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Influence of the use of bentonite as a riddling agent on foam quality and protein fraction of sparkling wines (Cava)

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Abstract

This paper studies how the use of bentonite to facilitate the riddling process affects the foam properties and the protein fraction of sparkling wines (Cava). In all monovarietal sparkling wines (Macabeu, Xarel.lo, Parellada, Chardonnay and Pinot Noir), the addition of bentonite produced a statistically significant diminution of the Mosalux parameters (HM and HS). This depreciation of the foam quality may be attributed to the direct interaction of bentonite with wine proteins. Concretely the use of bentonite caused a diminution of more of 80% of total the soluble protein. Gel filtration by FPLC showed that bentonite particularly affected the 60 kDa and 20–30 kDa protein fraction. The high molecular fraction, on the other hand, was not affected.

Keywords: Sparkling wine; Foam; Proteins; Bentonite; Mosalux; FPLC

1. Introduction

One of the major factors affecting the visual organoleptic characteristics of sparkling wines is the persistence of foam (Marchal, Bouquelet, & Maujean, 1996). The ability of sparkling wines to form a stable collar is considered by consumers to be a criterion of quality (Brissonet & Maujean, 1993). For this reason, winemakers are very interested in understanding the factors that affect the foamability of wine.

Of the different foam active substances, proteins seem to play a major role because of their surface properties (Bamforth, 1985; Moreno-Arribas, Pueyo, Nieto, Martin-Alvarez, & Polo, 2000). Proteins act as tensoactive substances, enhancing foam stability, and it seems that they contribute to film elasticity and film strength (Malvy, Robillard, & Duteurtre, 1994). In fact, some authors have found that there is a close relationship between protein concentration and foam quality in sparkling wines (Brissonet & Maujean, 1993; Pueyo, Martín-Alvarez, & Polo, 1995).

In the champenoise method, after the second fermentation, sparkling wines remain in contact with the yeast lees in the bottle. During this ageing period, the yeast releases proteins and other compounds that have a positive effect on foam stability (Martinez-Rodriguez, Carrascosa, Martin-Alvarez, Moreno-Arribas, & Polo, 2002; Todd, Fleet, & Henschke, 2000).

Although numerous authors have studied the protein fraction of white wines in recent years (Canals, Arola, & Zamora, 1998; Dambrouck et al., 2003; Dizy & Bisson, 1999; Dorrestein, Ferreira, Laureano, & Teixeira, 1995; Ferreira, Picarra-Pereira, Monteiro, Loureiro, & Teixeira, 2002; Kwon, 2004; Rodriguez-Delgado, Malovana, Montelongo, & Cifuentes, 2002; Vincenzi et al., 2005), very few have studied how the champagnisation process affects the soluble protein fraction (Luguera, Moreno-Arribas, Pueyo, & Polo, 1997; Luguera, Moreno-Arribas, Pueyo, Bartolomé, & Polo, 1998; Martinez-Rodriguez & Polo,

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2003). Besides, champagne and cava winemakers often add bentonite to the wine in order to facilitate the process of riddling (Poinsaut & Hardy, 1995). However, the electrostatic interaction of bentonite with proteins considerably decreases its concentration (Senee, Viaux, Robillard, Duteurtre, & Vignes-Adler, 1998) and it may, therefore, have an important effect on the foaming characteristics of sparkling wine.

During the last years, diverse studies have been published about the influence of the fining treatments with bentonite on the foam quality of base wines (Marchal, Chaboche, Douillard, & Jeandet, 2002; Maujean, Poinsaut, Dantan, Brissonet, & Cossiez, 1990; Poinsaut, 1991; Puig-Deu, Lopez-Tamames, Buxaderas, & Torre-Boronat, 1999; Vanrell et al., 2002; Vanrell, Esteruelas, Canals, & Zamora, 2005). Nevertheless, to our knowledge only one specific work exists that undertakes the study of the influence of the addition of bentonite to facilitate the riddling process in real conditions of champanisation (Martinez-Rodriguez & Polo, 2003).

The aim of this study was to determine how adding bentonite affects the soluble protein fraction and consequently the foam properties of sparkling wines. For this study, we selected Macabeu, Xarel.lo, Parellada, Chardonnay and Pinot Noir grapes because they are the varieties that are most often used to produce sparkling wines.

2. Materials and methods

2.1. Chemicals

All the products were of high purity and suitable for fast protein liquid chromatography (FPLC). All solutions were previously filtered through 0.22 μm acetate cellulose filters (Millipore GSE) and degassed using an ultrasonic water bath.

2.2. Sparkling wines

Wines based on Macabeu, Xarel.lo, Parellada, Chardonnay and Pinot Noir were made from the 2002 crop at the experimental vineyard of the Enology Faculty of the Rovira i Virgili University in the village of Constanti (Tarragona, Spain).

