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Foamability and Foam Stability of Molecular Reconstituted Model Sparkling Wines

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ABSTRACT: The present work aims at identifying the contribution of the different wine components to the foaming properties of wines. Twelve fractions were isolated from wine, and foam aptitude of each fraction was measured individually at the concentration at which it was recovered, using wine model solutions. For these concentrations, the maximum foam height (HM) was 8.4−11.7 cm, foam height on stability was 6.9−7.5 cm, and foam stability (TS) was 3.0−6.5 s. Moreover, foam measurements were also performed using 2-, 5-, and 10-fold concentrations of these compounds in wine. The HM increased linearly with the concentration of mannoproteins having low content of protein (MP1), and TS increased exponentially. The fractions that individually showed higher foaming properties were mixed in binary and ternary combinations, demonstrating that MP1 when mixed with low molecular weight hydrophobic compounds strengthens the air/water interface of these solutions, a characteristic that is on the basis of sparkling wines' foamability and foam stability.

KEYWORDS: foam, sparkling wines, polysaccharides, phenolic compounds, mannoproteins, arabinogalactans

INTRODUCTION

Foam characteristics are one of the most important organoleptic properties of sparkling wines. Several studies have searched to establish a correlation between their chemical composition and foam properties, namely, foamability and foam stability. Proteins were the first group of molecules to be proposed to explain sparkling wines' foam properties due to their surfactant characteristics. Some authors have correlated positively protein concentration and foamability,[1−4] but for foam stability the results are contradictory with both positive[2] and negative correlations.[5] Recent studies have shown that glycoproteins rather than proteins are the most prominent macromolecules responsible for the foam of sparkling wines.[6,7] Among the wine glycoproteins, the yeast mannoproteins have been associated with the improvement of the foam properties in sparkling wines.[8] The use of mannoproteins or cell wall extracts as additives for improving the foam properties of sparkling wines elaborated by the traditional method was also proposed.[6] Concerning carbohydrates, an oligosaccharide fraction with 2−3 kDa was correlated with foam stability, whereas the polysaccharides were related with foamability,[9] although this correlation has been only observed for neutral polysaccharides, not for the acidic ones.[10]

The main polysaccharides that are present in wines are the mannoproteins and the type II arabinogalactans.[11] Mannoproteins are neutral polysaccharides composed mainly of mannose and small amounts of glucose, associated with protein that can range from 2 to 36%.[11−14] Mannoproteins are composed of a highly branched, short chain structure, where most of the mannopyranose residues are terminally linked and 2,6-linked, together with 2- and 3-linked linear residues.[11,14] Type II arabinogalactans (AG) are also named arabinogalatan-proteins (AGP) due to the small proportion of protein sometimes present. These polysaccharides are composed mainly of a 3-linked galactopyranose backbone branched at C6 by galactose and arabinose residues. Glucuronic acid is also found as terminal nonreducing and 4-1linked[15] in amounts that can range between 3 and 20%.[15,16] The different contents in uronic acids confer on them characteristics of weak acidic or even acidic polysaccharides.

The influence of polysaccharides on the foam stability of *Champagne* wines was also inferred by the similarity of the adsorption layers of *Champagne* wines with those of reconstituted solutions containing the low molecular weight material and polysaccharide-rich fractions.[17] Sparkling wines' foam behavior results from the synergistic interaction between the different foam active compounds that due to aggregation or complex formation may modify their surface-active properties.[18] Thus, foaming properties not only are due to the presence or absence of a specific group of compounds but also are influenced by the net balance of the number and type of compounds ranging among different chemical structures.[1,5,19]

In a previous work it was shown that the better foam stability of a reconstituted sparkling wine was achieved by the synergistic effect of the combination of the high molecular weight (HMW) material with the hydrophobic low molecular weight fraction (MeLMW-F3).[20] In the present work, the HMW fraction previously isolated was fractioned into nine subfractions representing mannoproteins with different amounts of protein (5, 38, and 64%), arabinogalactans (one neutral and 2 acidic fractions), and three fractions with different amounts of polysaccharides, proteins, and phenolic compounds. Also, the lower molecular weight compounds (<12 kDa), composed of different amounts of carbohydrates, peptides, and phenolic compounds, were divided into 3 fractions according to their size and polarity. The foam parameters of the wine model solutions containing each one of these 12 fractions or a combination of selected fractions were evaluated.

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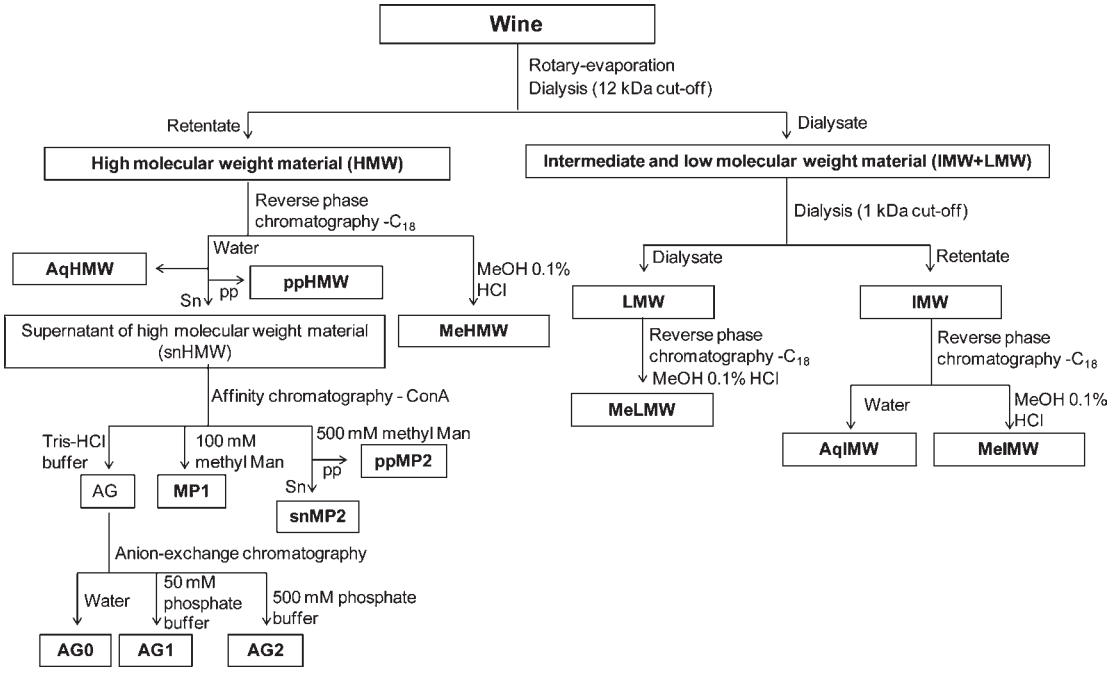
Scheme 1. Diagram of the Isolation Steps of Wine Biomolecules



