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## ABSTRACT

Foam is the first attribute observed when sparkling wine is served. Bentonite is essentially used to flocculate particles in sparkling base wines but can impair their foamability. Gums from *Acacia senegal* and *Acacia seyal* improved the foamability of different bentonite-treated base wines. Our main goal was to see how the supplementation with new fractions separated from *Acacia* gums by Ion Exchange Chromatography affected foamability of sparkling base wines, deepening the relation between foam behavior and characteristics of wine and gums. High molar mass fractions increased the maximal foam height and the foam height during the stability period in, respectively, 11 out and 8 out of 16 cases (69% and 50%, respectively). The properties of the supplementing gums fractions obtained by IEC and, although to a minor extent, the wine characteristics, affected positively and/or negatively the foam behavior. Wine foamability also depended on the relationship between wine and gums fractions properties.



*Keywords*: Sparkling base wine; foam; *Acacia* gums; Ion Exchange Chromatography; macromolecules; SEC-MALLS; hydrophobic score; volumetric properties

## 1. Introduction

Cava from Spain or champagne from France are amongst the most famous sparkling wines. When they are served, foam behavior is the first attribute observed by consumers. The wine selection to elaborate high quality sparkling wine is hence based, among others, on their foam behavior (Martínez-Rodríguez & Pueyo, 2009). In sparkling wines, foam is a high volume dispersion of gas into the liquid (Coelho, Reis, Domingues, Rocha, & Coimbra, 2011a). Its stability is closely linked to intermolecular forces and surface properties (Abou Saleh, Aguié-Beghin, Foulon, Valade, & Douillard, 2007). In the absence of surface-active components with high molar mass, the complex foam system is greatly unstable. This results in the thinning and consequent rupture of the liquid film because of the drainage and hence, collapsing of the foam (Blasco, Viñas, & Villa, 2011). The phenomenon of bubble coalescence also plays an important role in foam collapse. Adsorption of particular molecules reduces surface tension, modifying interaction forces and also interfacial rheological properties (Abdallah, Aguié-Béghin, Abou-Saleh, Douillard, & Bliard, 2010; Marchal et al., 2020). The shelf life of foam was improved therefore by stabilizing the film between bubbles.

Wine is a complex matrix containing many types of molecules, including polysaccharides, proteins and polyphenols. Polysaccharides in wine may be grouped into three major families: (i) polysaccharides rich in arabinose and galactose (PRAGs), (ii) those rich in rhamnogalacturonans (RG-I and RG-II), coming from the grapes, and (iii) mannoproteins (MPs) from yeasts during fermentation and the aging on lees. All these types of molecules can positively or negatively affect foamability. The impact of wine macromolecules, such as complex carbohydrates (Abdallah et al., 2010; Martínez-Lapuente, Guadalupe, Ayestarán, & Pérez Magariño, 2015) and proteins (Vanrell, Canals, Esteruelas, Fort, Canals, & Zamora, 2007; Coelho et al., 2011a) has been reported, although the conclusions were not always clear and sometimes contradictory. For example, Maujean, Poinsaut, Dantan, Brissonnet and

Cossiez (1990) observed that the protein content correlated positively with foam height but not, in any sense, with foam stability, whereas Pueyo, Martín Álvarez and Polo (1995) found that protein concentration in cava wines was linked positively to its stability but negatively to foam height. Another example might be the effect of MPs on wine foamability: they seem benefit it (Blasco et al., 2011), although Martínez-Lapuente et al. (2015) did not observed any effect of them on the maximum foam height or the foam stability height of sparkling wines. Foaming characteristics also seem to be influenced by the synergistic interaction of all active foam compounds, but the literature is not totally conclusive (Coelho et al., 2011a; Martínez-Lapuente et al., 2015; Apolinar-Valiente et al., 2020a).

Bentonite, a clay mineral, is usually added to wine in order to cause particle flocculation. Bentonite acts like a negatively charged structure which is able to exchange its cations with positively charged compounds of the wine (not only proteins) and also with uncharged but polar molecules (Martínez-Rodríguez & Pueyo, 2009). However, this process also leads to a drastic loss of foamability (Marchal, Chaboche, Douillard, & Jeandet, 2002; Vanrell et al., 2007) because of the adsorption of soluble proteins (Abdallah et al., 2010). The addition of *Acacia* gums has been demonstrated as a valuable tool to compensate this negative influence on foam (Apolinar-Valiente et al., 2020a). *Acacia* gums have both foaming and interface activity: they are suggested to migrate slowly to air/wine interfaces and make stable foams. The arabinogalactan-protein (AGP) nature of *Acacia* gums can explain their foaming ability. AGPs lead to reduce the surface tension providing the ability to form a film at the surface of the bubbles (Rodríguez Patino et al., 2007). *Acacia* gums are already authorized as additives in wine production, acting as a protective colloid which confers body to wine (Sanchez et al., 2018) and which also prevents coloring agents from precipitating in red wine (Nigen et al., 2019). This type of gums is exuded by several *Acacia* trees species (i.e. *Acacia senegal* –*Asen*G– and *Acacia seyal* –*Asey*G–), each having specific characteristics (Lopez-Torrez et al., 2015). A*sen*G presents higher content of proteins and larger molar mass compared to

A*sey*G. Moreover, A*sen*G macromolecules are more anisotropic and show a more branched structure than those of A*sey*G (Sanchez et al., 2018). *Acacia* gums have been traditionally separated in three classical fractions by hydrophobic interaction chromatography (HIC): (i) the arabinogalactan-peptide fraction (AGp or HIC-F1) with low protein content, low molar mass and with disk-like morphology, (ii) the arabinogalactan-protein fraction (AGP or HIC-F2) rich in protein, with high molar mass and with spheroidal structure, and (iii) the glycoproteins fraction (GP or HIC-F3) presenting the largest protein amount, with high molar mass and showing an assembly of ring-like modules (Sanchez et al., 2018). Apolinar-Valiente et al. (2019, 2020b) have recently fractionated *Acacia* gums by Ion Exclusion Chromatography (IEC) into two fractions: a minor fraction in weight (F1) showing great protein amount and high molar mass, and a major fraction in weight (F2) having low protein content. Conformational and structural differences were also observed, presenting F1 more anisotropic shape as well as more compressible and less hydrated structure than F2. Although IEC- and previously referenced HIC-fractions (Mejia Tamayo et al., 2018; Apolinar-Valiente et al., 2019, 2020b) showed several equivalences, fractions obtained by IEC maintained their own identity and characteristics. For example, F1 obtained by IEC from *Acacia senegal* gum (F1*sen*) presented higher weight average molar mass ($M_w$) (3 100 000 $g \cdot mol^{-1}$) and intrinsic viscosity values (88 $mL \cdot g^{-1}$) (Apolinar-Valiente et al., 2019) compared to HIC-F3, which contributed to 70% of F1*sen* ($M_w$: 1 600 000 $g \cdot mol^{-1}$; intrinsic viscosity: 55 $mL \cdot g^{-1}$, from Mejia Tamayo et al., 2018).

Our main goal was to investigate if the supplementation of IEC-fractions from different *Acacia* gums species (*Asen*G and *Asey*G) could partially restore the foamability of sparkling base wines after bentonite treatment. To this aim, we have hence separately supplemented eight well-differentiated base wines with fractions separated by IEC from *Asen*G (F1*sen* and F2*sen*) and from *Asey*G (F1*sey* and F2*sey*). The recovery of F1 fractions is however hard and costly, because the low yields of IEC fractionation (Apolinar-Valiente et al., 2019 and

2020b). The wines foamability was measured using the Bartsch Shaking Test (ST) (Bartsch, 1924) adapted by Marchal et al. (2020). During ST, a liquid (the base wine in our study) and its interface with a gas phase were vigorously agitated. As a result, the air was incorporated into the liquid leading to foam composed of small bubbles. Following comparison with the classical gas-sparging method (the so-called Mosalux) reported by Maujean et al. (1990), ST method needed six times less volume of wine to measure foamability, i.e. six times less amount of gums fractions. Knowing the difficulty to obtain a significant amount of F1 fractions by IEC, this point should be taken into serious consideration. This aspect could be moreover an essential factor in researching studies using micro-winemaking, reducing the amount of material resources involved. According to Drenckhan and Saint-Jalmes (2015), ST provided a good and fast estimation of the foamabilities of various samples at once, so it could be very easily and efficiently applied in wineries and oenological laboratories without complex and sophisticated systems.

The secondary aim was to gain more in-depth knowledge of the relationship between foam behavior and the characteristics of base wines and gums fractions. The more winemakers know the characteristics of wine and *Acacia* gums, the better they can manage to increase foamability of their sparkling wines using this valuable tool. Therefore, information about characteristics of wines and gums fractions was presented, being linked to our foamability results. From the consumer perspective, foam is perceived when serving sparkling wine but also when drinking it (Martínez-Rodríguez & Pueyo, 2009). For this reason, when assessing the correlations between foam height and characteristics of gums fractions and the base wines, we considered more valuable to open the perspective not only to particular moments but during the total ST. In our knowledge, this is the first work which investigates the impact of supplementation with new fractions separated by IEC from *Asen*G and *Asey*G on the foamability of sparkling base wines.