Some bottles of the different monovarietal base wines were conserved at 15 °C until the end of the experiment. The rest of monovarietal base wines were used for the elaboration of monovarietal sparkling wines (Cava) using the champanoise method. A preadapted yeast culture (DV10, Martin Vialatte) was used for the second fermentation in the bottle. Some of the bottles were prepared without bentonite and others were added with 30 mg/l of bentonite (Microcol, Laffort) to study the specific influence of this riddling agent. After 24 months of ageing, base wines and sparkling wines were used for foaming properties determination and for protein fraction analysis. All the analysis were done with five different bottles.

2.3. Yeast autolysis

A medium containing 100 g/l of glucose, 100 g/l of fructose, 6 g/l of tartaric adjusted at pH 3.5 with KOH was prepared. This medium was inoculated with 5×10^6 viable cells/ml of previously rehydrated yeast (DV10, Martin Vialatte), and 100 mg/l of commercial fermentation activators (Vitiamine; Martin Vialatte) were also added. Samples of the medium were extracted at 0, 50 and 100 days after inoculation and used only for protein analysis.

2.4. Sample preparation

All base wines and sparkling wines were previously degassed by magnetic stirring for 15 min and centrifuged at 4000*g* for 5 min at 4 °C. The supernatant was used directly for foaming properties determination (Maujean et al., 1990) and for protein fraction analysis (Canals et al., 1998).

Aliquots of 30 ml of the different samples (base wines and sparkling wines previously degassed and autolysis medium) were immediately put into three dialysis tubes of a molecular weight cut-off of 12 kDa. (SIGMA, dialysis tubing-cellulose membrane; D-9652) to remove salts and other low molecular weight compounds. The dialysed samples were lyophilised and preserved at −20 °C until the moment of analysis. On the day of the analysis, the lyophilised samples were resuspended in 600 μl of 0.3 M ammonium acetate solution adjusted at a pH 7.00. The samples were centrifuged at 12,000*g* for 2 min at 4 °C and the supernatant were used for FPLC analysis (Canals et al., 1998).

2.5. Measurement of foaming properties

The foam measurement of base wines and sparkling wines were carried out using the Mosalux procedure (Maujean et al., 1990; Poinsaut, 1991). A glass cylinder placed on a glass frit was filled with 100 ml of the sample. Carbon dioxide was injected into the glass cylinder through the glass frit with a constant gas flow of 115 ml/min under a constant pressure of 100 kPa. Foam height was measured by photoelectric cells (infrared beams).

Two parameters were measured: HM was the maximum height reached by the foam and HS was the stable height of the foam. HM represented the foamability and HS represented the persistence of the foam collar or the wine's ability to produce stable foam. Some authors also use to measure another parameter, TS that correspond to time needed for the foam to collapse after the gas flow has stopped (Maujean et al., 1990). This parameter also represents the foam stability but we have not determined it because the imprecision of its measurement.

Both parameters, HM and HS are expressed in mm. All measures were determined in triplicate.

2.6. Gel filtration chromatography on FPLC

The previous dialysed, lyophilised and resuspended samples from base wines, sparkling wines and autolysis medium were first separated by a Superdex 75 PC 3.2/30 column on a fast protein liquid chromatography system (Smart System, Pharmacia, Uppsala, Sweden). The samples (50 μl) were injected and eluted with a 0.3 M ammonium acetate solution with a flow of 40 μl/min. The column eluents were continuously monitored at 280 nm using a μPeak Monitor (Pharmacia, Uppsala, Sweden). The different fractions: F1, F2 and F3 were collected and lyophilised for subsequent analysis.

2.7. Cation exchange chromatography on FPLC

Fractions F1, F2 and F3, from base wines and sparkling wines were fractionated by cation exchange chromatography using the ion exchange column Mono S PC 1.6/5 (Pharmacia, Uppsala, Sweden). The initial buffer was 0.05 M acetic acid–HCl (pH 2.0). The elution buffer was the initial buffer with sodium chloride 1 M. The column was equilibrated with the initial buffer at a flow rate of 100 μl/min. The lyophilised samples were resuspended in the initial buffer (100 μl) and were loaded into the column. The bound proteins were eluted with a gradient from 0% to 100% of elution buffer and continuously monitored at 230 nm.

2.8. Anion exchange chromatography on FPLC

Fraction F1 from base wines and sparkling wines were fractionated by anion exchange chromatography using the ion exchange column Mono Q PC 1.6/5 (Pharmacia, Uppsala, Sweden). The initial buffer was 0.02 M Tris–HCl (pH 8.0). The elution buffer was the initial buffer with sodium chloride 1 M. The column was equilibrated with initial buffer at a flow rate of 100 μl/min. The lyophilised samples were resuspended in initial buffer (100 μl) and were loaded into the column. The bound proteins were eluted with a gradient from 0% to 100% of elution buffer and continuously monitored at 230 nm.