MATERIALS AND METHODS

Source of Wine Fractions. The high molecular weight (HMW) material was obtained from 35 bottles of base wine (26 L), e.g. a wine prepared to be doubly fermented to produce a sparkling wine in a stage where the second fermentation was not yet performed. These wines arose from Bical, Arinto, Fernão-Pires, and Baga, varieties used in Bairrada appellation to produce sparkling wines. The isolation sequence of the HMW material is illustrated in Scheme 1. The wines were rotary-evaporated under reduced pressure at 35 °C to eliminate the ethanol and concentrate the total solids. The material was then dialyzed (12 kDa cutoff membrane, Medicell) in order to remove tartaric acid and other small molecules. The retentate was concentrated, frozen, and freeze-dried, to give the wine HMW material as a powder.

The intermediate (IMW) and low (LMW) molecular weight material was obtained from 4 bottles of sparkling wine (3 L) produced by Estação Vitivinícola da Bairrada (EVB), from Fernão-Pires (FP), produced according to the traditional method, as described by Coelho et al.[20] The sparkling wines were rotary-evaporated under reduced pressure at 35 °C and dialyzed (12 kDa cutoff). The material that diffused through the dialysis membrane (dialysate) was recovered by concentration under rotary-evaporation and frozen for use in the isolation step described in Scheme 1. The fractions were the same as previously used by Coelho et al.[20]

Fraction of HMW Material. The fractions of polysaccharides were obtained by combining the use of different chromatographic supports to allow the separation of the different classes of polysaccharides (Scheme 1). The wine polymeric material was fractionated using a C_{18} solid-phase-extraction column (SPE-C_{18}, Supelco-Discovery, 10 g). The material was eluted with water, and the uncolored unbound material was recovered and concentrated. Upon concentration, the occurrence of a precipitate (ppHMW) that was separated from the supernatant (snHMW) was observed. During the elution with water a dark red band was observed in the C_{18} column and was recovered separately (AqHMW). The three fractions were frozen and freeze-dried. The bound fraction was recovered with acidic methanol (0.1% v/v HCl in MeOH), concentrated, frozen, and freeze-dried, presenting an intense red color (MeHMW).

The snHMW material was eluted through an affinity medium of concanavalin A (Con A) Sepharose 4B (GE Healthcare, Uppsala, Sweden) in a column with 30 cm length and 2 cm diameter, operated at 5 °C with a constant flow of 1 mL/min. Prior to elution, the column was prewashed with a solution of 1 M NaCl, 5 mM $MgCl_2$, 5 mM $MnCl_2$, and 5 mM $CaCl_2$, and equilibrated with a buffer solution of Tris-HCl 20 mM and 0.5 M NaCl at pH 7.4. The arabinogalactans (AG) were eluted with Tris-HCl buffer, and mannoprotein fraction (MP1) was desorbed with two bed volumes of the same buffer containing 100 mM methyl-α-D-mannopyranoside, as described by Vidal et al.[11] Due to the large amount of material handled, successive batches were done, always after regeneration of Con A resin with 0.1 M Tris buffer, 0.5 M NaCl at pH 8.5 followed by 0.1 M sodium acetate, pH 4.5, containing 1 M NaCl. The Con A resin was also eluted with 500 mM of methyl-α-D-mannopyranoside. Both fractions were dialyzed and freeze-dried. After the dialysis of the fraction recovered with 500 mM of methyl-α-D-mannopyranoside, the formation of a precipitate inside the dialysis membrane that was separated from the supernatant was observed. The supernatant gave origin to the fraction snMP2 and the precipitate to fraction ppMP2.

Anion-exchange chromatography was performed for the fraction rich in AG using a HyperSep SAX 10 g (Thermo Fisher Scientific, U.K.). Prior to elution, the column was conditioned with methanol followed by water and MeOH:water (5:95 v/v). The eluted AG rich fraction gave a neutral fraction (unretained) eluted with water (AG0), and two acidic fractions obtained in a stepwise elution using 50 mM (AG1) and 500 mM (AG2) phosphate buffer pH 6.5. Acidic fractions were dialyzed, and all fractions were freeze-dried, as described in Scheme 1.

Extraction and Isolation of IMW and LMW Material. The dialysate (the molecules that diffused throughout the membrane tube of 12 kDa) from sparkling wine sample was dialyzed with a cutoff of 1 kDa (Spectra/Por). The 1 kDa dialysate was added, under stirring, to a batch containing a C_{18} resin suspension, during 3 h, for sorption of the hydrophobic material. The resin was recovered by filtration, washed with water until the conductivity of the water was reached, and extracted with

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acidic methanol (MeOH 0.1% v/v HCl). This solution, which comprised the material with molecular weight lower than 1 kDa, gave origin to the fraction MeLMW. Using this procedure, the retentate, which comprised the material with intermediate molecular weight (between 1 and 12 kDa), gave origin to two fractions, the fraction of material not sorbed to the C_{18}, that remained in the water solution (AqIMW), and the fraction of material retained in the C_{18} resin and recovered with acidic methanol (MeIMW) (Scheme 1).

Measurement of Foam Properties. Foamability and foam stability were assessed using an adaptation of the Mosalux and Bikerman method.[3,4,18] Analytical grade CO_2 from a cylinder flowed through a glass-frit fitted in the bottom of a column (530 × 15 mm i.d.). The gas flow rate was controlled at 10 L/h by a flow meter (Cole-Parmer Instruments Company, IL, USA) and under a constant pressure of 1 bar. Foamability was evaluated as the increase in height of 10 mL of model wine solutions placed inside the glass column, after CO_2 injection through the glass-frit. Two parameters of foamability were measured: (1) HM (maximum height reached by foam after CO_2 injection through the glass frit, expressed in centimeters) represents the solution ability to foam. (2) HS (foam stability height during CO_2 injection, expressed in centimeters) represents the solution ability to produce stable foam persistence of foam collar. Foam stability time (TS) was evaluated as the time elapsed before bubble collapse until the liquid appears after the interruption of CO_2, and is expressed in seconds. The isolated fractions obtained from the wine were added independently or in mixtures to the wine model solution taking into account their average proportion in wines. For these solutions, the measurements of foam properties were done with 5 replicates.

