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Improvement of the foamability of sparkling base wines by the addition of *Acacia* gums

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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
wines. Polysaccharides have been also implicated in sparkling wine foam characteristics (Girbau-Sola et al., 2002; Abdallah et al., 2010; Martínez-Lapuente et al., 2015). Oligosaccharides are carbohydrates consisting of two to ten monosaccharide residues, and their composition and content of base wines can be influenced by winemaking process, grape variety and vintage (Jégou et al., 2017). However, there are very few works relating oligosaccharides with the foaming properties of wine. The synergistic interaction of the active foam compounds, such as peptides, proteins and complex carbohydrates, could modify their surface-active properties and, hence, the foaming properties (Martínez-Lapuente et al., 2015), but most of the studies present contradictory results (López-Barajas et al., 1997; Lao, Santamaria, López-Tamames, Bujan, Buxaderas & de la Torre- Boronat, 1999; Girbau-Sola et al., 2002).

Bentonite, a montmorillonite clay, is usually employed to prevent the protein haze in white wine. Champagne and Cava winemakers often add bentonite to the wine in order to facilitate the riddling process (Vanrell et al., 2007). Addition of bentonite carries a net negative charge at the pH of wine, interacting electrostatically with the positively charged wine proteins, and, therefore, causing their flocculation (Sauvage, Bach, Moutounet & Vernhet, 2010). Marchal, Chaboche, Douillard and Jeandet (2002) and Dambrouck, Marchal, Cilindre, Parmentier and Jeandet (2005) reported a loss of wine foamability after addition of bentonite, relating it to the drastic reduction of protein amount. Therefore, it seems obvious that the wine industry must search for new techniques to prevent or to reduce/compensate the undesirable effects of bentonite treatment on the quality of the wine foam.

Acacia gum is a natural highly glycosylated hydroxyproline-rich arabinogalactan-peptide and arabinogalactan-proteins exuded by Acacia trees species (i.e. Acacia senegal and Acacia 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·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 (*AsenG*) and *Acacia seyal* gum (*AseyG*) 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 *AsenG*, 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 AsenG 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·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
Reims University. The enological characteristics of the eight wines are within the classical values for base wines (alcoholic degree: between 10 and 13% v/v; titratable acidity: between 3 and 7 g·L⁻¹, expressed in sulfuric acid; pH: between 3.0 and 3.5). One part of all the eight base wines were treated with bentonite (20 g·hL⁻¹; Microcol Alpha®, Laffort), stirred gently for a few hours, kept in cold storage (10 days, 4°C), racked and filtered (1 μm). The obtained bentonite-treated wines were coded as CO (control wine) followed by its corresponding origin, resulting in COMA (control wine from Malaga), COSA (control wine from Saragossa), COTA (control wine from Tarragona), CONO1 and CONO2 (control wines from the cooperative winery Nogent l’Abbesse) and CORU1, CORU2 and CORU3 (control wines from the University of Reims) and forming the CO wines. A sample without bentonite was performed in each wine, and these non-bentonite-treated wines were coded as ORI (original wine) followed by its corresponding origin, resulting in ORIMA (original wine from Malaga), ORISA (original wine from Saragossa), ORITA (original wine from Tarragona), ORINO1 and ORINO2 (original wines from the cooperative winery Nogent l’Abbesse) and ORIRU1, ORIRU2 and ORIRU3 (original wines from the University of Reims) and forming the ORI wines.

2.2. Isolation of polysaccharide and oligosaccharide fractions from base wines

Following the methodology previously described (Jégou et al., 2017; Apolinar-Valiente, Ruiz-García, Williams, Gil-Muñoz, Gómez-Plaza & Doco, 2018), 5 mL of wine were partially depigmented onto a polyamide column, being eluted the not retained polysaccharides and oligosaccharides. High-resolution size exclusion chromatography was subsequently performed and polysaccharides and oligosaccharides were separately collected according to their elution time. Elution was performed using a Superdex-30 HR column (60 x
1.6 cm, Pharmacia, Sweden) with a precolumn (0.6 x 4 cm) equilibrated at 1 mL·min⁻¹ with 30 mM ammonium formiate (pH 5.6). The isolated fractions were freeze-dried, redissolved in water, and freeze-dried again four times to remove completely the ammonium salt.