2.9. Affinity chromatography on FPLC

The lyophilised samples of sparkling wines obtained without bentonite were resuspended in 600 μl of 0.3 M ammonium acetate solution. Fifty microliters were used directly for Gel filtration analysis. Another aliquot of 50 μl was diluted till 500 μl with the binding buffer (0.02 M Tris–HCl 20; 0.5 M NaCl 0.5; 0.001 M $MnCl_2$; 0.001 $CaCl_2$; pH 7.4) and loaded into the Concanavaline A column (HiTrap Con A-Pharmacia, Uppsala, Sweden). The binding buffer was eluted at a flow of 1 ml/min during 4 min. A non-bound fraction was collected. For the elution of bound fraction, an elution buffer (0.5 M Methyl-α-D-mannopyranoside; 0.02 M Tris–HCl; 0.5 M NaCl; pH 7.4) was used at a flow of 1 ml/min during 5 min. Both, bound and non-bound fractions, were dialysed, lyophilised and used for gel filtration analysis.

2.10. Determination of protein concentration

The protein concentration of the different molecular weights fractions was directly expressed in form of absorbance at 280 nm (A280) and not as protein concentration since A280 is not specific for proteins and therefore it can include polysaccharides and even phenolic compounds. Total soluble protein concentration was calculated by adding the A280 of the fractions F1, F2 and F3.

2.11. Statistical analysis

All the data are expressed as the arithmetic average ± standard deviation from five replicates. Statistical comparisons between means were established with Student's *t*-test using Statview (software for Macintosh).

3. Results and discussion

The foaming properties of the five monovarietal base wines and their corresponding sparkling wines elaborated with or without the addition of bentonite are shown in Fig. 1.

Although a slight diminution in foamability (HM) and the persistence of the foam (HS) in the sparkling wines elaborated without addition of bentonite respect to its corresponding base wines was detected, this one was not statistically significant in any case. On the contrary, when the sparkling wines were elaborated with addition of bentonite,

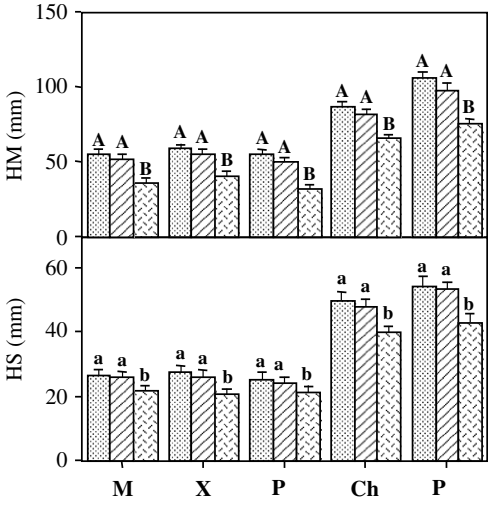

Fig. 1. Foaming properties of base wines and sparkling wines (Cava): ⬚: base wine; ▨: cava without bentonite; ⬚: cava with bentonite. M, Macabeu; X, Xarel.lo; Pa, Parellada; Ch, Chardonnay; and PN, Pinot Noir. Equal letters AB indicate the absence of statistically significant differences ($p > 0.05$) among the three samples examined for each wine.

a statistically significant decrease of HM and HS was observed respect to its corresponding base wines. This behaviour was detected in all the studied varieties.

Therefore, all these data seem to indicate that the addition of bentonite to facilitate the riddling process affects seriously the foam quality, whereas the champanisation process does not seem to have a great importance. These results are, in general terms, in agreement with the few data previously published on the subject (Martinez-Rodriguez & Polo, 2003).

In a previous study (Canals et al., 1998) we proposed a new method, which used FPLC, for separating white wine proteins into several fractions. In all the wines studied, three fractions were obtained by gel filtration (F1, F2 and F3). Subsequent analysis by SDS–PAGE and cationic exchange chromatography showed that F2 and F3 were presumably proteins. Specifically, fraction F1 has a molecular weight greater than 100 kDa and did not give an electrophoretic band. Fraction F2 was a single protein of a molecular weight of environs 60 kDa, which according to the literature is probably Invertase (Dambrouck, Marchal, Cilindre, Parmentier, & Jeandet, 2005). Finally, fraction F3 consisted of several different proteins with molecular weights between 20 and 30 kDa and probably contains the described pathogenic related proteins such as Thaumatin-like proteins and Chitinases (Pocock, Hayasaka, McCarthy, & Waters, 2000).

