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Isolation and Characterization of a Thermally Extracted Yeast Cell Wall Fraction Potentially Useful for Improving the Foaming Properties of Sparkling Wines

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Two different yeast cell wall extracts were obtained using enzymatic digestion and thermal treatment. The effects of the extracts obtained on the foaming properties of a model wine and two sparkling wines were studied. The model wine and sparkling wines, supplemented with the thermal extract, presented better foaming properties than did the samples supplemented with the enzymatic extract. The fractioning (Con A chromatography) and characterization (SDS-PAGE, SEC, GC, and RP-HPLC) of both extracts showed that the fraction responsible for the foaming properties is constituted by mannoproteins with a relative molecular weight between 10 and 30 kDa, presenting an equilibrated composition of the hydrophobic and hydrophilic protein domains. This thermal extract did not modify the protein stability in both the model wine and the sparkling wines. These results demonstrate that the enrichment of a sparkling wine with mannoproteins extracted by mild heat procedures will contribute to improving its foaming properties.

KEYWORDS: Sparkling wines; foaming properties; yeast; cell wall; mannoprotein; extraction methods.

INTRODUCTION

The elaboration process of sparkling wines by the traditionnelle method (like *champagne* in France and *cava* in Spain) involves some biological transformations and technological procedures that determine the organoleptic properties of the wine. Foam characteristics are one of the most important organoleptic properties of sparkling wines (*1*). They are the subject of many works focused on the variables involved in foam quality (*2*−*4*). Several studies have searched to establish a correlation between the chemical composition and the foam characteristics of sparkling wines. Proteins have been associated with wine foamability and foam stability (*5*), and diminution of their concentration by the use of technological adjuvants as bentonites has been studied (*6*). Also, some authors have found that polysaccharides can contribute to the foam properties of sparkling wines as well (*5*, *7*). However, recent studies have shown that glycoproteins rather than proteins are the most prominent macromolecules responsible for the foam of sparkling wines (*8*). Among the wine glycoproteins, the yeast mannoproteins, released during fermentation and autolysis, have been particularly studied in recent years for their ability, among others, to improve the tartaric salt stability (*9*, *10*) and to increase the stability of the wine against the protein haze (*11*, *12*). Also, mannoproteins have been associated with the improvement of the foam properties in sparkling wines (*13*). Because the mannoprotein concentration in wines is highly dependent on diverse technological variables (*13*, *14*), the development of commercial formulates of mannoproteins or cell wall preparations enriched in mannoproteins is an interesting goal in the field of enological research. To date, some yeast derivatives have been assayed as a source of mannoproteins in winemaking with different purposes (*15*, *16*). In general, there is a clear consensus between producers considering that the elaboration method of the yeast derivatives greatly influences their properties. Yeast derivatives are currently obtained by enzymatic or thermal procedures, or by a combination of them, and the method used can have a great influence on the characteristics of the mannoprotein fraction obtained. While some authors have described that enzymatically obtained yeast cell wall preparations are useful to avoid protein haze in white wines (*10*), others had considered that extracts obtained by heat treatment could be useful for the same purposes (*17*). To date, there are no studies available about the use of mannoproteins or cell wall extracts as additives for improving the foam properties in sparkling wines elaborated by the champenoise method. In this work, the main objective was to study the relationship between different procedures for obtaining a soluble yeast cell wall extract of mannoprotein with foaming properties.

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MATERIALS AND METHODS

Biological Material and Chemicals. A commercial yeast cell wall preparation (Laffort, Guipúzcoa, Spain) was used in the experimental trial for mannoprotein extraction. The material was supplied as a spray-dried powder. All of the chemicals used were analytical grade and were purchased from Sigma Aldrich (St. Louis, MO).

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Model Wine and Sparkling Wines. The model wine contained ethanol (10% v/v), tartaric acid (4 g/L), malic acid (3 g/L), acetic acid (0.1 g/L), potassium sulfate (0.1 g/L), and magnesium sulfate (0.025 g/L). The pH was adjusted to 3.0 with sodium hydroxide. Sparkling wines A and B were two different commercial sparkling wines. Wine A was elaborated with a mixture of three different base wines: Macabeo, Xarel-lo, and Parellada. Wine B was prepared from one monovarietal base wine (Parellada).