2.3. Complex carbohydrate analysis of base wines

Reported by Apolinar-Valiente et al. (2018), neutral monosaccharides were released after hydrolysis of the wine polysaccharides by treatment with TFA (120°C, 75 min) and quantified by gas chromatography (GC) analysis. The addition of all the neutral monosaccharides was used to calculate the total polysaccharide content. We have calculated the percentage of each polysaccharide family (mannoproteins (MPs), polysaccharides rich in arabinose and galactose (PRAGs), and rhamnogalacturonans type II (RG-II)) based on the neutral monosaccharide content, as previously reported (Apolinar-Valiente et al., 2018).

The total oligosaccharide content was calculated on the basis of the neutral and acidic sugar composition of the wine oligosaccharide fraction. It was determined after solvolysis with anhydrous MeOH containing 0.5 M HCl (80 °C, 16 h), by GC of their per-O-trimethylsilylated methyl glycoside derivatives (Doco, O’Neill & Pellerin, 2001).

2.4. Amino acid composition of base wine proteins

Following the method described by Lowry, Rosenbrough, Farr and Randall (1951), and modified by Potty (1969), 25 mL of trichloroacetic acid (TCA) at 10% were added to 10 mL of wine and were kept at 4°C for 2 hours to precipitate the proteins of base wines. The tubes were centrifuged (38 400 g, 20 min) and the supernatant liquid was removed. Four washes were realized with 1 mL of MilliQ water each one, and after transference to hydrolysis tubes the samples were freeze-dried.
Amino acid composition from freeze-dried samples was determined following the methodology previously described by Lopez-Torrez et al. (2015). Samples were hydrolyzed with 6 N HCl and heating at 110°C for 24h. The excess of acid was eliminated by washing twice with water (0.5 mL) and once with absolute ethanol (0.5 mL), and hydrolyzed samples were analyzed by liquid chromatography with a Biochrom 30 analyser (BIOCHROM 30, Cambridge, UK) using an ion-exchange column (Ultra-pac-8 lithium form; Amersham Pharmacia Biotech, Piscataway). Lithium citrate (0.2 M, pH 2.2) was used as eluent and norleucine as internal standard. The total amino acids content (TAAs) was calculated by adding the amount of all the amino acids from the hydrolysis of the wine proteins precipitated with TCA.

2.5. Acacia gum samples

Acacia gums from *Acacia senegal* trees (AsenG) (Lot: OF152413) and from *Acacia seyal* trees (AseyG) (Lot: OF110724) were provided by ALLAND & ROBERT Company – Natural and organic gums (Port Mort, France).

2.6. Fractionation of AsenG by Hydrophobic Interaction Chromatography (HIC)

Following the classical fractionation method (Renard et al., 2006), macromolecular fractions, HIC-F1, HIC-F2, and HIC-F3 were obtained from AsenG soluble powder by HIC performed at room temperature on one Phenyl-Sepharose CL-4B (Sigma, St. Louis, Mo) column (40 x 2.6 cm) equilibrated with degassed 4.2 M NaCl. AsenG was dissolved in water (100 g·L⁻¹), stirred overnight to allow the complete hydration, loaded and eluted successively by 4.2 M NaCl (fraction HIC-F1), 2 M NaCl (fraction HIC-F2), and finally water (fraction HIC-F3) at a flow rate of 1 mL·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 concentrated 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 AsenG, AseyG, 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, AsenG, AseyG, HIC-F1, HIC-F2 and HIC-F3 were separately added to synthetic wine (SYWI) at 600 mg·L⁻¹. On the other hand, AsenG and AseyG were separately added at 300 mg·L⁻¹ to the eight CO wines, whereas HIC-F1, HIC-F2 and HIC-F3 were also separately added (300 mg·L⁻¹) to two selected CO wines (COMA and CONO2). These wines treated with gums or AsenG fractions formed the CO-supplemented wines. In all the cases, gum or gum fraction powder was gently stirred (20 °C, 24 h).
2.9. *Sparging procedure (Mosalux method) to measure foaming parameters*

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

2.10. *Pictures*

Pictures of control SYWI and SYWI-treated samples were taken after four minutes of gas injection during the analysis by Mosalux in control and treated samples, with the objective of achieving a better observation and analysis of the foam quality.

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

3. Results and discussion

At first, the foaming parameters measured by Mosalux procedure in control SYWI and SYWI-treated samples are shown. The pictures taken after 4 minutes of gas injection to these samples get information about the foam aspect and the size of bubbles. Secondly, the foam features in ORI and CO wines, as well as in CO-supplemented wines are given. Finally, we explore the link between the sparkling base wine foamability and the complex carbohydrate and amino acid content of wines and gum treatments, as well as concerning some basic molecular and structural parameters of gum treatments.