The comparison between base wines and their respective sparkling wines obtained without adding bentonite shows that total protein decreases in Macabeu, Xarel.lo and Chardonnay, whereas no significant changes were found in Parellada and Pinot Noir (Table 1 and Fig. 2). This decrease in total protein concentration was mainly due to the F3 protein fraction while F1 and F2 seem to remain stable. The decrease in F3 observed in three monovarietal sparkling wines may be related to the increase in ethanol concentration during the second fermentation or with proteolytic activity originated during the contact with lees (Leroy, Charpentier, Duteurtre, Feuillat, & Charpentier, 1990). These data are in agreement with our previous work (Canals et al., 1998), which also showed that there was a decrease in proteins during alcoholic fermentation.

Otherwise, the addition of bentonite to facilitate the riddling process considerably decreases the protein fractions F2 and F3 that practically disappear in all the varieties studied (Table 1 and Fig. 2). Only fraction F1 seems to be non-affected by the presence of bentonite. According to these data, the presence of bentonite during the champagnisation process seems to eliminate more than 80% of total soluble protein. These results are in general terms in agreement with (Martinez-Rodriguez & Polo, 2003).

As it was stated in Section 1, proteins play a major role in foam quality of sparkling wines (Andres-Lacueva, Lopez-Tamames, Lamuela-Raventos, Buxaderas, & de la Torre-Boronat, 1996; Pueyo et al., 1995). Therefore, the drastic decrease of protein concentration caused by the addition of bentonite to facilitate riddling process is probably the reason for which the foam quality is affected.

Fig. 3 shows the chromatograms analysed by the cationic exchange of fraction F1 from Macabeu base wines and sparkling wines. The rest of varieties presented a similar behaviour. In all cases no significative peaks were detected, indicating that fraction F1 is not retained by our column. The fact that fraction F1 was not retained by a Mono S PC 1.6/5 column (Pharmacia, Uppsala, Sweden) when the pH was 2.0 suggests that fraction F1 does not have a positive electrical charge in these conditions or at the pH of the wine. This may be one of the reasons why the addition of bentonite does not affect fraction F1 in sparkling wines.

Figs. 4 and 5 show the cationic exchange chromatograms of fractions F2 and F3, respectively. Both figures present only the results for the base wines and the sparkling wines obtained without bentonite, because when it was added fractions F2 and F3 were nearly eliminated. In the case of Fig. 4, it is only shown the chromatogram corresponding to the Macabeu since the rest of varieties showed a similar behaviour. A single major peak and other of minor surface were detected for fraction 2 in all grape varieties.

Fig. 5 present the chromatograms corresponding to the five varieties, since slight differences of behaviour among them were observed. For fraction F3, several peaks are detected. In that case some differences between the different grape varieties are detected. Macabeu and Parellada base wines showed two or three peaks at the beginning of the chromatogram whereas in its final part nearly nothing was distinguished. On the contrary, Xarel.lo, Chardonnay and Pinot noir base wines presented several peaks throughout all the chromatogram.

On the other hand, the results of Fig. 5 show that the protein of all sparkling wines decrease respect to the base wines. It should be pointed out that the proteins at the end of the chromatogram were very much affected by the champagnisation process, indicating that proteins with a more positive electrical charge are more unstable. These data also agree with our previous results (Canals et al., 1998) and the proteins from fraction F3 in both studies behave in a similar way during the alcoholic fermentation of grape must.

On the other hand, after 24 months of ageing we did not detect the release of proteins described by some authors (Dupin et al., 2000; Todd et al., 2000) when the wine came into contact with the lees. However, other authors (Luguera et al., 1998) did not find any increase in proteins throughout the process of cava ageing. Fraction F1 is only greater in sparkling wines than their respective base wines in a few cases.

Bentonite may not affect fraction F1 for two reasons: the peak consists mainly of polysaccharides and therefore does not interact electrostatically with bentonite (or with the cation exchange column) or it consists of proteins (or glycoproteins) that do not have a positive electrical charge

Table 1
Effect of bentonite on protein concentration of sparkling wines (Cava)

Grape variety	Fraction	Base wine	Cava without bentonite	Cava with bentonite
Macabeu	F1	103 ± 25A	107 ± 24A	97 ± 18A
	F2	230 ± 28A	199 ± 27A	ND
	F3	459 ± 18A	288 ± 25B	ND
	Total protein	791 ± 19A	589 ± 35B	97 ± 18C
Xarel.lo	F1	172 ± 21A	199 ± 48A	209 ± 52A
	F2	370 ± 24A	294 ± 134A	ND
	F3	951 ± 66A	256 ± 147B	ND
	Total protein	1492 ± 83A	948 ± 150B	209 ± 52C
Parellada	F1	141 ± 35A	148 ± 21A	134 ± 28A
	F2	240 ± 59A	244 ± 4A	ND
	F3	319 ± 117A	322 ± 99A	ND
	Total protein	698 ± 107A	712 ± 49A	134 ± 28B
Chardonnay	F1	131 ± 28A	134 ± 18A	121 ± 17A
	F2	220 ± 27A	216 ± 35A	ND
	F3	1142 ± 70A	938 ± 56B	ND
	Total protein	1491 ± 83A	1286 ± 76B	121 ± 17C
Pinot noir	F1	114 ± 18A	131 ± 25A	124 ± 32A
	F2	370 ± 93A	309 ± 11A	ND
	F3	934 ± 175A	849 ± 76A	ND
	Total protein	1416 ± 247A	1287 ± 69A	124 ± 32B