Enzymatic or Thermal Extraction of Mannoproteins. The enzymatic extraction of mannoproteins was done according to the following procedure: 2 g of yeast cell walls was suspended in 100 mL of a citrate−phosphate buffer, pH 7.2. Cell walls were washed and centrifuged three times (5000*g* for 10 min) at 4 °C. The suspension obtained was incubated at 40 °C for 5 h with Glucanex 200G, a commercial preparation of β-glucanases (Novozymes, Dittingen, Switzerland). For the thermal extraction of mannoproteins, the same conditions as those used for preparing the cell wall suspension were used, followed by heating at 80−85 °C for 24 h under stirring. In both cases, the liquid phase obtained was ultrafiltered (pore size 10 kDa), freeze-dried, and kept for experimental analysis.

Instrumental Analysis of Foaming Properties. For analysis of the foaming properties, an apparatus developed at the Instituto de Fermentaciones Industriales (IFI) was used to determine the height increase occurring in a liquid when air is passed through it. The change in the height of the liquid was quantified by an ultrasound wave emitter−detector following the methodology described by Nuñez et al. (*13*). Three parameters were measured:

(a) Hpeak: the maximum height reached by the foam after air injection through a glass frit. Hpeak has been related to the wine's ability to foam.

(b) Hplateau: the foam height stability during air injection. This has been related to the average bubble lifetime.

(c) Sd300: standard deviation of foam measures in the last 300 points. This has been related to the effervescence of the wine.

The relative standard deviations ($n = 6$) for the parameters studied were 6.1% for Hpeak, 3.6% for Hplateau, and 2.9% for Sd300.

The extraction of foam for reversed-phase high-performance liquid chromatography (RP-HPLC) analysis was carried out following the same procedure, except extracting the foam formed using a glass cylinder coupled with a vacuum pump. Two fractions were obtained, separated, and collected for analysis: the foaming phase (FP), constituted by the foam obtained after the sample gasification, and the remaining phase (RP), or the sample obtained after the foam was removed.

Analytical Determinations for Compound Characterization. *Protein Fraction.* The concentration of the soluble protein fraction was determined according to the Bradford method (*18*), based on the reaction of the protein with Coomasie blue G-250. The absorbance was determined at 595 nm 15 min after the addition of the reactive. Results were expressed in milligrams of bovine serum albumin (BSA) per liter.

Polysaccharides. The concentration of neutral polysaccharides was determined by the phenol sulfuric method, according to Segarra et al. (*19*). The absorbance was determined at 490 nm. Results were expressed in milligrams of mannose per liter.

The procedure described by Segarra et al. (*19*) was used for the isolation of polysaccharides. A total of 5 mL of ethanol (96%, v/v) and 50 mL of HCl (1 N) were added to 1 mL of sample. After 18 h of incubation at 22 °C, the tubes were centrifuged (1800*g* for 20 min), after which the supernatant was discarded and the pellet was washed three times in ethanol (96%, v/v). The samples obtained were hydrolyzed at 100 °C for 24 h in a closed vial containing 1 mL of 2 M trifluoroacetic acid and 0.5 mL of myoinositol (0.1%, w/v, internal standard). After hydrolysis, the mixture was evaporated to dryness under vacuum. The dried hydrolyzed residue was silylated following the procedure of Troyano et al. (*20*). Briefly, the sample was dissolved in 100 μL of anhydrous pyridine, and 100 μL of (trimethylsilyl)imidazole, 100 μL of trimethylchlorosilane, 100 μL of *n*-hexane, and 200 μL of deionized water were sequentially added, with shaking of the sample during each step. The silylated derivatives present in the organic phase were immediately injected into the gas chromatograph. Trimethyilsilyl derivatives were analyzed on a Hewlett-Packard 6890 chromatograph, equipped with a flame ionization detector (FID) and a split/splitless injector. Samples were analyzed on a Carbowax 20M column (30 m × 0.25 mm) coated with a stationary phase of 0.25-μm thickness. Temperatures were as follows: injector and detector, 220 °C; oven, held at 40 °C for 10 min, then increasing 7 °C/min to 150 °C, and finally programmed at 30 °C/min to 210 °C. The carrier gas was helium (12.5 psi, split 1/15). Response factors were calculated with a series of pure standards at different concentrations using myoinositol as the internal standard. The identification of the compounds present in the samples was carried out by comparing the retention times of the peaks with those of pure standards.