SYWI was not the final aim of this work but it represented a good tool to obtain deeper knowledge which allows us achieving our objectives. For this reason, we consider that it could be interesting to test treatments on SYWI at a dose (600 mg·L\(^{-1}\)) greater than the permitted concentration (300 mg·L\(^{-1}\)) (OIV, 2019). However, and considering that the
primary aim of our work is focused in sparkling base wines, we preferred testing the maximum levels permitted in these samples.

3.1. Foaming parameters on SYWI after gum and HIC-fractions addition at 600 mg·L⁻¹

HM and HS of control synthetic wine (SYWI), as well as of SYWI formulated with AsenG, AseyG, HIC-F1, HIC-F2 and HIC-F3 (600 mg·L⁻¹) are presented in Figure 1. The fact that we have eliminated all the possible matrix effects allows us to focus only on the characteristics and composition of gum or gum fractions treatments. Both HM and HS increased considerably when AsenG and mainly AseyG were added to SYWI. On the other hand, these two foam parameters also improved when HIC-F1 was used, but they increased greatly when HIC-F2 or HIC-F3 was added to SYWI.

The varying hydrophobicity of studied HIC-fractions could affect their general influence on the foam height of SYWI. Onishi and Proudlove (1994) reported that the absolute level of hydrophobic polypeptide is important to enter into and stabilize foam, rather than the ratio of hydrophobic to hydrophilic polypeptides. We have therefore estimated the hydrophobic score (Table 1) through the amino acid composition data of gum and HIC-fractions (Table 1) and the hydrophobicity scale proposed by Monera et al. (1995), using the non-polar hydrophobic amino acids (alanine, isoleucine, leucine, phenylalanine, proline and valine). For every gum or HIC-separated fraction from AsenG, the content of each hydrophobic amino acid is divided by the total content of amino acids, and the result is multiplied by its corresponding coefficient from the hydrophobicity scale (footnote in Table 1). The sum of the values of all the hydrophobic amino acids corresponds to the hydrophobic score of gums or HIC-separated fractions from AsenG. The order of increased hydrophobicity of these gum fractions was HIC-F1<HIC-F2<HIC-F3 according to the principle of separation method
(Mejia-Tamayo et al., 2018) as well as to the calculated hydrophobicity score (Table 1), which corresponded with the order of the foamability. Brissonnet and Maujean (1993) suggested that hydrophobic proteins accessed easier into the foam than hydrophilic ones. According to these authors, polypeptides with a greatly hydrophobic exterior showed a higher trend to interact with other compounds of bubble wall (interface gas/liquid), whereas hydrophilic proteins presented a higher affinity for water than for bubble surfaces. Onishi and Proudlove (1994) observed a correlation between polypeptide molar mass, hydrophobicity and foam stabilizing activity in beer. In the same line, Moreno-Arribas, Pueyo and Polo (1996) reported that the hydrophobicity of the characterized peptides could account for the foamability of sparkling wine. Indeed, it is important to remark that the differences in hydrophobicity of gums and gum fractions were intimately linked to their total amino acid content, which followed the order HIC-F1<AseyG<AsenG<HIC-F2<HIC-F3 (Table 1). It can be hence deduced that the impact of hydrophobicity was strongly related with the amount of amino acids, resulting both of them in a great impact on the foam features of the different synthetic wines. As will be discussed below, the form and the aspect of the bubbles could be a reason for the reverse behavior of both Acacia gums when their foam parameters values (AsenG<AseyG) were compared to their hydrophobicity values (AseyG<AsenG).