## 2. Material and Methods

### *2.1. Wine samples*

Using the traditional white winemaking method, eight base wines were vinified. The origins of three base wines were three different regions from Spain: Malaga (MA) using Moscatel grapes, Saragossa (SA) and Tarragona (TA), both using Macabeo grapes. Five other base wines were elaborated in the French region of Champagne. Two of them were vinified at the cooperative winery Nogent l'Abbesse (NO1 and NO2) from Chardonnay grapes, while the rest of base wines were provided by Reims University, being elaborated from Pinot noir (RU1) and Chardonnay (RU2 and RU3) grapes. All of them showed proper values of the enological classical parameters for typical base wines (alcoholic degree: between 10 and 13%; pH: between 3.0 and 3.5; titratable acidity expressed in sulfuric acid: between 3 and 7 $g \cdot L^{-1}$). Bentonite (20 $g \cdot hL^{-1}$; Microcol Alpha®, Laffort) was added to a part of the base wines, which were consequently stirred gently for 10 days at 4° C and filtered (1 μm). These wines were named as CO (control wine) followed by its corresponding origin. This resulted in COMA, COSA, COTA, CONO1, CONO2, CORU1, CORU2 and CORU3. The wines non-treated with bentonite were named as ORI (original wine) followed by its corresponding place of origin. This resulted in ORIMA, ORISA, ORITA, ORINO1, ORINO2, ORIRU1, ORIRU2 and ORIRU3.

### *2.2. Wine composition analysis*

The methodology to obtain the total amino acids content (TAAs), the families of polysaccharides percentage, the total content of polysaccharides (TPs) and the total content of oligosaccharides (TOs) of the eight studied wines was previously reported (Apolinar-Valiente et al., 2020a).

The total polyphenol index (TPI) was calculated following the method of Ribéreau-Gayon, Glories, Maujean and Dubourdieu (2006) with some modifications. Briefly, 100 μL of wines

were diluted in 2.5 mL of water, being the absorbance measured (280 nm) in 1 cm cell using a spectrophotometer UV-1800 (Shimadzu, Kyoto, Japan).

According to Martínez-Lapuente et al. (2018), the weight average ($M_w$) and number average ($M_n$) molar masses as well as the molar mass distribution of polysaccharides isolated from the eight wines were determined. Five cumulative ranges for molar masses have then been delimited: range 1 (R1) = 2500−20 000 g·mol$^{-1}$; range 2 (R2) = 20 000−100 000 g·mol$^{-1}$; range 3 (R3) = 100 000−250 000 g·mol$^{-1}$; range 4 (R4) = 250 000−500 000 g·mol$^{-1}$; and range 5 (R5) = 500 000−1 000 000 g·mol$^{-1}$. These five ranges have been selected due to their correspondence with values obtained from different polysaccharide families by Size Exclusion Chromatography (SEC) analysis: RG-II monomer, $M_w$ = 5000 g·mol$^{-1}$; RG-II dimer, $M_w$ = 10 000 g·mol$^{-1}$; $MP_{0c}$, $M_w$ = 58 000 g·mol$^{-1}$; $AGP_2$, $M_w$ = 165 000 g·mol$^{-1}$; $MP_{0a}$, $M_w$ = 350 000 g·mol$^{-1}$; $MP_3$, $M_w$ = 1 000 000 g·mol$^{-1}$ (Vidal, Williams, Doco, Moutounet, & Pellerin, 2003). These data were obtained by coupling size exclusion chromatography with a multiangle light-scattering device (MALLS; Wyatt Technology Corporation, USA), a differential viscometer (Viscostar II, Wyatt Technology Inc., USA), and a differential refractive index detector (Optilab TrEX, Wyatt Technology Inc., USA). SEC elution was performed on OH-pack guard column followed by two serial Shodex OH-pack KB-804 and KB-805 columns (0.8 x 30 cm; Shodex Showa Denko, Japan) at 1 mL·min$^{-1}$ flow rate using 0.1 M $LiNO_3$ filtered (0.1 μm) mobile phase containing 0.02% $NaN_3$. A d*n*/d*c* classical value was employed (0.146 mL·g$^{-1}$).

*2.3. Fractionation of Acacia gums samples by Ionic Exchange Chromatography (IEC)*

*Asen*G and *Asey*G were kindly provided by ALLAND & ROBERT Company – Natural and organic gums (Port Mort, France). Following the IEC fractionation method of Apolinar-Valiente et al. (2019), macromolecular fractions F1*sen* and F2*sen* were obtained from *Asen*G soluble powder. The separation was performed at room temperature through a DEAE

Sephacel (Sigma Aldrich, St. Louis, Mo) column (54 x 20 cm), being equilibrated with degassed water. Dissolved *Asen*G (650 g dispersed in 6500 mL of water, i.e. 10 $g \cdot L^{-1}$) was loaded and eluted by water (~10 L; flow rate: 40 $mL \cdot min^{-1}$) to obtain fraction F1*sen*, corresponded to AGPs eluted during this linear phase. Later, a gradient from water to 2 M NaCl was performed (5 h; flow rate: 20 $mL \cdot min^{-1}$) following by a plate at 2 M NaCl (~ 20 L; flow rate: 20 $mL \cdot min^{-1}$). The fraction F2*sen* corresponded to AGPs eluted during the gradient and linear phases. Both fractions were separately heated at 50° C, and then concentrated and desalted against 10 volumes of water through a cross flow filtration system (ÄKTA flux, GE Healthcare) using a transmembrane pressure of 15 psi. The membrane used was a polysulfone hollow fiber (GE Healthcare) with a nominal cut off of 30 kDa. The fractions were spray-dried using a B-290 Mini Spray Dryer (BUCHI™). Similar procedure was applied to obtain F1*sey* and F2*sey* from *Asey*G soluble powder.

Previous works reported the neutral sugars, uronic acid and amino acid compositions and the structural parameters of F1 and F2 from *Asen*G (Apolinar-Valiente et al., 2019) and *Asey*G (Apolinar-Valiente et al., 2020b). All this information appears in the present work in a similar or adapted form.

### *2.4. Treatments*

Fractions separated from *Asen*G and *Asey*G by IEC were dispersed in water and gently stirred (20 °C, 24 h). The eight CO-wines were separately supplemented (300 $mg \cdot L^{-1}$) with gums fractions, resulting in the supplemented CO-wines. According to International Organisation of Vine and Wine (OIV, 2019), the dose used of *Acacia* gum shall not exceed this value.

### *2.5. Foaming parameters measurement*

Firstly, we compared ST and Mosalux methods. The foamability of the ORI-wines (*n*=8), the CO-wines (*n*=8) and the four types of supplemented CO-wines of two selected samples (Malaga and Champagne NO2 wines) (*n*=8) were analysed by both methods. Malaga and Champagne NO2 wines were selected by following two steps. Firstly, we performed an ascending hierarchical classification (AHC) by dissimilarities using parameters and foamabilities data of the eight CO-wines. This analysis resulted in several groups, whose more separated sub-groups were composed by (i) Champagne NO2 and Champagne RU3 wines, as well as by (ii) Malaga and Tarragona wines. Consequently, and taking into account both sub-groups obtained by AHC analysis, we selected the wines coming from different countries with the higher significant maximal differentials of foam height (ΔFH*max*) after fractions supplementations, according to Fig. 1A and 1B.

*2.5.1. Mosalux method*

Following Maujean et al. (1990), 100 mL of the sample was introduced in a glass cylinder with a glass frit at the bottom, injecting carbon dioxide gas through the glass frit (constant rate flow: 7 $L \cdot h^{-1}$) at constant pressure (1 bar). Foam height was monitored during gas injection for 5 min. The maximum foam height (HM-MOS) reached by the foam column (mm) and the foam stability height (HS-MOS, representing the height (mm) at which the foam stabilizes during gas injection) were measured. All the experiments were done in triplicate, being the room temperature controlled (18 ± 1° C).

*2.5.2. Shaking test (ST)*

According to the often referenced as "Bartsch shaking test" (Bartsch, 1924) with modifications (Marchal et al., 2020), 15 mL of each sample were introduced in tubes (internal diameter: 1 cm; height: 20 cm), and plugged by a bung. The distance between the wine surface and the bung was 9 cm. The tubes placed in a laboratory grid were vertically,

strongly and manually shaken 12 times (1 agitation/sec.). Then, pictures were taken at 5 sec. (T5) and every 10 sec. (T10, T20…) during 90 seconds after stopping the agitation of tubes. The foam height (mm) was consequently measured through a graduated scale positioned exactly behind the tubes during the picture taking. Their Maximum Foam Height measured by ST was abbreviated as HM. All tests were performed in triplicate, being all the tubes in each repetition agitated on the same rack and by the same operator to reduce the experimental error.

### *2.6. Statistical data*

Statistical analyses were applied to compare ST and Mosalux methods, and to analyze the statistical relationships between foamability and the characteristics of wines and gums fractions. Results according to a least significant difference (LSD) test and Pearson correlations were considered statistically significant only when the degree of significance ($p$) was smaller than 0.05. Regarding multiple regression analysis, we have used a maximum of two independent variables with the aim of strengthening statistics. Statgraphics Centurion XVI.I software (StatPoint Technologies, Inc., USA) was used to apply all these statistical analyses. Ascending hierarchical classification (AHC) and principal component analysis (PCA) were calculated using XL-STAT, which is a plug-in for Microsoft Excel developed by Addinsoft.

## 3. Results and discussion

### *3.1. Comparison of ST and Mosalux procedures*

Table 1 shows the foam height of the ORI-wines (original wines; $n$=8), the CO-wines (bentonite-treated wines; $n$=8) and the COMA and CONO2 supplemented wines ($n$=8) measured by ST. In this way the differences of wines were not only caused by the origin but also by varying oenological techniques. That has enabled us to ensure that the comparison of

ST and Mosalux procedures were done using a greater variability of samples. Because of the results obtained, as well as the sake of clarity and space reasons, only values at T5, T10, T70, T80 and T90 appear in Table 1. The rest of information is available in Supplementary Table 1. All these base wines (*n*=24) exhibited their HM at T5, followed by values at T10. On the other hand, we considered that the foam stability height determined by ST started when the foam height was not statistically different to the last measure (T90). This period began before or just at T70 for all these 24 samples, ensuring the appropriate duration to confirm an accurate stability of the wines.