All data are the mean ± sd of five different bottles and are expressed directly in absorbance units at 280 nm. F1, high molecular weight protein fraction (MW > 100 kDa); F2, intermediate molecular weight protein fraction (MW = 60 kDa); F3, low Intermediate molecular weight protein fraction (MW = 20–30 kDa). Statistical differences: equal letters ABC indicate the absence of statistically significant differences ($p > 0.05$). Different letters indicate the existence of statistically significant differences ($p < 0.05$).

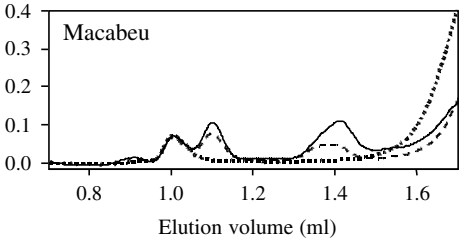

Fig. 2. Gel filtration chromatography of base wines and sparkling wines: —, base wine; ---, cava without bentonite; . . ., cava with bentonite. F1, high molecular weight protein fraction (MW > 100 kDa); F2, intermediate molecular weight protein fraction (MW = 60 kDa); F3, low molecular weight protein fraction (MW = 20–30 kDa).

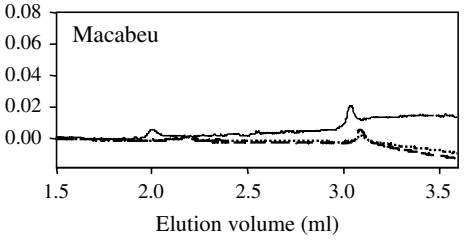

Fig. 3. Cationic exchange chromatography of fraction F1: —, base wine; ---, cava without bentonite; and . . ., cava with bentonite.

at the pH of wine. In both cases, as fraction F1 is really the only macromolecular fraction that can be found in a sparkling wine produced in real conditions (with the addition of bentonite), the macromolecular substances that may be

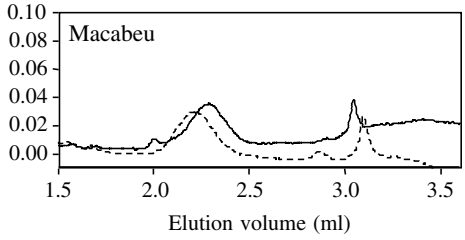

Fig. 4. Cationic exchange chromatography of F2: —, base wine and ---, cava without bentonite.

released by yeast autolysis must be searched for in this fraction.

Fig. 6 shows the anionic exchange chromatogram of fraction F1 from base wines and sparkling wines. In this case the chromatograms of all the varieties are shown and some differences among them are observed. Unlike cationic exchange chromatography, the column retained some peaks, indicating that this macromolecular fraction has a certain negative electrical charge in our working conditions. Although the resolution is not good, some differences between sparkling wines and base wines were found in nearly all grape varieties. In general terms, the total areas of the sparkling wines were greater than of their respective base wines, with the only exceptions of Xarel.lo and Chardonnay that had been produced with the addition of bentonite. In these two grape varieties, the addition of bentonite seems to decrease the total area and also to affect the shape of the anionic exchange chromatogram of frac-

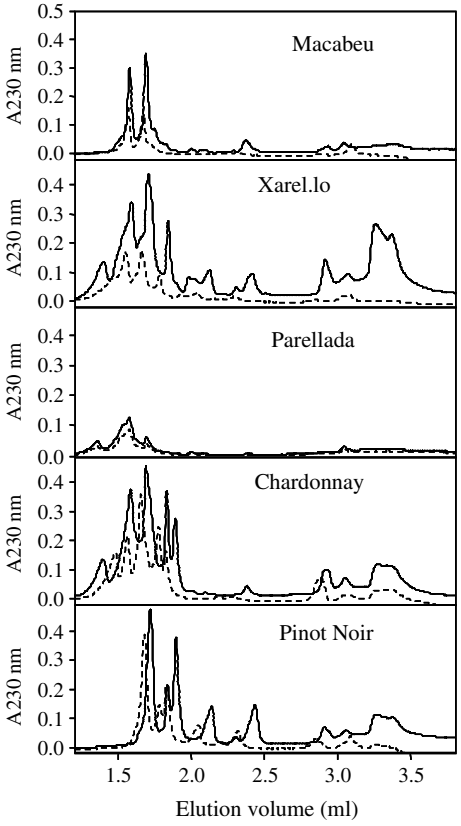

Fig. 5. Cationic exchange chromatography of F3: —, base wine and ---, cava without bentonite.