SDS-PAGE and Size-Exclusion Chromatography (SEC). For SDS-PAGE analyses, the extracts were mixed in a 10 mM Tris HCl buffer, pH 8.0, containing 2.5% sodium dodecyl sulfate (SDS), 10 mM ethylenediaminetetraacetic acid (EDTA), and 5.0% β-mercaptoethanol, and heated at 100 °C for 10 min. SDS-PAGE was conducted with the Phast-System Electrophoresis apparatus, precast PhastGels Homogeneous 20%, and PhastGels SDS buffer strips (Pharmacia, Uppsala, Sweden). Electrophoretic conditions and silver staining followed the procedures of the manufacturer. A protein marker kit (14400−96400 Da; Pharmacia, Uppsala, Sweden) was used as the molecular weight marker.

A Sephacryl S200 HR (Amersham Biosciences, Uppsala, Sweden; 1.6 cm × 60 cm) gel permeation column, equilibrated in 50 mM sodium phosphate buffer (pH 7.2) containing 150 mM NaCl at 0.8 mL/min, was used to study the molecular size distribution of the fractions. The eluant was monitored at 214 nm by a Pharmacia LKB absorbance detector (Pharmacia, Uppsala, Sweden). Molecular weights were estimated with a calibration curve using the standard protein marker kit described above.

Affinity Chromatography. The fractionation of the extracts was carried out by chromatography on concanavaline A (Con A) Sepharose 4B gel (Amersham Biosciences, Uppsala, Sweden), containing approximately 13 mg of immobilized Con A per milliliter of gel. Mannoproteins were eluted by 0.5 M α-D-methylmannoside added to the initial buffer (20 mM Tris HCl (pH 7.5) containing 200 mM NaCl, 1 mM $CaCl_2$, and 1 mM $MnCl_2$) and were detected by measuring the absorbance of the eluted fractions at 214 nm using a Pharmacia LKB (Uppsala, Sweden) absorbance detector.

RP-HPLC. RP-HPLC separations were carried out on a Beckman System Gold HPLC equipped with System Gold Software data acquisition system version 711 (Beckman Instruments, Fullerton, CA), using a C_4 Phenomenex Jupiter column (300-Å pore size, 250 × 4.6 mm i.d., 5-μm particle size; Phenomenex, Torrance, CA). Samples were eluted at room temperature using 0.1% (w/v) trifluoroacetic acid in water as solvent A and 0.085% (w/v) trifluoroacetic acid in water/acetonitrile (10:90, v/v) as solvent B. The elution was performed as follows: 0−5 min, 0% solvent B in isocratic mode, and then a linear gradient by increasing the concentration of solvent B from 0% to 50% in 55 min, followed by another linear gradient by increasing the concentration of solvent B from 50% to 100% in 15 min. The flow rate was 1 mL/min. The absorbance was recorded at 215 nm using a Beckman 166 UV detector.

Protein Stability Test. Different concentrations (0−0.5 g/L) of the extracts were used to supplement the model wine and the two sparkling wines previously degassed. Samples were heated at 85 °C for 30 min, followed by 15 min of incubation on ice. Differences in turbidity were estimated by nephelometry (2100N, Hach Co., Loveland, CO). Results were expressed as nephelometric turbidity units (NTU). The protein sample was considered stable if the difference in turbidity before and after the treatment did not exceed 2 NTU.