The foam pictures of control SYWI as well as SYWI with separate addition (600 mg·L⁻¹) of AsenG, AseyG and HIC fractions from AsenG after 4 minutes of gas injection using a sparging procedure are shown in Figure 2. The foam aspect presented two distinguishable regions in AseyG (Fig. 2). The first region was placed between 0.5 and 1.5 mm in height and presented similar foam aspect than the other treated samples, including AsenG. The second region was less compact and presented larger bubbles (between 2 and 4 mm in diameter at 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 AseyG was added in comparison with addition of AsetG, even although AseyG 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 AseyG 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% (AsenG) and 0.77% (AseyG) (Table 2), their addition at 300 mg·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·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·L⁻¹
Figure 3 shows HM (Figure 3C) and HS (Figure 3D) of CO wines (bentonite-treated wines), CO wines plus AsenG and CO wines plus AseyG. HM of all the Spanish CO wines was significantly increased by the AsenG (COMA: +24%; COSA: +6%; COTA: +6%) and also by the AseyG (COMA: +22%; COSA: +10%; COTA: +12%) treatments (Fig. 3C). Concerning the French CO wines, HM of CONO1 and CONO2 was significantly enhanced after AsenG (CONO1: +8%; CONO2: +11%) and AseyG (CONO1: +5%; CONO2: +7%) additions. Moreover, CORU2 also showed a rising value (+6%) of HM when AseyG was used (Fig. 3C). In short, AsenG addition increased HM in five out of eight CO wines, whereas AseyG did likewise in six out of eight CO wines.

HS parameter could represent the wine’s ability to produce stable foam or persistence of foam collar in a glass (Martínez-Lapuente et al., 2015). COMA, COSA and CONO2 increased their HS when AsenG (+17%, +7% and +6%, respectively) or AseyG (+21%, +4% and +8%, respectively) were used (Fig. 3D). In the case of COSA, the addition of AsenG increased HS even over the HS value of ORISA. The addition of AseyG to CORU3 enhanced its HS over the initial HS value (+9%). To resume, AsenG addition enhanced HS in three out of eight CO wines, whereas AseyG did the same in four out of eight CO wines.

3.4. Foaming parameters on two selected wines after separate addition of HIC-fractions at 300 mg·L⁻¹

The impact of the addition of AsenG HIC-fractions to selected COMA and CONO2 wines is presented in Figure 4. The first reason for the choice of these two wines was their different country of origin. Within each origin, we selected the wine with a better improvement of both foamability parameters (HM and HS) after separate Acacia gums additions, according to 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. AserG increased HM or HS in five out of eight CO wines (an efficiency of 63% of the cases), whereas AseyG 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 AseyG, 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·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·L⁻¹) and French CO wines (ranged between 78 and 98 mg·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 AseyG 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 AsenG and AseyG 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 AseyG 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.
bubbles gas/liquid interface. Besides, Aguié-Béguin et al. (2009) observed that the less the concentration of macromolecules of the wine, the lower rate of formation of the adsorbed layer at the air/champagne liquid interfacial. However, Abou Saleh et al. (2007) concluded that the structure of adsorption layer can change depending on unidentified factors. In our case, the addition of AseyG could play the role of this unidentified factor. Further physico-chemical studies should be hence realized in order to clear this point that seems at first sight contradictory. The influence of the size, the molecular weight; and the composition of wine polysaccharides on the wine foaming properties has been demonstrated (Coelho et al., 2011; Martínez-Lapuente et al., 2015). Moreover, and according to Martínez-Lapuente et al., (2015), the synergistic interaction of the foam active compounds, such as peptides, proteins and complex carbohydrates, could modify their surface-active properties and, hence, the foaming properties.

From all the results shown in the present and the previous sections, it seems necessary to take into consideration the composition and content of polysaccharides and oligosaccharides in the wines depending on the gum treatment in order to achieve a significant foamability improvement.

3.7. Effect of the amino acid composition of the wine proteins precipitated with TCA on the wine foaming properties after AsenG and AseyG separate additions

Table 2 shows that, between the CO wines, CONO2 presented the highest TAAs, whereas CORU3 showed the lowest value. However, we did not find any correlation between the composition and the content of amino acids and the foamability of CO wines after AsenG and AseyG separate additions. Maybe the very low protein content of wines after bentonite 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 ($R^2 = 99.0\%; p = 0.0102$),
serine ($R^2 = 96.5\%; p = 0.0352$) and threonine ($R^2 = 97.5\%; p = 0.0255$). Similarly, the variation percentage of HS was significantly correlated with the content of serine ($R^2 = 95.4\%; p = 0.0463$) and threonine ($R^2 = 95.4\%; p = 0.0456$). However, it is not easy explain the foamability of a wine considering an amino acid alone, even if a correlation existed. Otherwise, proteins are more consistent to biochemically explain a correlation with the foam.