Wine Model Solutions. Wine models were constructed from a hydroalcoholic base solution with 10% ethanol (v/v) and 0.5% tartaric acid (w/v) adjusted at pH 3.5 with NaOH solution. Glycerol and ethyl octanoate were added to this wine model in the concentrations of 0.7% (w/v) and 0.4% (w/v), respectively. The glycerol concentration used was in the range usually found in wines,[18] and previously used to prepare wine model solutions.[21] The ethyl octanoate concentration used was the concentration previously quantified in Bairrada sparkling wines.[22] The isolated fractions of wine were added individually to the model solutions and in combination with the other fractions. The fractions that individually were more contributive for foam parameters were mixed in binary and ternary combinations. For each experiment the foam parameters HM, HS, and TS were measured.

Sugar Analysis. Monosaccharides were released from cell wall polysaccharides by a prehydrolysis in 0.2 mL of 72% H_2SO_4 (w/w) for 3 h at room temperature followed by 2.5 h hydrolysis in 1 M H_2SO_4 at 100 °C. Neutral sugars were analyzed after conversion to their alditol acetates by GC, using 2-deoxyglucose as internal standard.[23,24] A Perkin-Elmer Clarus 400 gas chromatograph with split/splitless injector and a FID detector was used, equipped with a 30 m column DB-225 (J&W) with i.d. and film thickness of 0.25 mm and 0.15 μm, respectively. The oven temperature program used was as follows: 200 to 220 °C at 40 °C/min (hold 7 min at 220 °C) and to 230 at 20 °C/min (hold 1 min at 230 °C). The injector and detector temperatures were, respectively, 220 and 230 °C. The flow rate of the carrier gas (H_2) was set at 1 mL/min.

Uronic acids (UA) were quantified by a modification[23] of the 3-phenylphenol colorimetric method.[25] Samples were prepared by hydrolysis in 0.2 mL of 72% H_2SO_4 (w/w) for 3 h at room temperature followed by 1 h in 1 M H_2SO_4 at 100 °C. A calibration curve based on D-galacturonic acid as standard was used to calculate UA concentration.

Glycosidic-Linkage Composition of Polysaccharide Fractions. Glycosidic-linkage composition was determined by gas chromatography−quadrupole mass spectrometry (GC−qMS) of the partially methylated alditol acetates.[26] The sample (1−2 mg) was weighed into glass tubes and placed in a vacuum oven, at 40 °C, overnight in the presence of P_2O_5 (s). Afterward, it was dispersed in 1 mL of anhydrous dimethyl sulfoxide (DMSO) and stirred overnight for total solubilization. NaOH pellets (30 mg) were powdered under argon, added to the solution and kept stirring during 30 min. The polysaccharides were methylated with 80 μL of methyl iodide, added with a syringe into the closed tube with a cap with a silicone septum. The mixture was allowed to react for 20 min under stirring. Two mL of water was added, and the solution was neutralized with HCl 1 M. The methylated material was then extracted with 3 mL of CH_2Cl_2 and the aqueous phase was removed after centrifugation. The dichloromethane phase was then washed three times with 2 mL of water until the dichloromethane phase became limpid. The organic phase was transferred to a clean tube and dried by centrifugal evaporation (Univapo 100 ECH, UniEquip, Germany). This methylation procedure was repeated. The permethylated polysaccharides were hydrolyzed with 0.5 mL of 2 M trifluoroacetic acid (TFA) (1 h at 121 °C)[27] and dried by centrifugal evaporation. The reduction of monosaccharides was performed during 1 h at 30 °C with 20 mg of sodium borodeuteride (Isotec, Switzerland) in 300 μL of 2 M NH_3. The reaction was terminated by the addition of 0.1 mL of glacial acetic acid. The acetylation was performed with 3 mL of acetic anhydride using 450 μL of 1-methylimidazole as catalyst, during 30 min at 30 °C. Then, 3 mL of distilled water was added to decompose the acetic anhydride, and the acetylated sugars were extracted with 5 mL of CH_2Cl_2. The organic phase was washed three times with water and then dried by centrifugal evaporation. The partially methylated alditol acetates were dissolved in 70 μL of acetone, and 0.2 μL were injected and analyzed by GC−qMS on an Agilent Technologies 6890N Network gas chromatograph, equipped with a 30 m × 0.25 mm (i.d.), 0.1 μm film thickness DB-1 fused silica capillary column (J&W Scientific Inc., CA, USA), connected to an Agilent 5973 quadrupole mass selective detector. The oven temperature was programmed as follows: hold 5 min at 45 °C, to 140 at 10 °C/min (hold 5 min at140 °C), to 170 at 0.5 °C/min (hold 1 min at 170 °C) and then to 280 at 15 °C/min (hold 5 min at 280 °C). Helium carrier gas had a flow of 1.7 mL/min and a column head pressure of 2.8 psi. The mass spectrometer was operated in the electron impact mode (EI) at 70 eV scanning the range 40−500 m/z, in a full scan acquisition mode. Identification was achieved comparing the standard mass spectra and other spectra with a laboratory made database. Methylation analysis was assayed for MP1, AG0, AG1, and AG2.

For the permethylated fractions of AG0, AG1, and AG2, prior to acid hydrolysis, the dichloromethane solutions were split in two portions and a carboxyl reduction was performed. The permethylated polysaccharides were dried and dissolved in 1 mL of anhydrous tetrahydrofuran, and 20 mg of lithium aluminum deuteride (Aldrich, WI, USA) was added under argon. The suspension was kept at 65 °C during 4 h under stirring.[28] The reagent in excess was eliminated by adding 2−3 drops of ethanol and 2−3 drops of distilled water. The solution was neutralized by addition of 1 M HCl. Two mL of $CHCl_3$/methanol 2:1 (v/v) mixture was then added. The reduced polymers were removed from the white precipitate by centrifugation and washed thoroughly with the chloroform/methanol solution. The supernatant was collected and evaporated, and the carboxyl-reduced material was submitted to hydrolysis with TFA, reduction, and acetylation, as described above.

Protein Analysis. Protein quantification was based on the bicinchoninic acid (BCA) method using bovine serum albumin (BSA) as standard, using the Bicinchoninic Acid Protein Assay Kit from Sigma (Aldrich-Chemie, Steinheim, Germany). The samples were incubated in a water bath at 60 °C during 15 min. The absorbance was measured at 562 nm with a 6405 Jenway UV−vis spectrophotometer (U.K.) against a blank in the reference cell. The data were correlated with the calibration curve of BSA standard (concentration range of 0.05−0.40 mg/mL), also analyzed in the same conditions of the samples. At least three replicates of each concentration were carried out for all experiments. Protein analysis was assayed for all samples except AGs.