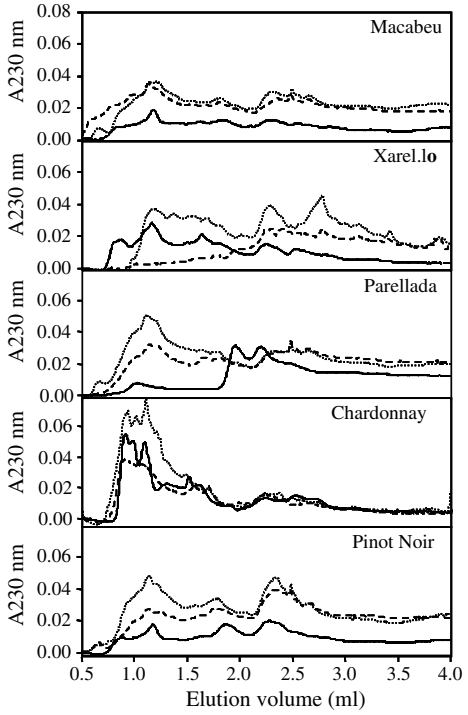

Fig. 6. Anionic exchange chromatography of F1: —, base wine; ---, cava without bentonite; and . . ., cava with bentonite.

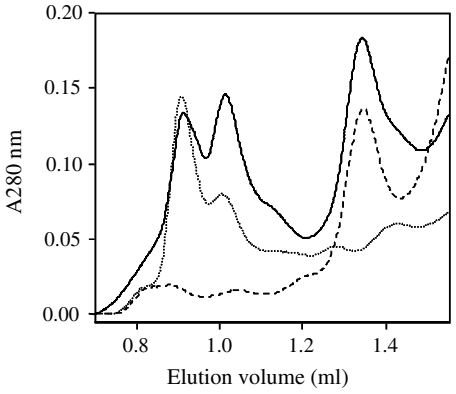

Fig. 7. Gel filtration chromatography of fractions obtained by concanavaline A affinity chromatography column: —, colloidal fraction of original cava; . . ., collodial fraction that bind to the affinity column; and ---, collidial faction that does not bind to the affinity column.

tion F1. Nevertheless, further studies are required if it is to be confirmed that these differences in the chromatogram profiles are due to the release of macromolecules by yeast autolysis.

Fig. 7 shows the gel filtration chromatogram of a Macabeu sparkling wine obtained without addition of bentonite in comparison with the equivalent analysis of the two fractions obtained by affinity chromatography. The rest of varieties showed a similar behaviour. Concanavaline A links specifically carbohydrates with terminal mannose or glucose and have been used for isolation of mannoproteins from other proteins (Waters, Pellerin, & Brillouet, 1994). These results show that fractions F1 and partially F2 are linked by concanavaline A. On the other hand fraction F3 is not linked to the affinity column.

The fact that fractions F1 and partially F2 were linked by the concanavaline A column indicates that they are mainly composed by polysaccharides or glycoproteins. As fraction F2 gives two peaks by cationic exchange chromatography, it may be considered that the major peak, which is probably invertase, is retained whereas the other one is not retained. This fact could explain because the peak F2 of the retained fraction presents a lower area than the peak F2 of the original cava. However, the non-retained fraction do not present a clear peak F2 probably because of the lower concentration of the minor component. On the other hand, fraction F1 does not give any peak by cationic exchange chromatography. Therefore, F1 is probably a fraction containing polysaccharides and/or mannoproteins (Waters et al., 1994). This glucidic composition may justify that this fraction has not positive electrical charge in wine conditions being unaffected by bentonite action.

Fig. 8 shows the gel filtration chromatogram of the synthetic medium fermented by the yeast. At the beginning of the process (day 0), only a very little fraction corresponding to same elution volume that the fraction F1 is detected. This fraction increases notably throughout the conservation time, indicating that contains the colloidal products

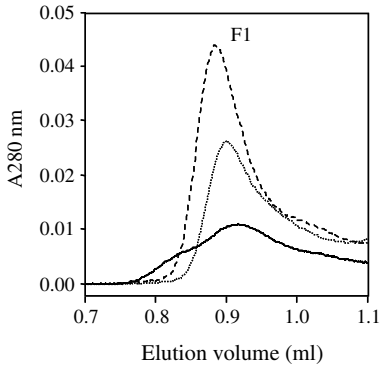

Fig. 8. Gel filtration chromatography of synthetic medium fermented by yeast: —, 0 days; . . ., 50 days; and ---, 100 days

of yeast autolysis. All this data confirms that colloidal autolysis products are present in fraction F1. However, no significant differences were found for F1 between the base wines and their corresponding sparkling wines (Table 1). This can be explained because the real process of sparkling wine elaboration is quite different from the experimental conditions developed in a synthetic medium. In fact, Nuñez, Carrascosa, González, Polo, and Martínez-Rodriguez (2005) have found that protein and polysaccharides concentration oscillates throughout the aging time. The authors attribute this behaviour to a balance between the release of these macromolecules and their disappearance by hydrolysis or precipitation.