In this connection, the variation percentage of HM was significantly correlated with the content of protein of gums and gum fractions ($R^2 = 96.7\%; p = 0.0327$). On the other hand, the variation percentage of HS was significantly correlated with $M_w$ ($R^2 = 95.0\%; p = 0.0498$) and the content of protein ($R^2 = 96.6\%; p = 0.0345$). These two correlations would explain the no effect previously mentioned on HM or HS after the HIC-F1 addition to COMA and CONO2, keeping in mind that this sample presents the lowest protein content (4.9 mg·g$^{-1}$ of sample) and $M_w$ values (3.5 x 10$^5$ g·mol$^{-1}$) (Table 1). Moreover, they also can explain the positive increase of HS in CONO2 and also of HM in both selected base wines when HIC-F2 was added to them. This fraction shows high protein content (63.1 mg·g$^{-1}$ of sample) and $M_w$ values (1.5 x 10$^6$ g·mol$^{-1}$) (Table 1). Several authors observed a close relationship between protein concentration and foam features in base (Maujean et al., 1990; Brissonnet & Maujean, 1993; Marchal et al., 2002) or sparkling (Vanrell et al., 2007; Martínez-Lapuente et al., 2015) wines. However, Puig-Deu et al. (1999) observed that lower levels of proteins may favor a higher stability time of foam.

The behavior of foamability after addition of HIC-F3 appears as much more complex and more difficult to understand and explain from this point of view. As stated above, the foamability parameters increased when it was added to CONO2, whereas they decreased after addition to COMA. According to the obtained correlations as well as the highest protein 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·L\(^{-1}\); CONO2: 62 mg·L\(^{-1}\)) and PRAGs (COMA: 41 mg·L\(^{-1}\); CONO2: 20 mg·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
ranges, being the result of complex poly-macromolecular associations leading to a network at
the gas/liquid interface rather than the result of a single component. Further experiments
should be hence realized in order to deepen our understanding of this essential aspect.
A positive Pearson correlation (+0.9372; \( p = 0.0106 \)) was also observed between the
variation percentage of HS and the percentage of non-polar amino acids of gums and gum
fractions. Non-polar amino acids were calculated subtracting from 100 the addition of the
percentages of polar and charged amino acids (arginine, aspartic acid, cysteine, glutamic
acid, glycine, histidine, hydroxyproline, lysine, serine, threonine and tyrosine). Martínez-
Lapuente et al. (2015) noted that amino acids with non-polar side chains presented greater
coefficients of correlation with foam parameters than amino acids with polar side chains.
Taking all these into account, the amino acid content and composition, the \( M_w \) and the
content of non-polar amino acids of the gums and the gum fractions showed therefore a main
role on the foamability features after their addition.
On the other hand, the differences between glycosyl composition of gums or \( AsenG \) fractions
added to the wines showed no effect on foam parameters. Maybe their high percentages of
carbohydrates (between 81.3% and 97.8%, from Mejia Tamayo et al., 2018) disabled the
potential influence of the variations of these compounds between gum samples.

4. Conclusions
In the light of all our results, we can say clearly that the addition of \( AsenG \) and \( AseyG \), which
are already authorized additives in enology, appears as a precious tool to recover/partially
restore wine foaming parameters after bentonite treatments. Foam parameters were increased
with efficiencies of 63% and 88% of the cases when, respectively, \( AsenG \) and \( AseyG \) were
added. For this purpose, \( AseyG \) was more effective gum than \( AsenG \). The foamability of
wines treated first with bentonite (CO wines) and subsequently with gum or gum fractions differed greatly depending not only on the gum or the gum fraction treatment, but also on the wine composition. Further research about the impact of enological practices on the increasing foamability after gum addition, as well as about other type of wines, gums and gums fractions, must be done to deepen our knowledge concerning the improvement of foam behaviour in base wines.

Acknowledgments

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Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

Compliance with ethics requirements

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

References


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 (Mₚ, g·mol⁻¹) and the hydrophobicity score of AsenG, AseyG and AsemG HIC-separated fractions.

<table>
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<th>Gum and gum fractions</th>
<th>Alaᵃ</th>
<th>Argᵃ</th>
<th>Aspᵃ</th>
<th>Cysᵃ</th>
<th>Gluᵃ</th>
<th>Glyᵃ</th>
<th>Hisᵃ</th>
<th>Hypᵃ</th>
<th>Ileᵃ</th>
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<th>Pheᵃ</th>
<th>Proᵃ</th>
<th>Serᵃ</th>
<th>Thrᵃ</th>
<th>Tyrᵃ</th>
<th>Valᵃ</th>
<th>TAAᵃ</th>
<th>Protein contentᵇ</th>
<th>Mₚᵇ</th>
<th>Hydrophobicity scoreᶜ</th>
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<td>137.7</td>
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</tr>
</tbody>
</table>

ᵃAla: alanine; Arg: arginine; Asp: aspartic acid; Cys: cysteine; Glu: glutamic acid; Gly: glycine; His: histidine; Hyp: hydroxyproline; Ile: isoleucine; Leu: leucine; Lys: lysine; Phe: phenylalanine; Pro: proline; Ser: serine; Thr: threonine; Tyr: tyrosine; Val: valine; TAA: total amino acids content.