Table 1 also gives the HM-MOS and HS-MOS values of ORI-wines and CO-wines (adapted from Apolinar-Valiente et al., 2020a) as well as COMA and CONO2 supplemented wines. Multiple regression analyses were performed trying to know if some correlation could be established between (i) the foam height values at T5 and T10 (two independent variables) by ST and (ii) the HM-MOS (dependent variable) of 24 varying wines (Table 1). T5 and T10 were selected as the two moments presenting the two higher foam height values. The following significant correlation was obtained: HM-MOS = 158.979 – 4.9781*T5 + 7.7887*T10 ($R^2$ = 79%; *p* (constant) = 0.0000; *p* (T5) = 0.0474; *p* (T10) = 0.0019). The HS-MOS (dependent variable) also correlated significantly with the foam height values at T70 and T90 (independent variables) by ST of 24 different wines (Table 1) through multiple regression analysis, although $R^2$ value was lower (HS-MOS = 122.387 – 20.4783*T70 + 24.2547*T90; $R^2$ = 72%; *p* (constant) = 0.0000; *p* (T70) = 0.0200; *p* (T90) = 0.0091). T70 and T90 were selected as the two moments when the foam stability period began and finished. For both multiple regression analysis, the obtained $R^2$ values (79% and 72%, respectively) may not enable us to make precise predictions equations, but they allow us ensuring consistent trends. In brief, ST was a valid, simple, fast and less costly method to measure the maximum foam height and the foam stability height of sparkling base wines.

312 *3.2. Base wine properties*

313 Table 2 shows TAAs, the families of polysaccharides percentages, TPs and TOs of the eight
314 studied CO-wines. All this information was adapted from data previously reported and
315 discussed by Apolinar-Valiente et al. (2020a). It was suggested that the grape origin
316 impacted highly on the composition of the eight base wines, although other several points
317 such as the cultivar grape, the maturity or the oenological treatments could also influence it.
318 Table 2 also includes TPI values that were higher in the studied Spanish wines (between 7.4
319 and 9.1) compared to the French wines values (between 4.5 and 5.4). Climate conditions
320 could impact the physiology of the plant, which would affect the accumulation of certain
321 phenolic compounds (Sun et al., 2017).
322 The structural characteristics (weight average (Mw) and number average (Mn) molar
323 masses) of polysaccharides from CO-wines were also obtained by SEC-MALLS analyses.
324 Several differences were observed concerning the molar mass distribution of polysaccharides
325 from CO-wines. R1 (low molar masses) ranged from 23% to 36%, whereas R5 (high molar
326 masses) varied from 3% to 6%. We have also observed differences of approximately 10%
327 between the lowest and the highest percentage values of the three other intermediate ranges
328 (R2, R3 and R4). Besides, differences were found between the $M_w$ and $M_n$ values. $M_w$ varied
329 from 118 000 g·mol$^{-1}$ to 164 000 g·mol$^{-1}$, while $M_n$ ranged between 24 700 g·mol$^{-1}$ and
330 41 400 g·mol$^{-1}$. Martínez-Lapuente et al. (2018) also found variations in the structural
331 parameters of polysaccharide fractions from sparkling wines elaborated with two different
332 cultivar grapes. We could hence conclude that, together with the compositional aspects, the
333 structural properties of polysaccharide fractions from the CO-wines studied were very
334 distinct. The well-differentiation of base wines was corroborated by PCA analysis
335 (Supplementary Figure 1A), explaining the first two principal components 72% of the
336 variability.
337

338 *3.3. Foaming parameters on ORI-wines and CO-wines measured by ST*

339 Tables 1A and 1B present the values of the HM (at T5) and those of the foam height during
340 the stability period (T70, T80 and T90) of the ORI-wines and the CO-wines. Similar
341 information concerning the rest of ST moments is given in Supplementary Table 1. As
342 expected, the eight ORI-wines presented the highest HM compared to their corresponding
343 CO-wines, in agreement with previous studies (Marchal et al., 2002; Vanrell et al., 2007).
344 The decrease of the TAAs of the proteins was estimated higher than 85% after treatment
345 with bentonite. This drastic decrease caused by the addition of bentonite would explain why
346 the HM was negatively affected with this treatment. On the other hand, and compared to
347 their corresponding CO-wines, ORIMA, ORISA, ORITA, ORINO2, ORIRU1, ORIRU2 and
348 ORIRU3 presented higher foam height values during the stability period in wines, whereas
349 ORINO1 wine showed similar values for this parameter. The tendency of bentonite to
350 remove specific proteins (Jaeckels et al., 2017) may be linked to the different features of
351 each wine, which could explain its different action on ORINO1 wine.

352

353 *3.4. Characteristics of fractions from Asen*G *and Asey*G

354 Table 3 shows the protein percentage of fractions from *Asen*G and *Asey*G (from Apolinar-
355 Valiente et al., 2019 and 2020b). F1*sen* and F1*sey* exhibited much greater protein
356 concentration (11.5% and 7.4%, respectively) than F2*sen* (1.6%) and F2*sey* (0.6%). The
357 intrinsic viscosity of the fractions (Table 3) was also notably higher in the case of F1
358 fractions (F1*sen*: 88 $mL \cdot g^{-1}$; F1*sey*: 36 $mL \cdot g^{-1}$) compared to F2 fractions (F2*sen*: 29 $mL \cdot g^{-1}$;
359 F2*sey*: 22 $mL \cdot g^{-1}$). Besides, the gums fractions showed a very different amino acid
360 composition (Supplementary Table 2), which should therefore give different hydrophobic
361 scores. Onishi and Proudlove (1994) reported that the absolute level of hydrophobic
362 polypeptide is important to stabilize foam in beer. This resulted from the more hydrophobic
363 amino acids, which were adsorbed at the air/liquid interfaces and consequently stablish

intermolecular nets. The drainage was then slowed, resulting in a higher stabilization of the liquid films and a longer foam lifespan. Following the procedure described by Apolinar-Valiente et al. (2020a), hydrophobic scores have been estimated (Table 3) through the non-polar hydrophobic amino acids (alanine, isoleucine, leucine, phenylalanine, proline and valine) and using the hydrophobicity scale proposed by Monera, Sereda, Zhou, Kay and Hodges (1995). The increasing order of the hydrophobic score resulted in: F2*sey* < F2*sen* < F1*sen* < F1*sey* (Table 3). Hydrophobicity has been demonstrated as a key factor on several structural and physicochemical properties of the gum (Mejia Tamayo et al., 2018; Sanchez et al., 2018).

Table 3 also gives other molecular characteristics such as molar mass distribution (R-I: range I = molar mass below 500 000 $g \cdot mol^{-1}$; R-II: range II = molar mass between 500 000 and 1 000 000 $g \cdot mol^{-1}$; and R-III: range III = molar mass above 1 000 000 $g \cdot mol^{-1}$), the weight average ($M_w$) and number average ($M_n$) molar masses of gums fractions (adapted from Apolinar-Valiente et al., 2019 and 2020b). These ranges were named using Roman numerals to clearly distinguish them from ranges for molar masses of CO-wines polysaccharides. F1*sey* and notably F1*sen* showed greater percentages values in range R-III (high molar masses) as well as much greater values of $M_w$ and $M_n$ compared to F2 fractions. Theoretically, molecules with higher $M_w$, so in our case F1 fractions, would migrate less easily to the interfaces. But F1 fractions also presented greater protein content than F2 fractions, which would result in diffusion to the upper interfaces. One of these two behaviors will act in a higher way than the other one, being in general the protein percentage the main factor. This point will be clarified with the foamability results obtained after gums fractions supplementation.

Table 3 includes, moreover, the coefficient of partial specific volume ($v_s°$) and the coefficient of partial specific adiabatic compressibility ($\beta_s°$) of gums fractions (from Mejia Tamayo et al., 2018). These two volumetric properties are directly related to the

compressibility and hydration of biopolymers (Gekko & Yamagami, 1991). The $v_s°$ coefficient was lower in both F2 fractions compared to F1*sey* and mainly to F1*sen*. The $\beta_s°$ coefficient was higher in F1*sen* and particularly in F1*sey* compared to F2 fractions. This data would imply lower hydrated and more flexible structure in F1 fractions compared with F2 fractions.

The well-differentiation of the gums fractions was reinforced by PCA analysis (Supplementary Figure 1B). The first two principal components explained together 86% of the variability of the data.

*3.5. Foamability of CO-wines after gums fractions supplementation*

The values of the HM (at T5) and those of the foam height during the stability period (T70, T80 and T90) of the CO-wines and the supplemented CO-wines are given in Tables 1C and 4. The information about the rest of ST moments is available on Supplementary Table 1. Moreover, Figure 1 gives the significant maximal differentials of foam height (ΔFH*max*). These differentials were calculated subtracting the foam height of CO-wines from the foam height of their corresponding supplemented CO-wines. This parameter must be taken into account, because the HM values not always matched to their corresponding ΔFH*max* values.