RESULTS AND DISCUSSION

Foam Properties of the Yeast Cell Wall Extracts. Figure 1 shows the effects of two soluble yeast cell wall extracts ($C =$ 0.25 g/L) on the foaming properties of a model wine. Three instrumental parameters are represented (Hpeak, Hplateau, and Sd300), which had been previously related with foam maximum height, foam stability, and effervescence, respectively (see

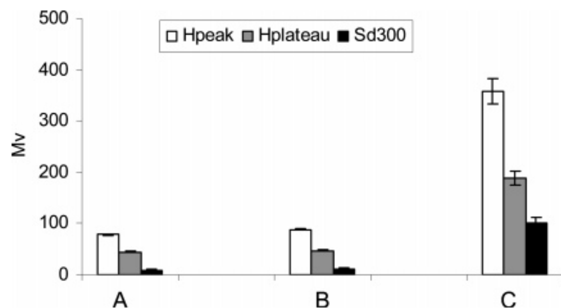

Figure 1. Effect of two soluble yeast extracts (0.25 g/L) on the foaming properties of a model wine. Data presented are the mean of three different experiments. (**A**) Model wine (control). (**B**) Model wine supplemented with the enzymatic extract. (**C**) Model wine supplemented with the thermal extract.

above). In all cases, the model wine supplemented with the thermal extract presented the best foaming properties, as compared with the model wine supplemented with the enzymatic extract, which had a behavior similar to that of the control model wine. Previous studies have tried to associate the method used for preparing the yeast cell wall extract with a specific purpose. For example, Dupin et al. (*17*) have found that mannoproteins extracted enzymatically are more useful in preventing protein haze in white wines that mannoproteins extracted by heat. Similar results were obtained by Moine-Ledoux and Dubourdieu (*10*) and Moine- Ledoux (*21*) in studying the properties of a mannoprotein fraction against the protein haze and the tartaric salt precipitation, respectively. However, Feuillat (*22*) observed that the thermal or enzymatic extraction of mannoproteins produced a similar stabilizing effect against tartaric salt precipitation, increasing the hypothesis that different kinds of mannoproteins or other yeast colloids could be involved in similar functions, independently of the extraction procedure. Until now, we have not found previous references about the influence of the mannoprotein extraction process on its foaming properties, and only few works had documented that mannoproteins released by yeast during aging can affect the foaming properties of the wines (*13*, *23*).

The response of the foaming parameters analyzed (Hpeak, Hplateau, and Sd300) after model wine supplementation with increasing concentrations of the thermal extract (0.05−0.4 g/L) is represented in **Figure 2**. There is a linear relationship between the extract addition and all of the foam parameters studied. It is worth noting that even a very low concentration of the additive (0.1 g/L) produced a remarkable increase in the values of the parameters studied, reflecting a general improvement in the foaming properties of the model wine.

Characterization of the Yeast Cell Wall Extracts. We tried to establish a relationship between the composition of the thermal extract and its role in wine foaming properties. After affinity chromatography on Con A, two fractions were obtained: F1 (eluted fraction) and F2 (retained fraction). Surprisingly, the eluted fraction presented higher foaming activity than F2 (**Figure 3**), thus indicating that presumably other colloids rather than mannoproteins could be involved in the foaming properties observed. **Table 1** shows the composition in proteins and polysaccharides of both extracts (enzymatic and thermal) and their fractions. The composition of each extract was dependent on the method used for extraction. Thus, the protein concentration was practically three times higher in the thermal extract than in the enzymatic preparation, confirming the prominent role of the protein fraction in the foaming properties in wine, which has been previously suggested by others (*5*, *6*,