Table 1. Yield of the Fractions Isolated from Wine, Sugar Composition, Total Sugar, Total Protein and Total Phenolic Content

		mol %										
fraction	yield (mg/L)	Rha	Fuc	Ara	Xyl	Man	Gal	Glc	Ur. Ac.	total sugars (%, w/w)	protein (%, w/w)	phenolics (%, w/w)
snHMW	255.2	2	0	25	0	26	32	7	7	75	8	
MP1	48.8	1	0	1	0	90	3	2	3	74	5	
snMP2	1.3	0	0	0	0	90	1	2	6	33	38	
ppMP2	2.2	1	0	0	0	83	4	3	9	29	64	
AG	85.2	2	0	28	0	5	45	12	8	93		
AG0	38.1	1	0	24	0	5	38	28	4	62		
AG1	32.0	3	0	28	0	7	47	3	11	77		
AG2	4.4	2	0	28	1	5	51	3	10	77		
ppHMW	38.6	2	0	8	1	17	10	9	53	3	0.2	0.1
AqHMW	4.8	3	2	23	4	21	16	14	17	12	80	8
MeHMW	64.0	7	1	38	1	8	16	11	18	65	40	10
AqIMW	17.7	7	1	6	3	30	11	10	32	53	19	18
MeIMW	23.9	3	1	15	6	10	4	45	18	9	*a*	*a*
MeLMW	356.3	2	1	10	5	20	4	49	10	7	39	6

[a] Fraction with high content of protein and phenolic compounds.

Determination of Total Phenolic Compounds. Total phenolic composition was determined by the Folin−Ciocalteu colorimetric method.[29] The samples were dissolved in hydroalcoholic solution (10% v/v of ethanol), and 0.125 mL of this solution was mixed with 0.5 mL of water and 0.125 mL of Folin−Ciocalteu reagent. After homogenization with a vortex, the sample was allowed to react during 5 min, and 1.250 mL of Na_2CO_3 (75 g/L) and 1.0 mL of water were added. The mixture was homogenized in a vortex, and reaction occurred during 90 min at room temperature. The absorbance was measured at 760 nm (6405 Jenway UV−vis spectrophotometer, U.K.). The calibration curve was built using gallic acid as standard in the concentration range 12.7−101.8 mg/L. At least three replicates of each concentration were carried out for all experiments. The analysis of total phenolic compounds was performed for the following samples: ppHMW, AqHWM, MeHMW, AqIMW, MeIMW, and MeLMW.

Statistical Analysis. To examine differences in foam parameters in the different wine model solutions, data were subjected to analysis of variance (ANOVA) and means were separated with a multiple range test, Tukeys's range test, $\alpha = 0.05$.

RESULTS AND DISCUSSION

Twelve different wine fractions were obtained according to their molecular weight, polarity, and solubility (Scheme 1). The nine polymeric fractions MP1, snMP2, ppMP2, AG0, AG1, AG2, MeHMW, ppHMW, and AqHMW were obtained from base wine; and the three fractions of intermediate and low molecular weight, AqIMW, MeIMW, MeLMW, were obtained from sparkling wine. These provided a wide range of molecules with different chemical properties allowing evaluation of their contribution to the foam properties of model solutions prepared using their combination in the proportions at which they are found in wines. In a previous study[20] it was reported that Bairrada sparkling wines contain polymeric material ranging from 349.6 to 550.6 mg/L, which is comparable with the amount of polymeric material recovered from the base wines used in this study (362.6 mg/L). Also, according to the sugar analysis performed, the proportion and structural features of mannoproteins and arabinogalactans in Bairrada sparkling wines and base wines are comparable. In the present study, to obtain a large amount of each polymeric fraction, the base wines prepared to produce these sparkling wines, using the same yeast strains, were used.

Characterization of Wine Isolated Fractions. The twelve different fractions obtained according to their molecular weight, polarity, and solubility (Scheme 1) were characterized concerning their abundance in the wines of origin, sugar composition, and content in protein and phenolic compounds (Table 1). Also, the snHMW material, that gave origin to the mannoprotein and arabinogalactan fractions, and AG, originating the three arabinogalactan fractions, were analyzed.

Concerning the three mannoprotein fractions, the most abundant was MP1 (48.8 mg/L of wine), contrasting with snMP2 and ppMP2, presenting only 1.3 and 2.2 mg/L, respectively. These fractions had different protein contents, 5%, 38% and 64% for MP1, snMP2, and ppMP2, respectively. All these fractions contained mannose as the main sugar. Glycosidic-linkage analysis of MP1 showed that 2,6-Man*p* (31.9 mol %), terminally linked Man*p* (29.8 mol %), 2-Man*p* (20.2 mol %), and 3-Man*p* (10.9 mol %) were the most abundant linkages (Table 2), confirming that they are mannoproteins from yeast origin.[11] The material not retained by the concanavalin A medium, accounting for 85.2 mg/L of wine, was very rich in sugars (93%), mainly galactose (45 mol %) and arabinose (28 mol %), containing only 5 mol % of mannose. This shows that this fractionation allowed separating the mannoprotein components from those arising from arabinogalactans (AG). The material recovered after fractionation through the concanavalin A medium accounted only for 57% of the material applied (snHWM). However, the recovery of mannose was 77% and arabinose and galactose were almost totally recovered (Table 1), showing that the main material lost through this purification step was protein. According to the protein content of the different fractions, it can be estimated a loss of approximately 50% of the protein applied.