*3.5.1. HM and the significant maximal differentials of foam height (ΔFHmax)*

As previously mentioned, HM was reached at T5 for the eight CO-wines (Table 1C) as well as for all the supplemented CO-wines (Tables 1C and 4). 11 out of the 16 supplementations with F1 fractions (69%) improved the HM for every wine at some moment during the ST. More specifically, HM was increased in 75% of the base wines (COMA, COSA, COTA, CONO2, CORU2 and CORU3) after supplementation with F1*sen*, and in 63% of the wines (COMA, COSA, COTA, CONO1 and CORU3) using F1*sey*. Therefore, F1 fractions improved HM in Spanish wines much more often (100% of the F1 supplementations) than in

French wines (50% of the F1 supplementations). In addition, both F1*sen* and F1*sey* supplementations enhanced foam height during all ST in the three Spanish wines (Supplementary Table 1). Instead, F2 fractions only increased punctually foam height. HM increased significantly in COMA wine after F2*sen* supplementation and in CONO2 wine using F2*sey*.

ΔFH*max* improved significantly in 100% of the wines using F1 fractions (Fig 1). Comparing both F1 supplementations, a higher ΔFH*max* for the same wine was more frequently obtained using F1*sey* (in 75% of the wines: COSA, CONO1, CONO2, CORU1, CORU2 and CORU3; Fig 1). By contrast, a greater ΔFH*max* for the same wine was less commonly found supplementing F1*sen* (in 25% of the remaining wines: COMA and COTA; Fig 1). This may suggest that F1*sen* increased more efficiently from a quantitative perspective the foamability in the studied Spanish wines, whereas F1*sey* was more successful supplementing these French wines. Furthermore, ΔFH*max* arrived sooner when F1*sey* was used (in 63% of the wines: COMA, COTA, CONO1, CORU1 and CORU3; Fig 1B) compared to F1*sen* (Fig 1A), whereas in the remaining 37% of the wines (COSA, CONO2 and CORU2) ΔFH*max* arrived at the same moment regardless of the F1 supplementation. Concerning supplementations with F2 fractions, they only enhanced punctually ΔFH*max*. This parameter was significantly improved using F2*sen* in COMA and COSA, and also after F2*sey* supplementation in COMA and CONO2 wines.

*3.5.2. Foam height during the foam stability period*

The foam height during the stability period increased in 63% of the base wines (COMA, COSA, COTA, CONO1 and CORU1) using F1*sen,* and in 37% of the base wines (COMA, COSA and COTA) after F1*sey* supplementation (Tables 1C and 4). Therefore, F1 fractions improved foam height during the stability period much more commonly in Spanish wines

441 (100%) compared to French wines (in 20% of the cases: CONO1 and CORU1 wines only
442 when they were supplemented with F1*sen*).
443 Summarizing, the separate supplementations with the gums fractions showed different
444 influences on the foamability of the eight base wines. These different impacts would depend
445 on the characteristics of the well-differentiated wines and the well-distinguished
446 supplementing fractions, but probably also on their complex relationships. This will be
447 discussed in sections 3.6 and 3.7.
448
449 *3.6. Impact of wine properties on foamability after gums fractions supplementation*
450 Using the data concerning base wine properties (Table 2) and foam height data of the CO-
451 wines and supplemented CO-wines (Tables 1B, 1C and 4, as well as Supplementary Table
452 1), we have calculated the significant Pearson correlation coefficients (Figure 2A).
453 Mannoproteins (MPs) percentage presented negative correlation with foam height when
454 wines were separately supplemented with F1 fractions at some points during ST. This
455 observation seems to be in contradiction with the fact that MPs of wine have been
456 demonstrated as major foam promoters (Blasco et al., 2011). However, Martínez-Lapuente et
457 al. (2015) did not find any impact of MPs on the maximum foam height or the foam stability
458 height of sparkling wines. Similarly, CO-wines of the present study did not show any
459 correlation between MPs percentage and foam height at any moment (Fig 2A). Therefore, it
460 seems logic to think that the separate supplementations with F1 fractions triggered the
461 negative effect of MPs on wine foamability. The synergistic interaction of the foam active
462 components, such as peptides, proteins and complex carbohydrates, could change their
463 surface-active properties and, thereby, their foaming properties (Martínez-Lapuente et al.,
464 2015). It could be coherent to conclude that F1 fractions were the compounds which may
465 modify the surface-active properties related to MPs in a negative way. Separate
466 supplementation with F1 fractions may play the role of the unidentified factor which,

according to Abou Saleh et al. (2007), could change the structure of the adsorption layer. In accordance with Blasco et al. (2011), protein fraction of MPs might interact with other proteins to form a more stable film by increasing its viscoelasticity. Could we hypothesize that the interaction [MPs-F1 fractions] may result in an excessive $M_w$ influencing negatively in the diffusion from the bulk to the interfaces? Further work to clarify this possibility should be carried out. We previously found (Apolinar-Valiente et al., 2020a) a correlation between the variation percentage of HM-MOS and the percentages of MPs and Total Polysaccharides content (TPs) after *Asey*G supplementation, appearing MPs also as a negative factor.

Positive correlations between foam height and PRAGs percentage of wines were observed at some ST moments after the supplementation of every gums fraction (Fig 2A). Any correlation between PRAGs percentage and foam height was noted in CO-wines. However, positive correlations were found between the foam height and the R3 percentage in CO-wines (from T50 to T90) and in CO-wines plus every fraction (from T20 to T90). Therefore, although there was a previous and positive impact of R3 on the foam height, the supplementation with fractions extended this increasing effect (from T20 to T50). A link between the positive correlations [R3–foam height] and [PRAGs–foam height] found in supplemented CO-wines may be suggested, because range R3 delimitates the molecules corresponding to the $AGP_2$. The positive effect of polysaccharides from grapes on foam stability has been previously assigned to PRAGs (Martínez-Lapuente et al., 2015), which concluded that foam stability could be explained also by their charges. PRAGs could interact with other molecules by, among others, hydrophobic forces, preventing coalescence of bubbles. The role of the hydrophobicity on wine foamability has been reported (Brissonnet & Maujean, 1993; Coelho et al., 2011a). According to Ferreira, Jorge, Nogueira, Silva and Trugo (2005), high hydrophobicity would be the better way for stabilizing the viscoelastic film around beer foam bubbles. Possible hydrophobic interactions between PRAGs and gums fractions could explain the observed positive correlations after its supplementation.

493 The greater hydrophobic scores in F1 fractions could explain their more frequent correlations
494 compared to their corresponding F2 fractions. Our observations would result of certain
495 complex poly-macromolecular associations leading to a network at the air/water interface
496 (Abdallah et al., 2010). Studying air/water interfacial properties of protein-*Acacia* gum
497 complexes, Schmitt et al. (2005) suggested that when foam was stabilized using protein-
498 polysaccharide complex, foam stability was higher compared to the solution containing the
499 protein (β-lactoglobulin) alone.
500 Moreover, a positive correlation was found between $M_n$ of wine polysaccharides and foam
501 height at T5 when F1*sey* supplemented the wines. Knowing that CO-wines did not show any
502 correlation between $M_n$ and foam height (Fig 2A), this behavior was evidently explained by
503 F1*sey* supplementation. Correlations between polypeptide molar mass, hydrophobicity and
504 foam stabilizing activity have been reported in beer (Onishi & Proudlove, 1994).
505 In short, after F1 supplementations, the MPs percentage in base wines affected negatively
506 their foamability, showing PRAGs and R3 percentages positive correlations. $M_n$ also
507 influenced positively the foamability of the base wines after F1*sey* supplementation.
508
509 *3.7. Impact of gums fractions properties on the foamability of base wines*
510 Pearson coefficient correlations (Fig 2B) were performed using the data about the fractions
511 properties (Table 3) and the foam height data of the supplemented CO-wines (Tables 1C and
512 4 and Supplementary Table 1).
513 Figure 2B shows that the properties of gums fractions impacted differently foamability at
514 varying points during the ST depending on the wine. It must be highlighted that at least two
515 properties of the gums fractions influenced positively on the foamability of every wine at
516 some moment during the ST. However, this number was usually higher than two.
517 Sugars percentage and foam height presented negative correlations in 1 out of the 8 studied
518 base wines at some moment during the ST period. These negative correlations did not

coincide with positive correlations between protein percentage and foam height most of the time. This suggested that the influence of sugars percentage could be independent of the effect of protein percentage. In a previous work, Coelho, Rocha and Coimbra (2011b) observed that among arabinogalactans from wine, the one with the lower percentage of sugars seemed to be the most relevant regarding the foam aptitude.

Positive correlations between foam height and protein percentage of fractions were observed at a given moment during the ST period in 5 out of the 8 studied base wines. Moreover, these moments were included, among others, within the foam stability period in 4 out of them. These observations would be linked with the fact that the greater the protein percentage of the fractions, the greater the protein supplementation of the base wines. This is in accordance with the fact that F1 fractions presented much higher impact on foamability than F2 fractions (Tables 1C and 4). Thanks to the greater content of proteins in F1 fractions, they would have better interfacial rheological properties, delaying the rupture of these films and hence stabilizing bubbles. A strong link between protein content and foam characteristics in base (Maujean et al., 1990; Marchal et al., 2002) or sparkling (Brissonnet & Maujean, 1993; Vanrell et al., 2007; Martínez-Lapuente et al., 2015) wines has been observed. Previously, we also found that the variation percentage of HS-MOS in base wines was significantly correlated with the protein content of gums and HIC-fractions from *Asen*G (Apolinar-Valiente et al., 2020a).