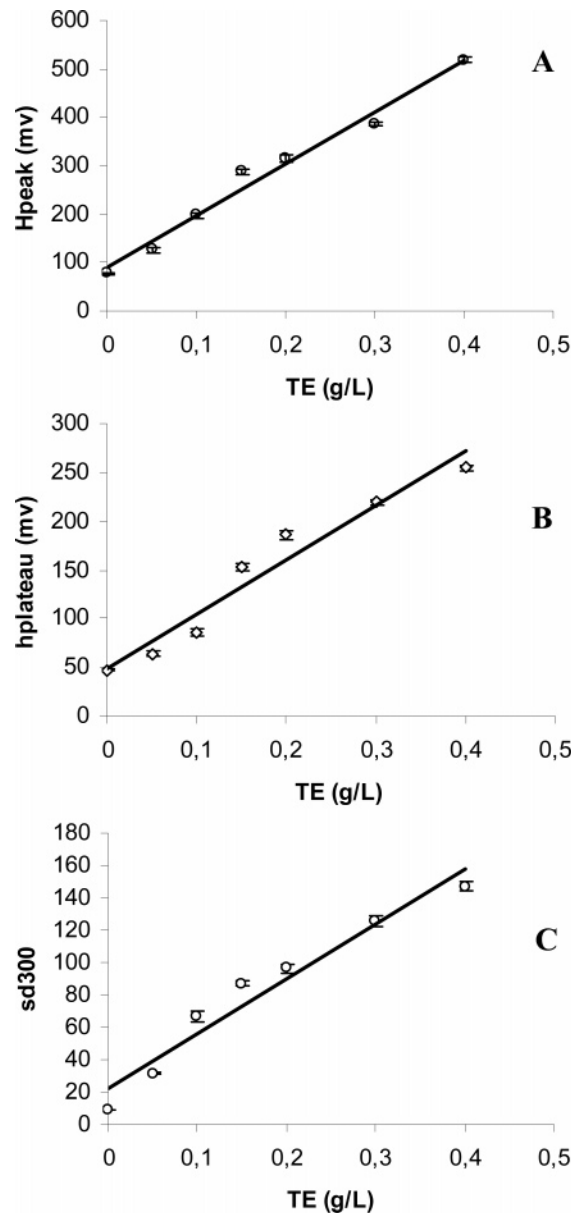

Figure 2. Response of the foaming parameters Hpeak (**A**), Hplateau (**B**), and Sd300 (**C**) analyzed after the supplementation of the model wine with different concentrations of the thermal extract (TE) (0.05–0.4 mg/L). Data presented are the mean of two different experiments.

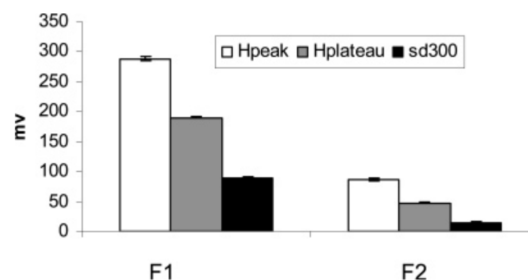

Figure 3. Foaming parameters of the eluted fraction (F1) and the retained fraction (F2) after Con A affinity chromatography of the thermal fraction. Data presented are the mean of three different experiments.

24). In contrast, the enzymatic extract was richer in polysaccharides than the thermal extract. In both cases, mannose was the main sugar present in the polysaccharides, confirming that, in spite of the procedure used for the extraction (enzymatic or thermal), mannoproteins were the main component of the two

Table 1. Composition (%) in Protein and Polysaccharides of the Enzymatic and Thermal Extracts (Total) and the Contribution (%) of Each Fraction (F1 and F2) to the Total Extract[a]

extract		% protein	% polysaccharide	% mannose	% glucose
	total	9.9 ± 0.2	90.9 ± 0.5		
enzymatic	F1	1.9 ± 0.3	14.5 ± 0.6	3.6 ± 0.2	10.9 ± 0.3
	F2	8.5 ± 0.9	76.0 ± 0.8	76.4 ± 0.4	0.0
	total	31.3 ± 0.1	66.1 ± 1.6		
thermal	F1	3.4 ± 0.3	21.2 ± 1.15	16.3 ± 0.8	4.9 ± 0.3
	F2	27.8 ± 0.8	45.0 ± 1.8	45.0 ± 1.2	0.0

[a] Data shown are the mean of three different experiments. F1: eluted fraction after Con A affinity chromatography. F2: retained fraction after Con A affinity chromatography.

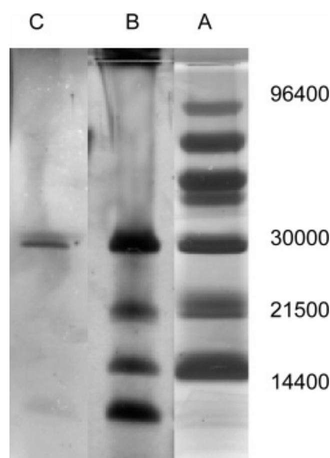

Figure 4. SDS-PAGE of the thermal and enzymatic yeast extracts: (**A**) molecular weight marker; (**B**) thermal extract; (**C**) enzymatic extract. Molecular weights of the standards are given on the right side of the gel.