Three fractions of arabinogalactans were recovered from wine: AG0, AG1, and AG2, accounting for 38.1 mg/L of wine, 32.0 mg/L, and 4.4 mg/L, respectively. These fractions were composed mainly of arabinose (24−28 mol %) and galactose (38−51%) (Table 1). Glycosidic-linkage analysis showed that the major linkages (Table 2) are 3,6-Gal*p* (25−35 mol %),

Table 2. Glycosyl Linkage Composition of MP1, AG0, AG1 and AG2 Fractions Isolated from Base Wine[a]

glycosyl linkage	MP1 (mol %)	AG0 (mol %)	AG1 (mol %)	AG2 (mol %)
T-Fuc*p*	tr[b]	tr	0.1	tr
2-Fuc*p*			0.2	tr
total	0.0	0.0	0.3	0.1
T-Rha*p*	0.1	0.1	0.3	0.3
4-Rha*p*		tr	0.4	
3-Rha*p*			0.1	
2,4-Rha*p*			0.3	0.1
total	0.1	0.2	1.1	0.3
T-Ara*f*	0.1	8.3	14.4	17.8
T-Ara*p*	tr	0.2	0.5	0.4
2-Ara*f*		0.2	0.4	0.6
3-Ara*f*		0.4	0.5	0.5
5-Ara*f*	0.3	4.5	4.4	2.2
3,5-Ara*f* = 3,4-Ara*p*		2.0	1.5	0.4
3-Ara*p*	0.2	0.2	0.3	0.1
total	0.6	15.7	21.9	22.0
4-Xyl*p*	0.24		0.1	
total	0.2		0.1	
T-Man*p*	29.8	0.9	0.7	0.3
2-Man*p*	20.2			
3-Man*p*	10.9			
6-Man*p*	2.9			tr
2,3-Man*p*	0.3	0.1	0.2	tr
2,4-Man*p*	0.1			
4,6-Man*p*	tr			
2,6-Man*p*	31.9	0.1		
3,6-Man*p*	1.3			
2,3,6-Man*p*	0.4			
2,3,4,6-man*p*	0.1			
total	96.0	1.0	0.9	0.4
T-Gal*p*	0.1	4.8	7.9	8.3
2-Gal*p*		0.5	0.8	0.2
4-Gal*p*	0.3	0.3	0.6	0.4
3-Gal*p*	0.1	5.3	6.7	8.7
6-Gal*p*	0.1	9.4	12.8	11.7
4,6-Gal*p*		1.4	2.2	1.7
3,6-Gal*p*	0.2	25.2	31.4	34.7
3,4,6-Gal*p*		4.5	7.3	6.9
2,3,6-Gal*p*		0.1	0.2	0.3
2,3,4,6-Gal*p*			tr	0.1
total	0.8	51.5	70.0	72.9
T-Glc*p*	0.4	0.5	0.2	0.1
3-Glc*p*		0.2	0.1	0.1
4-Glc*p*		19.4	1.4	1.3
6-Glc*p*	0.2	10.2	0.3	0.2
3,4-Glc*p*	tr	0.8	1.9	2.0
3,6-Glc*p*			0.1	
4,6-Glc*p*		0.3		
2,3,6-Glc*p*		tr	0.1	tr
2,3,4,6-Glc*p*		0.1	tr	tr
total	0.6	31.5	4.1	3.8
T-Glc*p*A		0.6	0.8	0.4
4-Glc*p*A		0.1	0.2	
4-Gal*p*A		tr	0.4	
total		0.7	1.3	0.4

[a] The molar ratios are the means of two repetitions. [b] Trace amounts.

6-Gal*p* (9−13 mol %), 3-Gal*p* (5−9 mol %), terminally linked Gal*p* (5−8 mol %), terminally linked Ara*f* (8−18 mol %), and 5-Ara*f* (2−5 mol %), together with the occurrence of terminally linked glucuronic acid. This composition is consistent with the presence of arabinogalactans.[15] The neutral fraction (AG0), in addition to the arabinogalactan, contained a glucan, identified by the presence of glucose (28 mol %) (Table 1) and 4-Glc*p* and 6-Glc*p* (Table 2). Comparing the amount of AG material eluted through the anion-exchange chromatography and the amount of material recovered in the three fractions, it was possible to observe a recovery of 88% of the material, with no significant difference for the recovery of polysaccharides (Table 1).

The fraction containing the hydrophobic high molecular weight material (MeHMW), accounting for 64.0 mg/L of wine, was rich in sugars (65%), mainly arabinose (38 mo%), uronic acids (18 mol %), and galactose (16 mol %), a sugar composition characteristic of a highly branched pectic polysaccharide.[30] It is possible that the retention of this fraction in the C_{18} resin may be due to the linkage of the polysaccharides to hydrophobic material, namely, phenolic compounds and/or protein. According to Table 1, this fraction is also rich in protein and phenolic compounds. However, because the colorimetric methods used to determine these compounds interfere one with the other when the concentrations of protein and phenolic compounds are both high, the values achieved may be overestimated. The fraction of the high molecular weight material not retained by the C_{18} stationary phase and that precipitate upon concentration (ppHMW) accounted for 38.6 mg/L wine. This white powder recovered was shown only to contain 3% of sugars, no phenolic compounds, and no protein (Table 1). The high molecular weight material fraction that was slightly sorbed in C_{18} stationary phase (AqHMW) accounted for only 4.8 mg/L, and was mainly composed by protein (80%); sugars account for 12% and phenolic compounds 8% (Table 1).

The fraction of low molecular weight sorbed in C_{18} resin (MeLMW) was the largest fraction recovered, accounting for 356.3 mg/L of sparkling wine. It was composed of peptides (39%), sugars (7%), and phenolic compounds (6%) (Table 1). The fraction of intermediate molecular weight retained in the C_{18} resin (MeIMW) accounted for 23.9 mg/L. It showed a high amount of phenolic compounds and protein, preventing their accurate estimation with the methodology used. Sugars accounted for only 9%, whereas glucose was the major sugar (45 mol %), probably resultant from the glycosylation of phenolic compounds. The fraction of intermediate molecular weight not sorbed in the C_{18} resin (AqIMW), accounting for 17.7 mg/L, was composed mainly of sugars (53%), protein (19%), and phenolic compounds (18%). The main sugar residues were uronic acids (32 mol %) and mannose (30 mol %) (Table 1), indicating that this fraction should be a mixture of degraded pectic polysaccharides and mannoproteins.