The hydrophobic score showed positive correlations with the foam height in 5 out of the 8 studied base wines at some moment during the ST period. Positive correlation was also found at some moments of the foam stability period in 2 out of these 5 base wines. Wierenga, Meinders, Egmond, Voragen and de Jongh (2003) reported that the adsorption of proteins to the air/water interface improved with increased hydrophobicity. They also observed the improving effect of the hydrophobicity on the foamability of non-alcoholic systems. This trend is, however, similar in beverages with moderate ethanol content. In base

545 sparkling wines, hydrophobic proteins seemed to contribute more to foam constitution than
546 hydrophilic proteins (Brissonnet & Maujean, 1993). Besides, Apolinar-Valiente et al.
547 (2020a) reported that the increasing order of the foamability of a synthetic wine after
548 supplementation with *Acacia* gums and *Asen*G HIC-fractions corresponded with the
549 enhancing order of their hydrophobicity. In beer, the correlation between hydrophobicity and
550 foam stability has also been reported (Onishi & Proudlove, 1994). It must be also highlighted
551 that both hydrophobic scores and protein contents are correlated with foam height in only 1
552 out of 8 wines. This apparent discrepancy may be explained not only by the protein content
553 but also by its composition, which play a key role in their hydrophobicity. For example, in
554 the present work, F1*sen* presented higher protein content (11.5%) but lower hydrophobic
555 score (1.528) compared to F1*sey* (7.4% and 2.761, respectively). The different protein
556 composition could also explain, together with other factors such as the employed
557 methodologies to measure the protein content, the unequal results about protein content and
558 foam behaviors previously mentioned in the introduction (Maujean et al., 1990; Pueyo et al.,
559 1995).
560 Positive correlations were also found between foam height and (i) the $M_w$ (in 6 out of the 8
561 base wines), (ii) the $M_n$ (in 3 out of the 8 base wines) and (iii) the cumulative molar mass
562 percentage of R-III (high molar masses range) (in 4 out of the 8 base wines) values of gums
563 fractions at some point during the ST period. These positive correlations were included
564 within the foam height stability period in 2 (in the case of $M_w$), 3 (in the case of $M_n$) and 2
565 (in the case of R-III) out of them. On the other hand, negative correlation between foam
566 height and R-I (low molar masses range) were found in 1 out of 8 base wines. From this it
567 can be concluded that, in general, high molar masses increased foamability of base wines.
568 However, as previously mentioned, molecules with greater $M_w$ would migrate less easily to
569 the interfaces. Therefore, we can hypothesize that the "higher protein content" factor which
570 was found in F1 fractions would influence in a greater way on the affinities and the diffusion

to the interfaces. Moreover, Lopez-Torrez et al. (2015) suggested that in *Acacia* gum solutions with $M_w$ values about 2–3 x $10^6$ g·mol$^{-1}$, aggregates were always present. F1*sen* appears as a fraction greatly rich in aggregates composed of proteins (Apolinar-Valiente et al., 2019). It has been also observed that after fractionation from *Acacia* gum by HIC, only the HIC-F3 fraction, contributing 70% to F1*sen* separated by IEC, presented a significant proportion of aggregates after centrifugation (reviewed by Sanchez et al., 2018). These observations, together with their similar fractionation methodology and their similar trends concerning $M_w$, $M_n$, R-I and R-III values suggest that F1*sey* would also present high content of aggregates. All this along with our results would be in coherence with observations made by Rullier, Novales and Axelos (2008), showing that protein aggregates participate to a better foam stabilization, although always conditioned to a minimum and obligatory presence of non-aggregated proteins. We previously reported that the variation percentage of HS-MOS of base wines correlated with $M_w$ value of gums and HIC-fractions from *Asen*G (Apolinar-Valiente et al., 2020a).

Intrinsic viscosity of gums fractions correlated positively with foam height in 3 out of 8 base wines at some point during the ST period. These correlations were within the foam height stability period in 2 out of them. As previously mentioned, drainage is one of the principal disruptive processes in foams. It implies the flow of continuous phase liquid through the thin films and Plateau borders (the intersection of three thin liquid films) of foam matrix, mainly by gravity. But in the case of wine foam, the Plateau borders are unusual; consequently, in our samples, drainage practically only takes place through the films of bubbles remaining essentially spherical. Drainage may be delayed improving the viscosity of the liquid phase. This action delays the foam film thinning and bubbles are separated by much thicker films. It may be suggested that the addition of F1 fractions, which showed great intrinsic viscosity values, would decrease the drainage process and, hence, the foam disruption. Carp, Wagner,

Bartholomai and Pilosof (1997) demonstrated the improvement of foam drainage stability of enhancing bulk viscosity through addition of xanthan gum.

The $v_s°$ coefficient correlated positively with foam height at some moment during the ST period in 5 out of 8 base wines. Furthermore, foam height gave positive Pearson correlations with the $\beta_s°$ coefficient at certain moment during the ST period in all the 8 base wines studied. The volumetric $v_s°$ and $\beta_s°$ coefficients depend mainly on the intrinsic contribution of the solute and its hydration. They can be linked to solvent-solute interactions and have been used to predict the structure and flexibility of macromolecules (Gekko & Yamagami, 1991). Flexible proteins decrease surface tension earlier and faster than rigid proteins (Martin, Grolle, Martin, Stuart, & Vliet, 2002), presenting a higher foaming capacity (Damodaran 2008). The higher values of $v_s°$ and $\beta_s°$ coefficients observed in F1 fractions compared to those of F2 fractions would corresponded to lower hydrated and more flexible structures. Since, as mentioned, proteins molecule flexibility have been clearly related to their interfacial properties (Gekko & Yamagami, 1991), F1 fractions will have better interfacial properties compared to F2 fractions, including better foaming properties.

Finally, we note here that the demonstrated higher anisotropy of F1 fractions compared to F2 fractions (Apolinar-Valiente et al., 2019 and 2020b) could also favor foamability of base wines, which is in coherence with our results. According to Dickinson (2016), a transient of elongated particles showed more effectiveness as steric barrier in the spaces between bubbles. This would imply higher inhibiting bubble coalescence in systems with highly elongated particles compared to less elongated particles.

Summarizing, the hydrophobic score, the R-III range (high molar masses range), the $M_w$ parameter, the intrinsic viscosity and the $v_s°$ and $\beta_s°$ coefficients of the gums fractions showed therefore an evident positive influence on foamability features after fractions supplementation. All these factors are strongly related to the protein percentage of gums fractions which, as expected, also played an important role on foamability (in 63% of the

base wines at some point during the ST). On the other hand, the sugars percentage and the R-I range (low molar masses range) affected negatively the foamability of one wine.

## 4. Conclusions

The shaking test (ST) was a valid and simple method to measure Maximum Foam Height and, albeit with less accuracy, foam stability of sparkling base wines. ST may be very easily used in wineries and oenological laboratories. HM was improved in 11 out of the 16 supplementations (69%) with F1 fractions, which were the fractions with high protein amount and high molar mass. F1 fractions increased HM as well as foam height during the stability period in Spanish wines much more commonly than in French wines. The differentials of foam height (ΔFH) between "supplemented CO-wines" and CO-wines enhanced significantly in all the studied wines at several moments after supplementations with F1 fractions. HM did not always match to its corresponding maximal ΔFH (ΔFH*max*). ΔFH*max* increased significantly in all the studied wines (100%) after F1 separate supplementations. When French wines were supplemented with F1*sey*, a greater significant increase of ΔFH*max* was observed compared to F1*sen*. In contrast, ΔFH*max* was higher in 2 out of 3 Spanish wines supplemented with F1*sen*. F2 fractions gave only small and punctual enhancing effects on foam behavior. It can be hence concluded that the supplementation of F1 fractions from *Acacia senegal* and *Acacia seyal* gums partially restored the foamability of some sparkling base wines after bentonite treatment.

Improvement of base wines foamability was positively influenced by the PRAGs and R3 percentages and the $M_n$ values of base wines, as well as by the protein and R-III (high molar masses range) percentages, the hydrophobic score, the $M_w$ and $M_n$ values, the intrinsic viscosity and the $v_s°$ and $\beta_s°$ coefficients of gums fractions at some point during the ST. However, foamability was negatively affected by the MPs percentage in wines as well as the sugars and the R-I (low molar masses range) percentages of gums fractions sometime in the

course of ST. Therefore, it must be concluded that foam behavior strongly depended on the properties of the supplementing gums fractions obtained by IEC and, although to a minor extent, on wine characteristics, as well as on their relationships. Further studies about these unclear and complex relationships as well as about the possible macromolecular complexes at the air/liquid interface must be done. Moreover, analysis should be carried out to deepen on the concentration of *Acacia* gums treatments and the addition of other types of gums on foam behavior in base and sparkling wines.

## CRediT authorship contribution statement

**Rafael Apolinar-Valiente**: Conceptualization, Investigation, Validation, Formal analysis, Writing - original draft, Writing - review & editing. **Thomas Salmon**: Methodology, Investigation. **Pascale Williams**: Conceptualization, Methodology, Investigation, Writing review & editing. **Michaël Nigen**: Resources, Visualization, Writing review & editing. **Christian Sanchez**: Resources, Visualization, Writing - review & editing. **Thierry Doco**: Conceptualization, Methodology, Validation, Writing - review & editing, Supervision. **Richard Marchal**: Conceptualization, Methodology, Validation, Writing - review & editing, Supervision.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests of personal relationships that could have appeared to influence the work reported in this paper.