ᵇFrom Mejía Tamayo et al. (2018)

ᶜFrom 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.
Table 2. Composition of studied wines: families of polysaccharides (%), total content of polysaccharides, (mg·L⁻¹) and total content of oligosaccharides (mg·L⁻¹) from CO wines, as well as total amino acids content from the hydrolysis of the wine proteins precipitated with TCA (mg·L⁻¹) of ORI and CO wines.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Wine/Origin</th>
<th>Malaga (MA)</th>
<th>Saragossa (SA)</th>
<th>Tarragone (TA)</th>
<th>Champagne NO1</th>
<th>Champagne NO2</th>
<th>Champagne RU1</th>
<th>Champagne RU2</th>
<th>Champagne RU3</th>
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<td>27</td>
<td>36</td>
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<td>CO wines</td>
<td>145</td>
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<td>133</td>
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<td>221</td>
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<tr>
<td>TOs⁴</td>
<td>CO wines</td>
<td>144</td>
<td>148</td>
<td>134</td>
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<td>ORI wines</td>
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<td>3.9</td>
<td>1.0</td>
<td>1.1</td>
<td>0.7</td>
</tr>
</tbody>
</table>

⁴ MPs: mannoproteins; RG-II: rhamnogalacturonans type II; PRAGs: polysaccharides rich in arabinose and galactose; TPs: total polysaccharide content; TOs: total oligosaccharide content; TAAs: total amino acids content.

The analyses were done in duplication.
Figure 1: Maximum Foam Height (HM, ■; mm) and Foam Stability (HS, □; mm) of control SYWI and SYWI with separate additions of AsenG, AseyG and HIC-fractions from Asen (600 mg·L⁻¹).

Different letters in the same colour column represent significant differences according to an LSD test ($p < 0.05$).

Each bar represents the average value of three samples.
Figure 2. Foam pictures of control SYWI (A) and SYWI with separate addition (600 mg·L⁻¹) of AsenG (B), AseyG (C) and HIC-F1 (D), HIC-F2 (E) and HIC-F3 (F) fractions from AsenG after 4 minutes of gas injection.
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 wines. C: Maximum Foam Height (HM; mm) of the CO wines and CO wines with separate addition of AsenG and AseyG at 300 mg·L⁻¹. D: Foam Stability (HS; mm) of the CO wines and CO wines with separate addition of AsenG and AseyG at 300 mg·L⁻¹.

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 RU3: Reims University 3) represent significant differences according to an LSD test ($p < 0.05$). Each bar represents the average value of three samples.
Figure 4. Maximum Foam Height (HM; mm) and Foam Stability (HS; mm) of ORIMA and ORINO2 wines, COMA and CONO2 wines and COMA and CONO2 wines with separate addition of *A sen*G, *Asey*G, HIC-F1, HIC-F2 and HIC-F3 fractions at 300 mg·L⁻¹.

Different letters in the same colour column represent significant differences according to an LSD test (*p* < 0.05).

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·L\(^{-1}\)) and families of polysaccharides (mg·L\(^{-1}\)) from CO wines; D: Glycosyl composition (%) and total content (mg·L\(^{-1}\)) of oligosaccharides from CO wines.

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<thead>
<tr>
<th>Wine/Amino acid</th>
<th>Cys ac(^a)</th>
<th>Ala(^a)</th>
<th>Arg(^a)</th>
<th>Asp(^a)</th>
<th>Cys(^a)</th>
<th>Glu ac(^a)</th>
<th>Gly(^a)</th>
<th>His(^a)</th>
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<th>Ile(^a)</th>
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<sup>a</sup>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.

<sup>b</sup>Ara, arabinose; Rha, rhamnose; Fuc, fucose; Gal, galactose; Glc, glucose; Man, mannose; Xyl, xylose; 2-OMeFuc, 2-O-CH₃-fucose; 2-OMeXyl, 2-O-CH₃-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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