Evaluation of Foam Aptitude of the Fractions Isolated from Wine. The twelve different fractions isolated from wine were individually used to prepare wine model solutions containing 10% ethanol and 0.5% of tartaric acid at pH 3.5. The amount of material used was that recovered for each fraction, as shown in the yield column in Table 1. A model solution containing the snHMW material, that gave origin to the mannoprotein and arabinogalactan fractions, was also prepared. All these solutions were tested to evaluate their foam aptitude, namely, the maximum height reached by foam after CO_2 injection through the glass frit, expressed in centimeters (HM), the foam stability height during CO_2 injection, expressed in centimeters (HS), and the foam stability time,

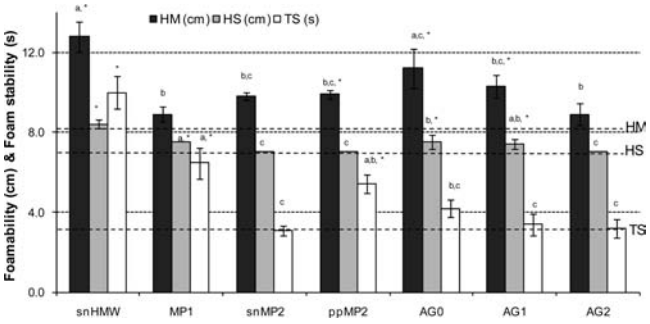

Figure 1. Foamability, HM and HS, and foam stability, TS, measured for snHMW (supernatant of high molecular weight material) and all fractions obtain from that (3 mannoprotein fractions with different protein contents, namely, MP1, snMP2, and ppMP2; and 3 arabinogalactans fractions, one neutral and two acidic fractions, namely, AG0, AG1, and AG2). All fractions were in recovered concentration in the model solution. Bars with the same character are not significantly different ($p < 0.05$). Dashed lines represent the control model wine foam values. * Significantly different ($p < 0.05$) from model solution.

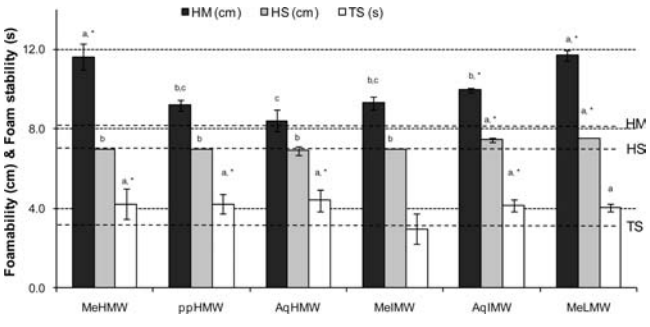

Figure 2. Foamability, HM and HS, and foam stability, TS, measured for six fractions: 3 polymeric (MeHMW, ppHMW, AqHMW), 2 of intermediate molecular weight compounds (MeIMW and AqIMW), and one of low molecular weight compounds MeLMW). All fractions were in recovered concentration in the model solution. Bars with the same character are not significantly different ($p < 0.05$). Dashed lines represent the model wine foam values. * Significantly different ($p < 0.05$) from model solution.

expressed in seconds (TS), as shown in Figures 1 and 2 and Table 3.

The HM ranged between 8.4 and 12.8 cm, being the maximum HM observed for snHMW material, followed by MeHMW, MeLMW, and AG0, and the minimum was observed for AqHMW and AG2 fractions. As the analysis of the blank wine model solution, composed only by ethanol and tartaric acid, at pH 3.5, showed a HM of 8.2 cm, with a standard deviation of 0.4 cm (5 replicates), it can be inferred that fractions snHMW, ppMP2, AG0, AG1, MeHMW, AqIMW, and MeLMW are contributing to the HM properties of these solutions increasing foamability 56%, 21%, 37%, 26%, 41%, 21%, and 43%, respectively, in relation to the control model solution. The higher values observed for snHMW than those observed for all six fractions obtained from it allows one to conclude that when the different mannoprotein or arabinogalactan-rich material are assayed individually, they have lower HM values than when they are assayed together in a mixture. This can be explained by the higher amount of polymeric material used in the solutions of snHMW than in the others (Table 1). Although MP1 was the fraction with higher amount of material from these six, it was not the fraction with higher HM, showing that other parameters than the concentration of a specific type of molecule should also be involved in HM. For example, for proteins such as β-casein, β-lactoglobulin, and glycinin, their unfolding rate at the air/water interface is the most important parameter for foam formation.[31] At wine concentration, the high and low molecular weight fractions retained in C_{18} and recovered with acidic methanol are important for HM. These two fractions are rich in protein/peptides (40% w/w, Table 1), so it is possible that they have the ability to unfold at the air/water interface strengthening the interfacial film formed.

The HS of the fractions analyzed at wine concentration varied only between 6.9 and 7.5 cm. Although these values are close to those measured for the blank wine model solutions (7.0 cm), significant differences were found for snHMW, MP1, AG0, AG1, AqIMW, and MeLMW, increasing foamability 20%, 7%, 7%, 6%, 6%, and 7%, respectively, in relation to the control model solution. Concerning TS, it varied between 3.0 and 10.0 s, whereas the blank solution was 3.2 s. The higher TS was observed for snHMW, which was very much higher than that observed for the fractions derived from it (6.5 to 3.1 s), and its addition increased TS 2-fold in relation to the control model solution. Nevertheless, fractions MP1 and ppMP2 were those that, among the 12 fractions under study, presented higher TS, showing that the mannoproteins are relevant molecules to explain the foam stability characteristics of wine. MP1 doubled the TS of the control model solution, and ppMP2 increased it 70%. Mannoproteins are not studied as foam stabilizers, but many other proteins, namely, β-lactoglobulin and β-casein have the ability to create layers with high surface elasticity that prevent coalescence of bubbles by their strong adsorption and unfolding at the air/water interface.[31] Other fractions that contribute to the TS are MeHMW, ppHMW, AqHMW, AqIMW, and MeLMW, that increase foam stability 32%, 32%, 38%, 30%, and 27%, respectively, in relation to the control model solution, showing that the AG fractions are not relevant to explain the wine foam stability. Studies on emulsification properties of gum arabic components (AG, glycoprotein, and AGP) showed that the AGP was the relevant component for explaining the surface activity of this hydrocolloid.[32] As the AG fractions used in this study contain a low percentage of protein, it can be expected that their contribution to the TS could be low.

The foam properties observed for snHMW were higher in all three parameters measured (HM, HS, and TS) than those of the fractions derived from it. As the concentration of the solution of snHMW was 4−195 times more concentrated than the other ones, it shows that, although not explaining all the foam properties, the concentration of the compounds in each model solution cannot be neglected. Also, considering that the concentration used for reconstitution of the fractions may be underestimated due to the manipulation and natural loss of material during the fractionation process, resulting in more diluted solutions than those present in real wines, it is a requirement to study the effect of the concentration of each fraction on foam properties.

Effect of Concentration of Wine Isolated Fractions in Foam Aptitude. In order to evaluate the concentration effect, the foam aptitude of model solutions up to 10-fold the wine concentration was measured for all 12 fractions. However, for MeLMW, the maximum concentration possible to dissolve in the wine model solution was 2-fold its concentration in wine.