extracts. F1, the main fraction responsible for the foaming properties, only represented a minor part of the product, being constituted by 3.4% of the total amount of the protein fraction (31.3%) and 21.2% of the polysaccharides (66.1%). The molecular characterization of the compounds, carried out using SEC and SDS-PAGE, showed in both cell wall extracts (enzymatic and thermal) a protein band corresponding to a relative molecular mass of 30 kDa (**Figure 4**). In addition, three bands were detected in the thermal extract with relative molecular masses between 10 and 21.5 kDa, which were absent in the extract obtained enzymatically. These results show that thermal extraction produces more heterogeneous protein fractions than do the enzymatic procedures. This is in agreement with the action mode of the enzyme preparation, which has the capacity cleaving the glucans in the anchor points with the cell wall. On the contrary, mild thermal procedures for mannoprotein extraction could facilitate the disruption of the noncovalent bonds (*17*). Moine-Ledoux and Dubourdieu (*10*) have found, using the same enzyme preparation, that enzymatic extraction of mannoproteins from yeast cell walls releases a mannoprotein with a MW of around 30 kDa. This mannoprotein has been identified as a fragment of the yeast invertase, being implicated in the improvement of protein stability. Here, a similar protein band (30 kDa) appeared in both extracts, showing that it is not responsible for the foaming properties observed.

To assess this point, the two fractions obtained from Con A chromatography for the thermal extract (F1 and F2) were analyzed by SDS-PAGE. We observed that the fraction responsible for foam activity (F1) only contains the compounds with relative molecular masses between 10 and 21,5 kDa, while the

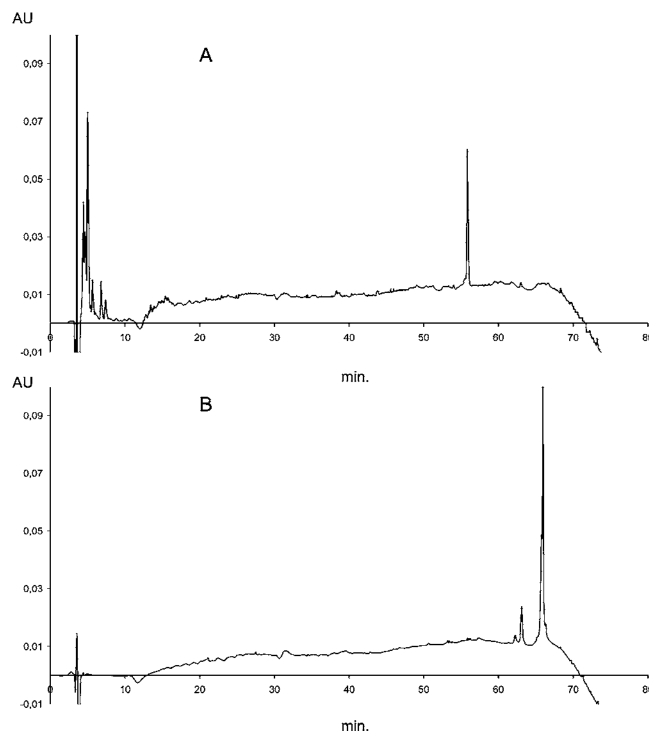

Figure 5. RP-HPLC of the eluted fraction F1 (**A**) and the retained fraction F2 (**B**) after Con A affinity chromatography of the thermal extract. AU: absorbance units.

other fraction (F2) includes the band corresponding to 30 kDa, also present in the enzymatic extract (data not shown). This result confirmed the hypothesis described above.