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**Table 1.** Foam height values (mm) of **(A)** ORI-wines (original wines), **(B)** CO-wines (control wines) and **(C)** Malaga and Champagne NO2 supplemented CO-wines with F1*sen*, F2*sen*, F1*sey* and F2*sey* (300 mg·L$^{-1}$), measured by Shaking Test (ST; at T5, T10, T70, T80, T90) and Mosalux (MOS) procedures.

| **A** | ST Time[a]/ORI-wines | ORIMA[b] | ORISA[b] | ORITA[b] | ORINO1[b] | ORINO2[b] | ORIRU1[b] | ORIRU2[b] | ORIRU3[b] |
|---|---|---|---|---|---|---|---|---|---|
| | T5 | 48.7 ± 0.6 | 36.3 ± 1.5 | 53.0 ± 1.8 | 53.0 ± 3.0 | 48.3 ± 1.8 | 54.3 ± 1.2 | 53.0 ± 2.7 | 51.0 ± 0.9 |
| | T10 | 45.0 ± 0.9 | 28.3 ± 1.5 | 48.3 ± 2.7 | 46.0 ± 3.6 | 41.7 ± 1.5 | 47.3 ± 1.2 | 42.7 ± 1,5 | 41.0 ± 2.7 |
| | T70 | 30.0 ± 1.0 | 9.7 ± 2.1 | 34.3 ± 1.2 | 3.6 ± 0.3 | 18.7 ± 1.5 | 23.0 ± 3.6 | 12.0 ± 2.9 | 16.3 ± 3.7 |
| | T80 | 28.3 ± 2.1 | 9.3 ± 2.3 | 34.3 ± 0.6 | 3.6 ± 0.3 | 18.0 ± 1.5 | 22.0 ± 3.6 | 11.3 ± 3.2 | 15.7 ± 3.2 |
| | T90 | 27.7 ± 1.5 | 8.7 ± 2.1 | 34.0 ± 1.0 | 3.3 ± 0.3 | 18.0 ± 1.5 | 21.0 ± 4.6 | 10.3 ± 2.7 | 15.0 ± 2.6 |
| | HM-MOS[c] | 260.0 ± 10.0 | 201.7 ± 2.9 | 366.7 ± 5.8 | 276.7 ± 5.8 | 225.0 ± 5.0 | 253.3 ± 10.4 | 180.0 ± 5.0 | 210.0 ± 5.0 |
| | HS-MOS[c] | 200.0 ± 20.0 | 130.0 ± 0.0 | 280.0 ± 26.5 | 136.7 ± 5.8 | 127.7 ± 2.5 | 150.0 ± 0.0 | 115.0 ± 0.0 | 120.0 ± 5.0 |

| **B** | ST Time[a]/CO-wines | COMA[d] | COSA[d] | COTA[d] | CONO1[d] | CONO2[d] | CORU1[d] | CORU2[d] | CORU3[d] |
|---|---|---|---|---|---|---|---|---|---|
| | T5 | 28.3 ± 1.2 | 21.0 ± 1.0 | 36.3 ± 1.2 | 36.7 ± 3.5 | 10.7 ± 2.1 | 46.7 ± 3.5 | 10.3 ± 0.6 | 23.0 ± 1.0 |
| | T10 | 18.0 ± 2.6 | 10.3 ± 0.6 | 25.3 ± 2.9 | 22.7 ± 3.5 | 2.7 ± 0.9 | 36.7 ± 1.5 | 1.3 ± 0.6 | 7.7 ± 0.6 |
| | T70 | 5.3 ± 1.5 | 1.3 ± 0.6 | 7.3 ± 1.5 | 4.3 ± 1.4 | 0.0 ± 0.0 | 8.0 ± 1.8 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| | T80 | 5.0 ± 1.0 | 1.3 ± 0.0 | 7.3 ± 1.5 | 4.0 ± 1.4 | 0.0 ± 0.0 | 7.3 ± 1.5 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| | T90 | 4.3 ± 1.2 | 1.0 ± 0.0 | 6.7 ± 1.5 | 3.3 ± 1.2 | 0.0 ± 0.0 | 7.0 ± 1.5 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| | HM-MOS[c] | 172.7 ± 2.5 | 130.0 ± 5.0 | 185.0 ± 5.0 | 155.0 ± 0.0 | 116.7 ± 2.9 | 166.7 ± 7.6 | 115.0 ± 0.0 | 131.7 ± 2.9 |
| | HS-MOS[c] | 127.7 ± 7.5 | 125.0 ± 0.0 | 160.0 ± 10.0 | 141.7 ± 2.9 | 111.7 ± 2.9 | 148.3 ± 2.9 | 111.7 ± 2.9 | 118.3 ± 2.9 |

| **C** | Supplemented CO-wines | Malaga (MA) | | | | Champagne NO2 | | | |
|---|---|---|---|---|---|---|---|---|---|
| | ST Time[a]/Fraction | F1*sen* | F2*sen* | F1*sey* | F2*sey* | F1*sen* | F2*sen* | F1*sey* | F2*sey* |
| | T5 | **37.3* ± 2.3** | **32.7* ± 2.3** | **40.0* ± 1.2** | 30.7 ± 0.0 | **19.7* ± 3.1** | 9.7 ± 1.3 | 32.7 ± 2.1 | **13.0* ± 2.3** |
| | T10 | **31.7* ± 2.0** | 21.7 ± 2.5 | **34.3* ± 1.5** | **24.0* ± 1.0** | **8.3* ± 2.1** | 2.7 ± 0.9 | **20.3* ± 2.6** | 3.7 ± 0.9 |
| | T70 | **21.0* ± 0.0** | 6.7 ± 1.5 | **13.0* ± 2.7** | 6.7 ± 1.2 | 0.7 ± 0.3 | 0.0 ± 0.0 | 1.3 ± 0.3 | 0.0 ± 0.0 |
| | T80 | **20.7* ± 0.6** | 6.7 ± 1.5 | **13.0* ± 2.7** | 6.7 ± 1.2 | 0.3 ± 0.0 | 0.0 ± 0.0 | 1.0 ± 0.2 | 0.0 ± 0.0 |
| | T90 | **20.0* ± 1.0** | 6.0 ± 1.5 | **12.3* ± 2.1** | 6.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.7 ± 0.2 | 0.0 ± 0.0 |
| | HM-MOS | 215.0 ± 5.0 | 183.3 ± 5.8 | 200.0 ± 0.0 | 175.0 ± 5.0 | 141.7 ± 5.8 | 118.3 ± 2.9 | 160.0 ± 0.0 | 125.0 ± 0.0 |
| | HS-MOS | 160.0 ± 0.0 | 126.7 ± 7.6 | 145.0 ± 15.0 | 127.7 ± 2.5 | 118.3 ± 2.9 | 113.3 ± 2.9 | 130.0 ± 0.0 | 120.0 ± 0.0 |

[a]Time numbers indicate the seconds after the beginning of ST to which the foam height was measured.

[b]ORIMA: Malaga original wine; ORISA: Saragossa original wine; ORITA: Tarragona original wine; ORINO1: Champagne NO1 original wine; ORINO2: Champagne NO2 original wine; ORIRU1: Champagne RU1 original wine; ORIRU2: Champagne RU2 original wine; ORIRU3: Champagne RU3 original wine.
[c]HM: the Maximum Foam Height (mm); HS: the Foam Stability Height during $CO_2$ injection (mm); data adapted from Apolinar-Valiente et al. (2020a).
[d]COMA: Malaga control wine; COSA: Saragossa control wine; COTA: Tarragona control wine; CONO1: Champagne NO1 control wine; CONO2: Champagne NO2 control wine; CORU1: Champagne RU1 control wine; CORU2: Champagne RU2 control wine; CORU3: Champagne RU3 control wine.
Data in bold with asterisks statistically indicate significant differences compared to their corresponding CO wine.
Values are the average of three replicates.

**Table 2.** Total amino acids content (TAAs; $mg \cdot L^{-1}$), families of polysaccharides (MPs; RG-II; PRAGs; %), total content of polysaccharides ($mg \cdot L^{-1}$), total content of oligosaccharides ($mg \cdot L^{-1}$), total polyphenols index as well as cumulative ranges for molar masses (R1, R2, R3, R4, R5; %), molecular parameters ($M_w$, $M_n$, $g \cdot mol^{-1}$) and intrinsic viscosity (I.V.; $mL \cdot g^{-1}$) by SEC MALLS of polysaccharides from studied CO-wines.