4. Conclusions

All these data confirm that the addition of bentonite to facilitate the riddling process affects seriously the foam quality, whereas the champanisation process does not seem to have a great importance This addition cause a statistically significant decrease of foamability (HM) and foam persistence (HS), and eliminates nearly all the proteins from fractions F2 and F3, reducing the total soluble protein concentration by more than 80%. As proteins really play a major role in foam quality, the drastic decrease of protein concentration caused by the addition of bentonite is probably the reason for which the foam quality is affected. Therefore, wine industry must search for new riddling agents (or new combinations of riddling agents) that do not have such an effect on the protein fraction of sparkling wines. However, fraction F1 is nearly unaffected by bentonite probably due to its glucidic composition. On the other hand, yeast autolysis in synthetic medium produces the apparition of a gel filtration fraction at similar elution volume that F1. These results suggest that the yeast colloidal autolysis products must be presents in the high molecular weight fraction.

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References

Andres-Lacueva, C., Lopez-Tamames, E., Lamuela-Raventos, R. M., Buxaderas, S., & de la Torre-Boronat, M. C. (1996). Characteristics of sparkling base wines affecting foam behavior. *Journal of Agricultural and Food Chemistry, 44*, 989–995.

Bamforth, C. W. (1985). The foaming properties of beer. *Journal of the Institute of Brewing, 91*, 370–383.

Brissonet, F., & Maujean, A. (1993). Characterization of foaming proteins in a champagne base wine. *American Journal of Enology and Viticulture, 44*, 297–301.

Canals, J. M., Arola, L., & Zamora, F. (1998). Protein fraction analysis of white wine by FPLC. *American Journal of Enology and Viticulture, 49*, 383–388.

Dambrouck, T., Marchal, R., Marchal-Delahaut, L., Parmentier, M., Maujean, A., & Jeandet, P. (2003). Immunodetection of proteins from grapes and yeast in a white wine. *Journal of Agricultural and Food Chemistry, 51*, 2727–2732.

Dambrouck, T., Marchal, R., Cilindre, C., Parmentier, M., & Jeandet, P. (2005). Determination of the grape invertase content (using PTA-ELISA) following various fining treatments versus changes in the total protein content of wine. Relationships with wine foamability. *Journal of Agricultural and Food Chemistry, 53*, 8782–8789.

Dizy, M., & Bisson, L. F. (1999). White wine protein analysis by capillary zone electrophoresis. *American Journal of Enology and Viticulture, 50*, 120–127.

Dorrestein, E., Ferreira, R., Laureano, O., & Teixeira, A. (1995). Electrophoretic and FPLC analysis of soluble proteins in four portuguese wines. *American Journal of Enology and Viticulture, 46*, 235–242.

Dupin, I. V. S., McKinnon, B. M., Ryan, C., Boulay, M., Markides, A. J., Jones, G. P., et al. (2000). Saccharomyces cerevisiae mannoproteins that protect wine from protein haze: their release during fermentation and lees contact and a proposal for their mechanism of action. *Journal of Agricultural and Food Chemistry, 48*, 3098–3105.

Ferreira, R. B., Picarra-Pereira, M. A., Monteiro, S., Loureiro, V. B., & Teixeira, A. R. (2002). The wine proteins. *Trends in Food Science and Technology, 12*, 230–239.

Kwon, S. W. (2004). Profiling of soluble proteins in wine by nano-high-performance liquid chromatography/tandem mass spectrometry. *Journal of Agricultural and Food Chemistry, 52*, 7258–7263.

Leroy, M. J., Charpentier, M., Duteurtre, B., Feuillat, M., & Charpentier, C. (1990). Yeast autolysis during champagne aging. *American Journal of Enology and Viticulture, 41*, 21–28.

Luguera, C., Moreno-Arribas, V., Pueyo, E., Bartolomé, B., & Polo, M. C. (1998). Fractionation and partial characterization of protein fractions present at different stages of the production of sparkling wines. *Food Chemistry, 63*, 465–471.

Luguera, C., Moreno-Arribas, V., Pueyo, E., & Polo, M. C. (1997). Capillary electrophoretic analysis of wine proteins. Modifications during the manufacture of sparkling wines. *Journal of Agricultural and Food Chemistry, 45*, 3766–3770.

Malvy, J., Robillard, B., & Duteurtre, B. (1994). Influence des protéines sur le comportement de la mousse des vins de Champagne. *Science des Aliments, 14*, 87–98.