Table 3. HM, HS and TS of All Fractions Isolated Measured in 1-, 2-, 5-, and 10-fold Their Average Recovered Concentration

n[wine]	MP1	snMP2	ppMP2	AG0	AG1	AG2	MeHMW	ppHMW	AqHMW	MeIMW	AqIMW	MeLMW
HM (cm)												
1	8.9 ± 0.4	9.8 ± 0.2*	9.9 ± 0.2*	11.2 ± 1.0*	10.3 ± 0.6*	8.9 ± 0.5	11.6 ± 0.7*	9.2 ± 0.3	8.4 ± 0.5	9.3 ± 0.3*	10.0 ± 0.1*	11.7 ± 0.3*
2	11.0 ± 0.5[b]											13.8 ± 0.4*
5	13.0 ± 1.6[c]											
10	19.3 ± 0.5*	10.1 ± 0.2	9.5 ± 0.4	10.9 ± 0.7	9.8 ± 0.3	11.8 ± 0.6*	13.4 ± 1.2	10.8 ± 0.7*	11.0 ± 0.6*	13.0 ± 0.4*	9.0 ± 0.0*	
HS (cm)												
1	7.5 ± 0.0*	7.0 ± 0.0	7.0 ± 0.0	7.5 ± 0.4*	7.4 ± 0.2*	7.0 ± 0.0	7.0 ± 0.0	7.0 ± 0.0	6.9 ± 0.2	7.0 ± 0.0	7.4 ± 0.1*	7.5 ± 0.0
2	8.1 ± 0.1*											12.9 ± 0.4*
5	8.7 ± 0.3*											
10	8.1 ± 0.3*	7.3 ± 0.1*	7.9 ± 0.1*	7.5 ± 0.0	7.4 ± 0.1	7.7 ± 0.4*	7.6 ± 0.2*	7.5 ± 0.1*	7.4 ± 0.2*	7.1 ± 0.1	7.0 ± 0.0*	
TS (s)												
1	6.5 ± 0.8*	3.1 ± 0.2	5.4 ± 0.5*	4.2 ± 0.4	3.4 ± 0.5	3.2 ± 0.4	4.2 ± 0.7	4.2 ± 0.5*	4.4 ± 0.5*	3.0 ± 0.7	4.2 ± 0.3*	4.1 ± 0.2
2	5.2 ± 0.3											11.5 ± 0.4*
5	10.3 ± 1.6*											
10	54.3 ± 19.1*	4.8 ± 0.1*	7.0 ± 0.4*	5.9 ± 0.7*	5.4 ± 0.6*	5.0 ± 0.4*	8.0 ± 0.9*	4.3 ± 0.5	4.8 ± 0.3	3.1 ± 0.6	3.6 ± 0.3*	

[*] Each fraction at recovered concentration is significantly different ($p < 0.05$) from wine model solution (data in the text); for the concentrations where $n > 1$[wine], it is significantly different ($p < 0.05$) from the fraction at immediately lower concentration. [b] Significantly different ($p < 0.05$) from model solution. [c] Significantly different ($p < 0.05$) from $n = 1$[wine].

Concerning the HM, only 5 of the 11 fractions studied showed an increase of this foam parameter when 10 times concentrated solutions of each individual fraction were used (Table 3). MP1 increased more than twice, from 8.9 to 19.3 cm, whereas AG2, ppHMW, AqHMW, and MeIMW increased 17−40% for a concentration increase of 1000%. Testing the HM for fraction MP1 using the intermediate concentrations of 2- and 5-fold its wine concentration, a linear increasing with concentration (HM (cm) = 1.10 *n*[wine] + 8.17, with an $R^2 = 0.96$) was observed. A comparable increasing was observed for fraction MeLMW at 2-fold wine concentration (from 11.7 to 13.8 cm), reaching a HM value higher than MP1 for 2-fold, and also higher than those observed for all other fractions assayed at 10-fold their wine concentrations. The most important factor for foam formation is the adsorption rate of the proteins to the bubble film, that differs with protein concentration, molecular weight, structure, and pH.[31] These results show that MP1 can adsorb in a higher amount within the time scale of foam production than the other compounds in the fractions studied. The fractions that promoted a significant increase in HS were MP1, snMP2, ppMP2, AG2, MeHMW, ppHMW, AqHMW, and MeLMW. Although the latter was only tested for the 2-fold wine concentration, it achieved a HS higher than the other fractions at 10-fold wine concentration (Table 3).

The foam parameter TS was shown to increase in 7 of the 11 fractions tested with concentrations 10 times higher. A 7.4 times increase was observed for MP1, whereas snMP2, ppMP2, AG0, AG1, AG2, and MeHMW increased 28−90%. When tested for the intermediate concentrations of 2- and 5-fold wine concentrations, fraction MP1 showed an exponential increasing with concentration (TS (s) = 3.77 $e^{0.257(n[\text{wine}])}$, with an $R^2 = 0.95$), allowing one to conclude that the concentration of the mannoproteins with 5% of proteic material influences foam stability of the wine model solution. The increase in concentration could lead to the formation of aggregates that in specific conditions promote higher foam stability.[33,34] Also, when the aggregates adsorb at the air/water interface, they can cross-link the two thin films of adjacent bubbles, leading to more stable films.[33,34] Therefore, it is possible that the exponential increase in foam stability observed is related to the formation of aggregates. The increasing of foamability and foam stability with the increasing of the mannoprotein concentration was also observed by other authors, where sparkling wines were supplemented with increasing concentrations of yeast extracts.[6] Concerning fraction MeLMW, the 2-fold increasing in concentration allowed an increase from 4.1 to 11.5 s, a value higher than those observed for all fractions, with the exception of MP1 at 10-fold (Table 3), showing that the low molecular weight hydrophobic material is also relevant to explain the foam stability of the solutions.

These results allowed concluding that not all fractions presented the same contribution to wine model solution foam properties. Nevertheless, the foam aptitude is influenced by the concentration of some wine constituents. From all fractions isolated, MP1 (mannoproteins), MeLMW (low molecular weight hydrophobic material), and AG0 (neutral arabinogalactans) seem to be the most relevant ones. All these fractions were recovered in high yield from wine and presented a significant impact on foam properties of the wine model solutions. These three fractions were selected to build wine model solutions where binary and ternary combinations were performed in order to evaluate any possible synergistic effect at the average concentration they occur in wine.

Evaluation of Foam Aptitude of Binary and Ternary Combinations of MP1, AG0, and MeLMW Fractions. Figure 3 shows the foam aptitude of the wine model solutions containing the different combinations of MP1, AG0, and MeLMW fractions.