| Feature/ Wine Origin | Malaga (MA) | Saragossa (SA) | Tarragona (TA) | Champagne NO1 | Champagne NO2 | Champagne RU1 | Champagne RU2 | Champagne RU3 |
|---|---|---|---|---|---|---|---|---|
| TAAs[a,b] | 1.2 ± 0.0 | 1.6 ± 0.1 | 1.6 ± 0.1 | 2.5 ± 0.2 | 3.9 ± 0.3 | 1.0 ± 0.1 | 1.1 ± 0.1 | 0.7 ± 0.0 |
| MPs[a,b] | 37 ± 3 | 37 ± 2 | 34 ± 3 | 58 ± 1 | 62 ± 1 | 49 ± 2 | 62 ± 2 | 48 ± 1 |
| RGII[a,b] | 21 ± 2 | 36 ± 1 | 30 ± 1 | 17 ± 1 | 19 ± 1 | 16 ± 1 | 18 ± 1 | 19 ± 1 |
| PRAGs[a,b] | 41 ± 3 | 27 ± 1 | 36 ± 3 | 26 ± 1 | 20 ± 1 | 34 ± 2 | 20 ± 1 | 33 ± 1 |
| TPs[a,b] | 145 ± 10 | 174 ± 14 | 168 ± 11 | 133 ± 8 | 140 ± 9 | 221 ± 14 | 114 ± 7 | 219 ± 15 |
| TOs[a,b] | 144 ± 12 | 148 ± 14 | 134 ± 11 | 78 ± 6 | 84 ± 8 | 80 ± 8 | 85 ± 7 | 98 ± 8 |
| TPI[a] | 9.1 ± 1.2 | 8.5 ± 0.9 | 7.4 ± 0.9 | 4.6 ± 0.6 | 5.3 ± 0.9 | 5.0 ± 0.7 | 4.5 ± 0.6 | 5.4 ± 0.5 |
| R1[a] | 26.5 ± 0.3 | 36.4 ± 0.4 | 29.2 ± 0.5 | 23.3 ± 0.2 | 23.4 ± 0.2 | 19.9 ± 0.2 | 27.1 ± 0.6 | 24.8 ± 0.4 |
| R2[a] | 38.6 ± 0.3 | 35.1 ± 0.2 | 35.7 ± 0.4 | 42.3 ± 0.7 | 37.5 ± 0.4 | 43.3 ± 0.6 | 36.6 ± 0.6 | 44.8 ± 0.3 |
| R3[a] | 20.7 ± 0.5 | 13.1 ± 0.5 | 21.8 ± 0.6 | 18.3 ± 0.5 | 16.5 ± 0.3 | 18.3 ± 0.5 | 16.6 ± 0.4 | 16.6 ± 0.4 |
| R4[a] | 10.4 ± 0.4 | 11.1 ± 0.2 | 9.9 ± 0.4 | 12.8 ± 0.3 | 18.7 ± 0.6 | 12.8 ± 0.4 | 15.2 ± 0.3 | 10.2 ± 0.3 |
| R5[a] | 3.8 ± 0.1 | 4.2 ± 0.2 | 3.4 ± 0.2 | 3.3 ± 0.1 | 4.9 ± 0.2 | 5.7 ± 0.2 | 4.6 ± 0.4 | 3.6 ± 0.3 |
| $M_w$[a] | 131 000 ± 11 300 | 118 100 ± 9 200 | 127 800 ± 10 100 | 127 600 ± 10 800 | 144 400 ± 11 900 | 164 000 ± 12 100 | 134 500 ± 10 700 | 117 500 ± 9 400 |
| $M_n$[a] | 33 920 ± 2 130 | 24 720 ± 2 160 | 29 840 ± 1 890 | 34 460 ± 2 110 | 35 040 ± 2 300 | 41 370 ± 3 210 | 29 540 ± 2 180 | 31 850 ± 2 390 |
| I.V.[a] | 18.2 ± 0.9 | 17.8 ± 0.6 | 18.8 ± 0.8 | 20.0 ± 0.9 | 21.9 ± 0.8 | 20.4 ± 1.0 | 21.5 ± 0.6 | 19.2 ± 0.8 |

[a]TAAs : total amino acid content; MPs: mannoproteins; RG-II: rhamnogalacturonans type II; PRAGs: polysaccharides rich in arabinose and galactose; TPs: total polysaccharide content; TOs: total oligosaccharide content; TPI: total polyphenols index; R1: range 1 = molar mass between 2500 and 20 000 $g \cdot mol^{-1}$; R2: range 2 = molar mass between 20 000 and 100 000 $g \cdot mol^{-1}$ ; R3: range 3 = molar mass between 100 000 and 250 000 $g \cdot mol^{-1}$; R4: range 4 = molar mass between 250 000 and 500 000 $g \cdot mol^{-1}$; R5: range 5 = molar mass between 500 000 and 1 000 000 $g \cdot mol^{-1}$; $M_w$: weight average molar mass; $M_n$: number average molar mass; I.V.: intrinsic viscosity.
[b]data from Apolinar-Valiente et al. (2020a).

**Table 3.** Protein percentage (%), hydrophobic score, cumulative ranges for molar masses (R-I, R-II, R-III; %), molecular parameters ($M_w$, $M_{n;}$ g·mol$^{-1}$) and intrinsic viscosity (IV; mL·g$^{-1}$) by SEC MALLS and the partial specific volume ($v_s°$; cm$^3$·g$^{-1}$) and the coefficient of partial specific adiabatic compressibility ($\beta_s°$; $10^{11}$ x Pa$^{-1}$) of F1*sen*, F2*sen*, F1*sey* and F2*sey*.

| Fraction/Property | Proteins (%)[a] | Hydrophobic score[b] | R-I[a,c] | R-II[a,c] | R-III[a,c] | $M_w$[a,c] | $M_n$[a,c] | IV [a] | $v_s°$ [a] | $\beta_s°$ [a] |
|---|---|---|---|---|---|---|---|---|---|---|
| F1*sen* | 11.5 | 1.528 | 0.0 | 2.6 | 97.4 | 3 100 000 | 2 500 000 | 87.8 | 0.610 | -9.4 |
| F2*sen* | 1.6 | 1.448 | 70.3 | 18.2 | 11.5 | 530 000 | 280 000 | 29.2 | 0.582 | -12.9 |
| F1*sey* | 7.4 | 2.761 | 12.3 | 6.3 | 81.4 | 3 100 000 | 1 200 000 | 35.6 | 0.607 | -7.4 |
| F2*sey* | 0.6 | 1.073 | 41.8 | 33.8 | 24.4 | 810 000 | 470 000 | 22.2 | 0.582 | -12.4 |

[a]data from Apolinar-Valiente et al. (2019 and 2020b).
[b] Values estimated 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.
[c]R-I: range I = molar mass below 500 000 g·mol$^{-1}$; R-II: range II = molar mass between 500 000 and 1 000 000 g·mol$^{-1}$; R-III: range III = molar mass above 1 000 000 g·mol$^{-1}$; $M_w$: weight average molar mass; $M_n$: number average molar mass; IV: intrinsic viscosity.

**Table 4.** Values of foam height (mm) from Shaking Test (ST) of Saragossa, Tarragona, Champagne NO1, Champagne RU1, Champagne RU2 and Champagne RU3 CO-wines separately supplemented with F1*sen*, F2*sen*, F1*sey* and F2*sey* (300 $mg \cdot L^{-1}$).

| Supplemented CO-Wine | Fraction/Time | T5[a] | T70 | T80 | T90 |
|---|---|---|---|---|---|
| Saragossa (SA) | F1*sen* | **28.0 ± 1.6*** | **6.0 ± 1.0*** | **6.0 ± 1.0*** | **5.7 ± 1.5*** |
| | F2*sen* | 20.0 ± 2.6 | 2.0 ± 0.9 | 2.0 ± 0.9 | 1.7 ± 0.9 |
| | F1*sey* | **33.0 ± 1.0*** | **5.7 ± 1.9*** | **4.7 ± 1.1*** | **4.7 ± 1.1*** |
| | F2*sey* | 19.7 ± 2.1 | 1.3 ± 0.0 | 1.0 ± 0.0 | 1.0 ± 0.0 |
| Tarragona (TA) | F1*sen* | **41.0 ± 1.7*** | **22.7 ± 0.6*** | **21.7 ± 0.6*** | **21.7 ± 0.6*** |
| | F2*sen* | 38.7 ± 1.5 | 9.7 ± 1.9 | 9.7 ± 1.9 | 9.7 ± 1.9 |
| | F1*sey* | **41.0 ± 1.7*** | **13.0 ± 2.6*** | **13.0 ± 2.6*** | **12.3 ± 3.1*** |
| | F2*sey* | 38.3 ± 3.5 | 9.0 ± 1.6 | 8.9 ± 1.3 | 8.7 ± 1.3 |
| Champagne CONO1 | F1*sen* | 39.0 ± 2.0 | **9.3 ± 1.8*** | **9.0 ± 1.5*** | **9.0 ± 1.5*** |
| | F2*sen* | 37.7 ± 1.5 | 7.7 ± 1.5 | 7.0 ± 1.0 | 6.7 ± 1.2 |
| | F1*sey* | **44.7 ± 3.5*** | 7.0 ± 1.6 | 7.0 ± 1.6 | 6.0 ± 1.6 |
| | F2*sey* | 36.7 ± 4.5 | 5.3 ± 1.9 | 4.7 ± 1.5 | 4.3 ± 1.2 |
| Champagne CORU1 | F1*sen* | 46.3 ± 4.5 | **12.7 ± 2.1*** | **11.7 ± 2.1*** | **11.3 ± 1.8*** |
| | F2*sen* | 46.0 ± 1.0 | 7.0 ± 0.0 | 6.3 ± 0.6 | 6.0 ± 1.0 |
| | F1*sey* | 49.7 ± 3.1 | 10.3 ± 1.8 | 9.3 ± 2.1 | 8.3 ± 1.2 |
| | F2*sey* | 43.0 ± 2.6 | 8.3 ± 1.8 | 7.7 ± 1.8 | 7.0 ± 1.6 |
| Champagne CORU2 | F1*sen* | **16.0 ± 1.0*** | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| | F2*sen* | 13.0 ± 1.7 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| | F1*sey* | 36.7 ± 2.1 | 2.0 ± 1.1 | 1.3 ± 0.9 | 1.0 ± 0.6 |
| | F2*sey* | 12.3 ± 0.6 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| Champagne CORU3 | F1*sen* | **29.0 ± 2.6*** | 1.3 ± 0.9 | 1.0 ± 0.6 | 0.7 ± 0.6 |
| | F2*sen* | 23.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| | F1*sey* | **37.0 ± 2.0*** | 2.3 ± 1.1 | 1.3 ± 0.6 | 1.0 ± 0.6 |
| | F2*sey* | 23.0 ± 1.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |

[a]Time numbers indicate the seconds after the beginning of ST to which the foam height was measured.
Data in bold with asterisks indicate statistically significant differences compared to their corresponding CO wine.
Shaking Test values are the average of three replicates.