Marchal, R., Bouquelet, S., & Maujean, A. (1996). Purification and partial biochemical characterization of glycoproteins in a champenois Chardonnay wine. *Journal of Agricultural and Food Chemistry, 44*, 1716–1722.

Marchal, R., Chaboche, D., Douillard, R., & Jeandet, P. (2002). Influence of lysozyme treatments on champagne base wine foaming properties. *Journal of Agricultural and Food Chemistry, 50*, 1420–1428.

Martinez-Rodriguez, A. J., Carrascosa, A. V., Martin-Alvarez, P. J., Moreno-Arribas, V., & Polo, M. C. (2002). Influence of the yeast strain on the changes of the amino acids, peptides and proteins during

sparkling wine production by the traditional method. *Journal of Industrial Microbiology and Biotechnology, 29*, 314–322.

Martinez-Rodriguez, A. J., & Polo, M. C. (2003). Effect of the addition of bentonite to the tirage solution on the nitrogen composition and sensory quality of sparkling wines. *Food Chemistry, 81*, 383–388.

Maujean, A., Poinsaut, P., Dantan, H., Brissonet, F., & Cossiez, E. (1990). Étude de la tenue et de la qualité de mousse des vins effervescents II. Mise au point d'une téchnique de mesure de la moussabilité, de la tenue et de la stabilité de la mousse des vins effervescents. *Bulletin de l'OIV, 711–712*, 405–426.

Moreno-Arribas, V., Pueyo, E., Nieto, F. J., Martin-Alvarez, P. J., & Polo, M. C. (2000). Influence of the polysaccharides and the nitrogen compounds on foaming properties of sparkling wines. *Food Chemistry, 70*, 309–317.

Nuñez, Y. P., Carrascosa, A. V., González, R., Polo, M. C., & Martínez-Rodriguez, A. J. (2005). Effect of accelerated autolysis of yeast on the composition and foaming properties of sparkling wines elaborated by a champenoise method. *Journal of Agricultural and Food Chemistry, 53*, 7232–7237.

Pocock, K. F., Hayasaka, Y., McCarthy, M. G., & Waters, E. J. (2000). Thaumatin-like proteins and chitinases, the haze-forming proteins of wine, accumulate during ripening of grape (Vitis vinifera) berries and drought stress does not affect the final levels per berry at maturity. *Journal of Agricultural and Food Chemistry, 48*, 1637–1643.

Poinsaut, P. (1991). Le mosalux appareil de mesure du pouvoir moussant du vin. *Revue des Oenologues, 59*, 36–43.

Poinsaut, P., & Hardy, G. (1995). Bentonites. III. Utillisation des bentonites en oenologie. *Revue des Oenologues, 77*, 29–34.

Pueyo, E., Martín-Alvarez, P. J., & Polo, M. C. (1995). Relationship between foam characteristics and chemical composition in wines and cavas (sparkling wines). *American Journal of Enology and Viticulture, 46*, 518–524.

Puig-Deu, M., Lopez-Tamames, E., Buxaderas, S., & Torre-Boronat, M. C. (1999). Quality of base and sparkling wines as influenced by the type of fining agent added pre-fermentation. *Food Chemistry, 66*, 35–42.

Rodriguez-Delgado, M. A., Malovana, S., Montelongo, F. J., & Cifuentes, A. (2002). Fast analysis of proteins in wines by capillary gel electrophoresis. *European Food Research and Technology, 214*, 536–540.

Senee, J., Viaux, L., Robillard, B., Duteurtre, B., & Vignes-Adler, M. (1998). The endogenous particles of a sparkling wine and their influence on the foaming behaviour. *Food Hydrocolloids, 12*, 217–226.

Todd, B. E. N., Fleet, G. H., & Henschke, P. A. (2000). Promotion of autolysis through the interaction of killer and sensitive yeasts: potential application in sparkling wine production. *American Journal of Enology and Viticulture, 51*, 65–72.

Vanrell, G., Cabanillas, P., Albet, S., Canals, J. M., Arola, L., & Zamora, F. (2002). Étude des composés influençant la mousse des Cavas. *Revue Française d'Oenologie, 196*, 30–36.

Vanrell, G., Esteruelas, M., Canals, J. M., & Zamora, F. (2005). Influence du type de calrification du vin de base et des adjuvants de tirage sur la qualité de la mousse des vins effervescents. *Revue des Oenologues, 114*, 28–30.

Vincenzi, S., Mosconi, S., Zoccatelli, G., Pellegrina, C., Veneri, G., Chignola, R., et al. (2005). Development of a new procedure for protein recovery and quantification in wine. *American Journal of Enology and Viticulture, 56*, 182–187.

Waters, E. J., Pellerin, P., & Brillouet, J. M. (1994). A Saccharomyces mannoprotein that protects wine from protein haze. *Carbohidrate Polymers, 23*, 185–191.