	2.6	10.9	5.3	6.6	8.2	12.1	7.2	1.7	6.2	117.8	137.7	1.6×10^6	1,864

Compound	Wine/Origin	Malaga (MA)	Saragossa (SA)	Tarragone (TA)	Champagne NO1	Champagne NO2	Champagne RU1	Champagne RU2	Champagne RU3
MPs[a]	CO wines	37	37	34	58	62	49	62	48
RGII[a]	CO wines	21	36	30	17	19	16	18	19
PRAGs[a]	CO wines	41	27	36	26	20	34	20	33
TPs[a]	CO wines	145	174	168	133	140	221	114	219
TOs[a]	CO wines	144	148	134	78	84	80	85	98
TAAs[a]	ORI wines	16.2	35.9	16.8	45.0	30.4	70.3	57.5	46.9
TAAs[a]	CO wines	1.2	1.6	1.6	2.5	3.9	1.0	1.1	0.7