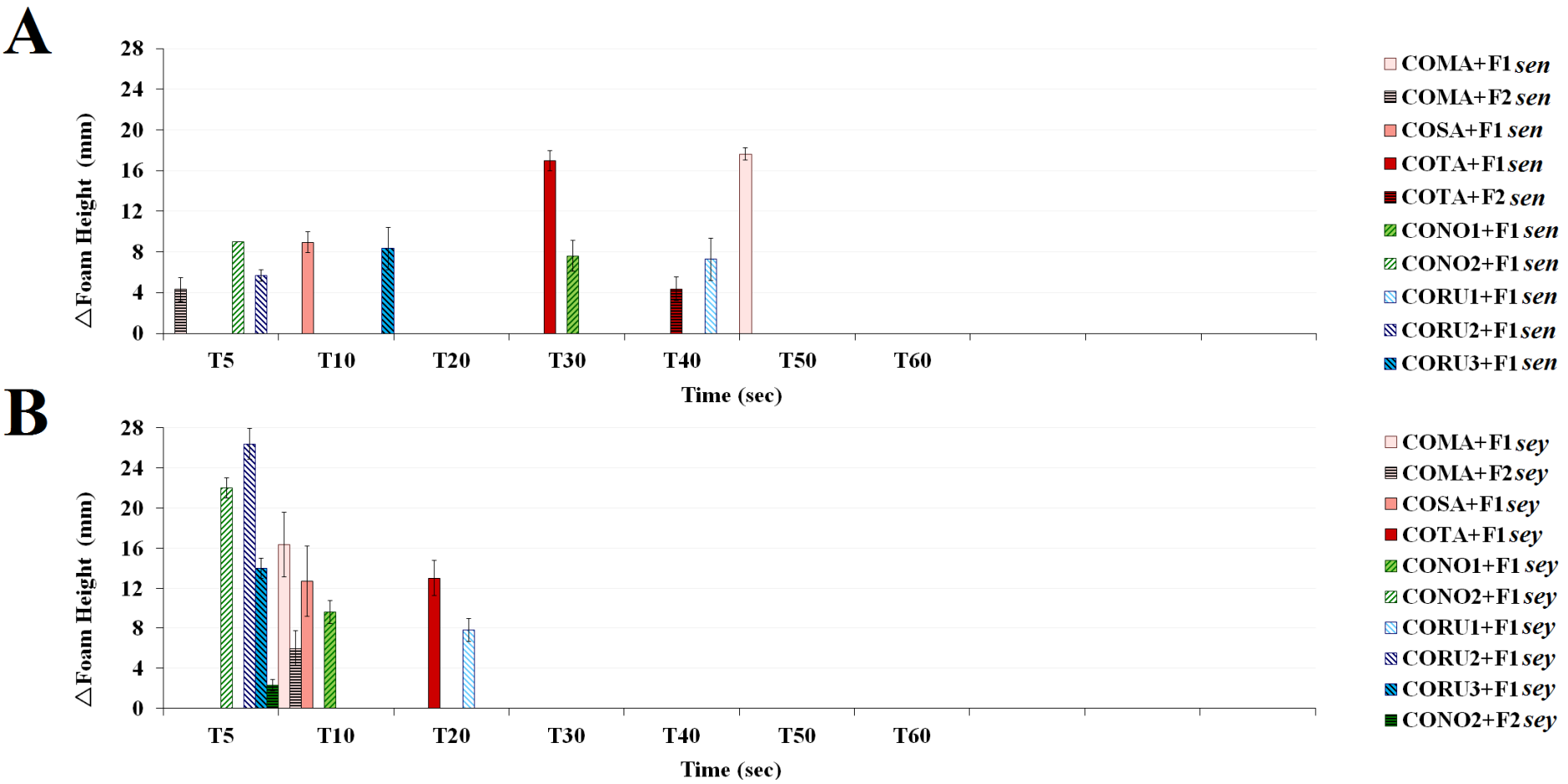
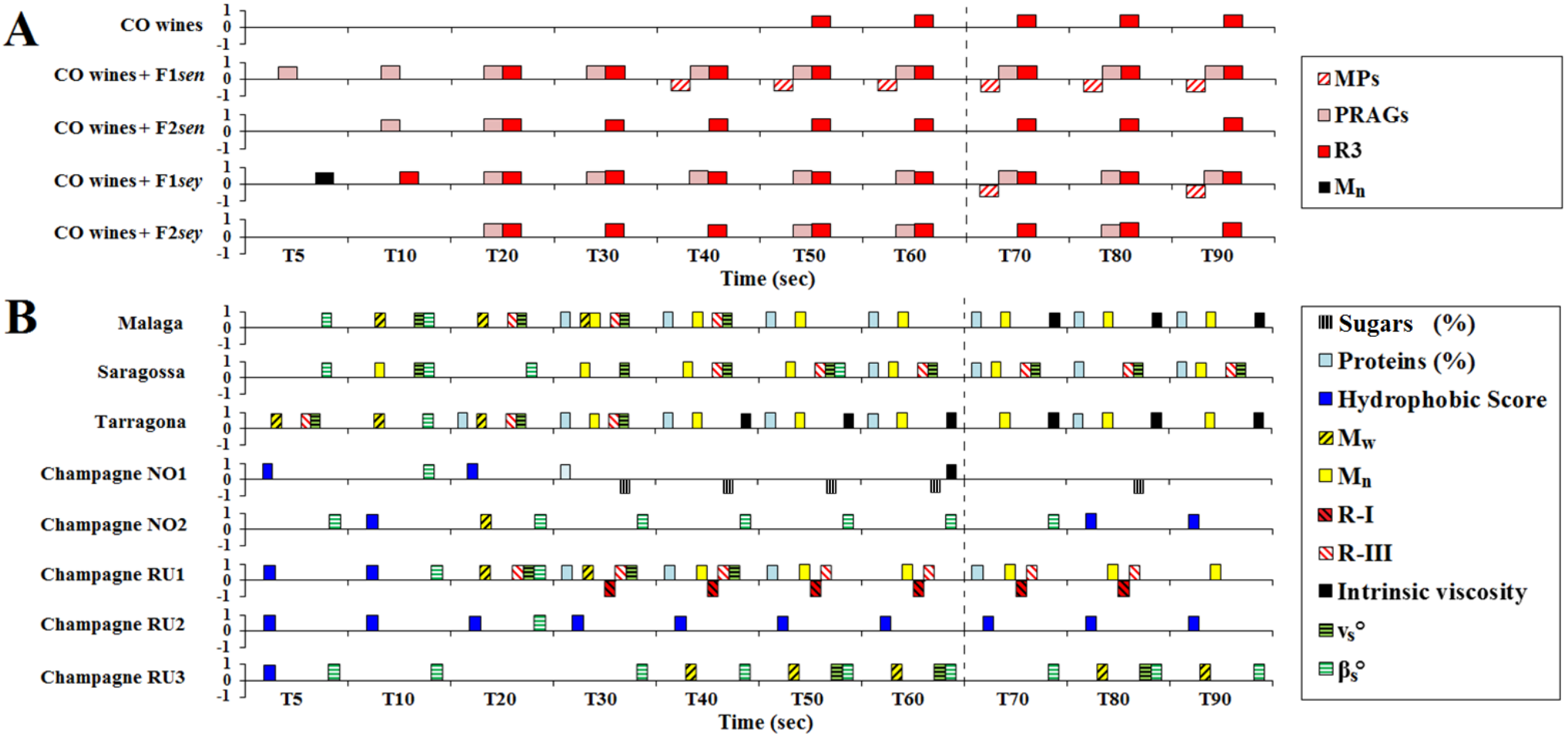



**Figure 1.** Shaking test: significant maximal differentials (ΔFH*max*) between foam height values (mm) of CO-wines (control wines) subtracted from foam height values (mm) of their corresponding supplemented CO-wines (300 mg·L$^{-1}$) with fractions from (**A**) *Asen*G and (**B**) *Asey*G.

COMA: Malaga control wine; COSA: Saragossa control wine; COTA: Tarragona control wine; CONO1: Champagne NO1 control wine; CONO2: Champagne NO2 control wine; CORU1: Champagne RU1 control wine; CORU2: Champagne RU2 control wine; CORU3: Champagne RU3 control wine.
Only the supplementations with fractions showing at least one statistical different moment have been included in this Figure.
Time numbers indicate the seconds after the beginning of Shaking Test to which the foam height was measured.
Shaking Test values are the average of three replicates.

**Figure 2.** Significant Pearson correlation coefficients ($p < 0.05$) between (**A**) the foam height (mm) of CO-wines (control wines) and supplemented CO-wines and the characteristics of wines and (**B**) the foam height (mm) of supplemented CO-wines and the properties of F1*sen*, F2*sen*, F1*sey* and F2*sey*.




MPs: mannoproteins (%); PRAGs: polysaccharides rich in arabinose and galactose (%); R3: range 3 = molar mass from polysaccharides of CO-wines between 100 000−250 000 g·mol$^{-1}$; $M_n$: number average molar mass (g·mol$^{-1}$); R-I: range I = molar mass from gums fractions below 500 000 g·mol$^{-1}$; R-III: range III = molar mass from gums fractions above 1 000 000 g·mol$^{-1}$; $M_w$: weight average molar mass (g·mol$^{-1}$).
Time numbers indicate the seconds after the beginning of Shaking Test to which the foam height was measured.
Shaking Test values are the average of three replicates.