We studied the hydrophobicity of the mannoproteins present in both fractions (F1 and F2) obtained from the thermal extract. **Figure 5** represents the results obtained. Several hydrophilic peaks and one hydrophobic peak were obtained for the F1 fraction, while the major fraction (F2), which was poorly involved in the foaming properties, only presented three peaks, more hydrophobic than those obtained for F1. Although hydrophobic proteins have been preferably isolated from the wine foam (*24*, *25*), it is known that yeast mannoproteins are related with the foam properties observed in the sparkling wines (*8*, *13*). Mannoproteins present a protein moiety with hydrophobic and hydrophilic domains and sugar moieties, which are usually hydrophilic (*8*). The presence of an equilibrated composition of hydrophobic and hydrophilic protein domains in F1 may be related with its foaming properties, contributing to create points of adsorption to the gas−liquid interface of the bubbles and in this way increasing its stability. About this point, we decided to study the foam formed after the gasification of the thermal extract. Two fractions were obtained, separated, and collected for analysis: the foaming phase (FP) and the remaining phase (RP). After RP-HPLC analysis, we found that the protein peaks corresponding to F1 (with hydrophilic and hydrophobic protein domains) were isolated in the FP, corroborating their implication in the foaming behavior observed (**Figure 6A**). The very hydrophobic peak constituents of F2 were also isolated in the FP, while no protein peaks were obtained in the RP (**Figure 6B**). In addition, we collected the six peaks obtained for F1 after RP-HPLC. Each sample was hydrolyzed, dried, and prepared individually for gas chromatography. Except for peak 5, which was not analyzed because of its low concentration, the presence of mannose was demonstrated in all peaks, confirming that they were effectively mannoproteins. We have no explanation for the fact that this fraction was not retained

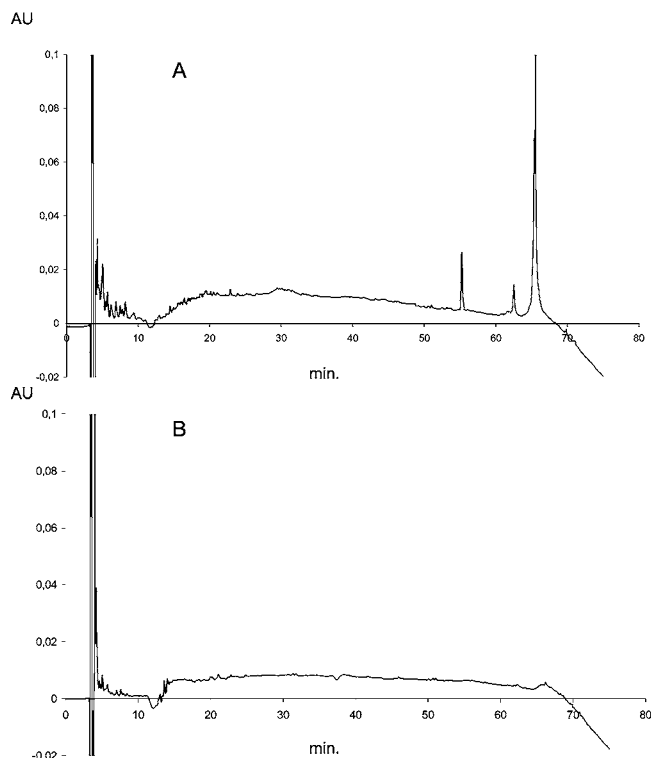

Figure 6. RP-HPLC of FP (**A**) and RP (**B**) obtained after model wine supplementation with the thermal extract. AU: absorbance units.

Table 2. Foam Parameters (Hpeak, Hplateau, and Sd300) Obtained for Two Different Commercial Sparkling Wines (A and B) Supplemented with Different Concentrations of the Thermal Extract (TE)[a]

		foam parameters (mV)		
wine	TE (g/L)	Hpeak	Hplateau	Sd300
A	0	423 ± 1.2	341 ± 2.3	1.5 ± 0.1
	0.10	471 ± 0.8	357 ± 1.1	2.6 ± 0.1
	0.20	748 ± 2.6	406 ± 2.9	3.7 ± 0.3
	0.30	865 ± 1.2	486 ± 3.2	4.4 ± 0.1
	0.40	998 ± 3.1	512 ± 2.2	4.9 ± 0.2
	0.50	1127 ± 1.2	533 ± 2.3	5.3 ± 0.2
B	0	583 ± 3.3	250 ± 1.2	10.0 ± 1.6
	0.05	637 ± 1.2	282 ± 0.8	14.5 ± 0.9
	0.10	946 ± 1.4	287 ± 2.2	18.5 ± 2.3
	0.15	1171 ± 2.8	292 ± 0.8	25.9 ± 1.4
	0.20	1394 ± 1.2	326 ± 2.9	36.1 ± 1.4

[a] Data shown are the mean of two different experiments.

on Con A chromatography. A possible explanation may be that F1 has a lower affinity for the column than F2 because of its low glycosilation. Further experiments will be developed to clarify this point. The presence of mannose was also confirmed in the hydrophobic peak isolated from the F1 fraction.