Concerning MP1, its combinations, both binary and ternary, all showed significant increases in HM (28−36%) and HS (8−10%) when compared with the fraction alone. This shows a synergistic effect of the mannoproteins with other wine components concerning foamability. However, for TS, no significant differences were observed for MP1 + MeLMW nor for the ternary mixture, when compared to the MP1 fraction alone. However, for MP1 + AG0, a significant decrease of 16% was observed, allowing concluding that the presence of both mannoprotein and arabinogalactan prevents a higher value for foam stability. The observation that there is a balance in wine between

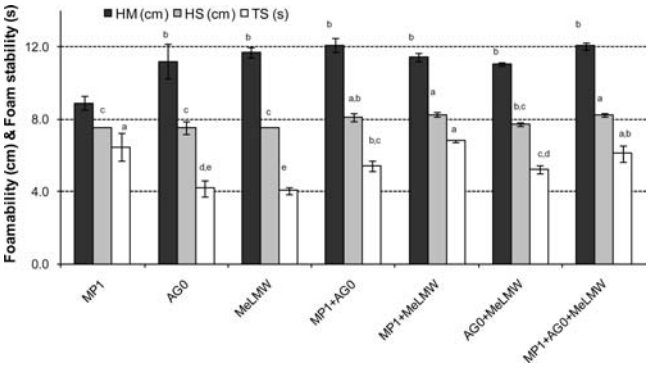

Figure 3. Foamability, HM and HS, and foam stability, TS, measured for the three fractions that contribute more to the foamability and stability individually and in binary combination and the tertiary combinations of these fractions. All fractions were in recovered concentration in the model solution. Bars with the same character are not significantly different ($p < 0.05$).

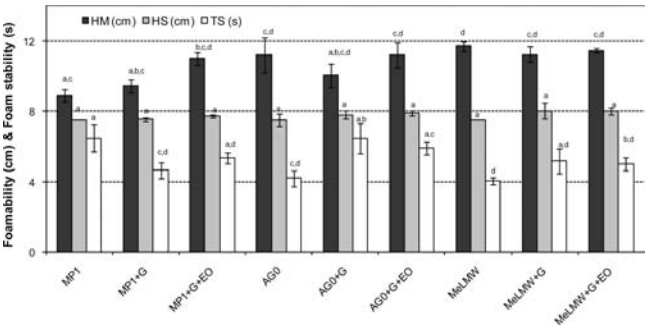

Figure 4. Foamability, HM and HS, and foam stability, TS, measured for the three fractions that contribute more to the foamability and stability in three different model solutions. All fractions were in recovered concentration in the model solution. Bars with the same character are not significantly different ($p < 0.05$).

constituents that act negatively and positively on foam has already been stated by Viaux et al.,[35] namely, the particles or macromolecular complexes retained by filter with 0.45 μm cutoff are able to destroy foam stability. This is consistent with the fact that arabinogalactans, due to their highly branched structure that confers them high solubility, are molecules with the highest molecular weight when compared with all other soluble polysaccharides present in wines.[15,16]

Concerning the AG0 fraction, its addition to the other fractions promotes a significant increase in the TS (29−46%) of the mixtures MP1 + AG0 and MP1 + AG0 + MeLMW when compared with the single fraction. HM parameter is not significantly different, and HS showed a slight increase for the combination MP1 + AG0 (8%) and for the ternary combination (10%). Regarding the fraction MeLMW, its combination with the other fractions increases significantly the HS and TS parameters (10% and 28−68%, respectively), with the exception of HS value for the combination with AG0. For all combinations, HM was not significantly different from MeLMW fraction alone.

In most cases, the ternary combination showed better foam parameters than the fractions individually. These results show that the foam stability of sparkling wines seems to be mainly influenced by mannoproteins with low content of protein (5%) and the foamability by arabinogalactans and a hydrophobic low molecular weight fraction (<1 kDa). The binary combination of MP1 and MeLMW presents a synergistic effect where all foam parameters were improved. This MeLMW fraction was shown in previous study to be composed by tensioactive molecules that seem to be involved in foam stabilization.[20] Comparable results have been obtained when the tensioactive molecules propylene glycol and triglycerol stearates were added to aerated food products, improving the interfacial properties of the gas/water interface, namely, the dynamic surface tension, interfacial viscosity, and permeability of the adsorption layer.[36] Along the time, the surfactant concentration at the bubble surface increases, lowering the gas diffusion and, consequently, increasing the foam stability.[36]

Other major wine components are also relevant to explain sparkling wine foam properties. For example, the presence of glycerol and glycerol plus ethyl octanoate also influences the foam parameters, namely, HM and TS (Figure 4). Glycerol represents almost 0.7% of wine composition[18] and is known to contribute to the viscosity of the solution. Also, ethyl esters of fatty acids have been positively correlated with foamability.[37] From these, ethyl octanoate was the major ester present in these sparkling wines.[22] In most experiments shown in Figure 4, the supplementation of the wine model with ethyl octanoate did not increment HS and TS when compared with the incorporation of glycerol, but the addition of ethyl octanoate decreased the relative standard deviation of the foam parameters of these solutions.

In conclusion, this work shows that the foam properties of model solutions of sparkling wines are ruled by a large number of molecules that act in a synergistic way. Nevertheless, some compounds are more relevant than others to explain their foam properties. The synergistic effect of mannoproteins with low content of protein (5%) and the components present in the low molecular weight fraction (<1 kDa), shown to contain surfactant compounds,[20] play a key role in strengthening the air/water interface that are based on the foamability and foam stability properties of sparkling wines.

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ABBREVIATIONS USED

AG0, neutral arabinogalactan; AG1, acidic arabinogalactan (eluted with 50 mM phosphate buffer); AG2, acidic arabinogalactan (eluted with 500 mM phosphate buffer); AqHMW, aqueous high molecular weight material; AqIMW, aqueous intermediate molecular weight material; EO, Ethyl octanoate; G, glycerol; HMW, high molecular weight material, higher than 12 kDa; IMW,

intermediate molecular weight material, between 1 and 12 kDa; LMW, low molecular weight material, less than 1 kDa; MeHMW, hydrophobic high molecular weight material; MeIMW, hydrophobic intermediate molecular weight material; MeLMW, hydrophobic low molecular weight material; MP1, mannoprotein with 5% of protein; ppHMW, precipitate of high molecular weight material; ppMP2, mannoprotein with 64% of protein; snHMW, supernatant of high molecular weight material; snMP2, mannoprotein with 38% of protein

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