Stability of the Thermal Extract and Foaming Activity in Two Different Sparkling Wines. The results presented above show that the thermal extract increases the foaming properties in a model wine. Then, this result suggests the possibility of using this compound as an additive to improve the foaming properties of a sparkling wine. Two different sparkling wines, previously degassed, were used to study the effect of this supplementation on foam. In **Table 2**, we present the foaming parameters obtained in each case. Although the two wines studied were different, in all cases the supplementation produced an improvement in the foaming parameters studied. For example, supplementation of wine B with 0.05−0.20 mg/L of the thermal extract gave a foam improvement similar to that obtained by supplementation of wine A with 0.10−0.50 mg/L, showing the influence of the wine matrix on the foam activity. The relationship between the wine composition and its foaming properties has been documented by others (see above), showing that wines are very complex solutions that can be considered as a multicomponent system containing diverse compounds that may show foaming activity by themselves or by association with other compounds. An interesting point observed here is that, in spite of the wine matrix, a positive contribution of the mannoprotein extract to the foaming properties of the wine was observed. This response was obtained for different concentrations of the additive, showing that very low concentrations of the product produce a significant enhancement of the foaming properties. This is particularly interesting when considering that wine macromolecules are not far from their solubility limit and precipitations may happen and aggregates can be formed if the macromolecule concentration increases in excess. After 2 months of incubation at 4 °C, the samples were analyzed again, and similar results were obtained, showing the stability of the effect observed at least in the conditions assayed.

Table 3. Stability Against the Protein Haze of the Model Wine (MW) Supplemented with Different Concentrations (g/L) of the Thermal Extract[a]

sample	NTU	sample	NTU
MW	0.09 ± 0.10	MW + 0.3 g/L	0.15 ± 0.14
MW + 0.1 g/L	0.09 ± 0.07	MW + 0.4 g/L	0.08 ± 0.08
MW + 0.2 g/L	0.22 ± 0.10		

[a] Data shown are the mean of two different experiments. NTU: nephelometric turbidity units.

The thermal extract described here contains glycoproteins presenting relative molecular weights between 10 and 21.5 kDa. Wine proteins with MWs around these values have been considered in some cases as an instability factor because they could be implicated in the protein haze in white wines. We have used an analytical method to study the stability of the protein fraction composing the thermal extract. **Table 3** shows the results obtained. The model wine supplemented with different concentrations of the thermal fraction was completely stable even for the highest concentrations used, showing the feasibility of using this compound as an additive. The same results were obtained in the supplementation of two sparkling wines (see above) with the thermal extract (data not shown). These results show that, in spite of their low molecular weight, mannoproteins constituting the thermal extract are stable, coinciding with the results of other authors, who indicate that the majority of low molecular weight proteins responsible for haze formation in wines derived from grape (*12*).

In summary, the results obtained in this study show that the thermal extract obtained by using the mild conditions described before is responsible for the improvement of the foam properties in the model wine and in the two different sparkling wines studied. The thermal extract is mainly constituted of mannoproteins, presenting a higher and more heterogeneous protein content than the enzymatic extract.

After Con A chromatography, we obtained a fraction (F1) presenting mannoproteins with relative molecular weights between 10 and 21.5 kDa that appear to be responsible for the improvement of the foam properties in a model wine. There are mannoproteins with hydrophobic and hydrophilic domains, which can contribute to foam quality and stability. This mannoprotein extract was stable against protein haze, in both model wine and sparkling wines.

The enrichment of a sparkling wine with mannoproteins extracted by mild heat procedures will contribute to the improvement of its foam properties. As far as we know, this is the first report considering the use of yeast mannoproteins extracted by heat as additives for this purpose. Further studies are necessary in order to know how mannoproteins can influence other organoleptic properties of sparkling wines.

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