EFFECT OF BENTONITE FINING DURING FERMENTATION ON PROTEIN CONTENT IN MACABEU WINES : COMPARISON OF PILOT- AND INDUSTRIAL-SCALE EXPERIMENTS

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Abstract

Aim : This work is aimed to study the effectiveness of the use of bentonite at different stages of the vinification process (pilot and industrial scales) in relation to wine protein stability. The effect of wine storage and ageing on protein content and stability is also studied.

Methods and results : The experimental trials were made with a Macabeu wine (vintage 2011) and included the following treatments : bentonite addition to must only, bentonite addition during fermentation (beginning, middle and end), and no treatment (control). The results show no effect of scale in fermentation kinetics. At both scales, the wines treated with bentonite during fermentation had lower total protein concentrations as compared to the control wines (10-17 %) and the wines obtained from must treated with bentonite (7-14 %), which were the most unstable.

Conclusion : This study shows that the fermenter size (industrial and pilot scale) has no significant effects on alcoholic fermentation, indicating that, from a practical point of view, pilot-scale fermentations satisfactorily reproduce those performed at industrial scale. Moreover, all the wines treated with bentonite during fermentation present a lower protein concentration and a higher stability.

Significance and impact of the study : The results obtained in pilot-scale fermentations are representative of industrial-scale fermentations and therefore can be used reliably to study protein stability and stabilization in white wines.

Key words : wine, bentonite, protein, scale, fining

Résumé

Objectif : Ce travail a pour but d'étudier l'efficacité de l'utilisation de la bentonite sur la stabilité des protéines dans différentes étapes de la vinification (échelles pilotes et industrielles). L'effet du vieillissement et du stockage du vin sur la teneur en protéines et leur stabilité est aussi étudié.

Méthodes et résultats : Les essais expérimentaux ont été réalisés avec un vin de Macabeu (millésime 2011) et comprennent les traitements suivants : ajout de la bentonite au moût, ajout de la bentonite au cours de la fermentation (début, milieu et fin) et traitement sans bentonite (contrôle). Les résultats ne montrent pas d'effet du changement d'échelle sur la cinétique de fermentation. Les vins traités pendant la fermentation, et ce aux deux échelles, avaient une plus faible concentration de protéines totales par rapport aux vins contrôles (10-17 %) et aux vins obtenus à partir de moût clarifié avec la bentonite (7-14 %), qui étaient les plus instables.

Conclusion : Cette étude montre que la taille du fermenteur (échelle industrielle et pilote) n'a aucun effet significatif sur la fermentation alcoolique et que d'un point de vue pratique, les fermentations à échelle pilote reproduisent avec succès les fermentations à échelle industrielle. De plus, les vins traités avec la bentonite pendant la fermentation présentent une plus faible concentration de protéines et une meilleure stabilité.

Signification et impact de l'étude : Les résultats de fermentations obtenus à échelle pilote sont représentatifs des fermentations à échelle industrielle et peuvent ainsi être utilisés de manière fiable dans les études de stabilité des protéines pour les vins blancs.

Mots clés : vin, bentonite, protéine, échelle, collage

manuscript received 27th August 2012 - revised manuscript received 21 January 2013

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J. Int. Sci. Vigne Vin, 2013, **47**, n°3, 221-226

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INTRODUCTION

Protein stability in white wines is a prerequisite for quality, and for this reason, wines are treated prior to bottling in order to avoid subsequent haze formation (Waters *et al.*, 2005). The most widely used technique for achieving this is bentonite treatment, a non-selective method that removes unstable proteins in a discontinuous process. However, bentonite treatments show negative effects such as removal of aromatic molecules or excessive protein removal, which negatively affects the foaming properties of the base wines used to produce sparkling wines (Salazar *et al.*, 2010). The possibility to develop alternative treatments or adjust the technique to avoid these consequences has been largely investigated, without finding any efficient and appropriate method (Waters *et al.*, 2005).

The addition of bentonite is usually done at the end of the alcoholic fermentation, after determining the dose needed to achieve protein stability. However, in certain wineries the bentonite treatment is conducted in two stages, adding a first dose during fermentation and then completing the stabilizing dose at the end of fermentation. This procedure can reduce the total dose that would be required with the usual single-addition treatment and moderate the negative effects that this practice has on wine. Recent works show that the best time for bentonite treatment is during (Pocock *et al.*, 2011) or before (Lambri *et al.*, 2012) fermentation. The experiments made to find the right time for bentonite dosage yielded different results according to various parameters (grape variety, wine type, foamability, aromatic composition, type of bentonite, etc.), hindering the task of establishing a standard protocol of action. For example, Achaerandio *et al.* (2001) point out the importance of ethanol concentration on the adsorption capacity of bentonite.

On the other hand, keeping dry wines in contact with their lees seems to be a good alternative to achieve major, if not total, stability, thereby reducing the dose of bentonite needed before bottling. The definition of wine lees given by EEC regulation No. 337/79 is "the residue that forms at the bottom of recipients containing wine, after fermentation, during storage or after authorized treatments, as well as the residue obtained following the filtration or centrifugation of this product". The effect of keeping the wine on lees on protein stability seems to be due to the presence of yeast mannoproteins, which can be released naturally during fermentation or by autolysis during wine ageing (Pérez-Serradilla and Luque de Castro, 2008). As a matter of fact, mannoproteins have been shown to protect wines from protein precipitation (Moine-Ledoux and Dubourdieu, 1998; Waters *et al.*, 1993; Waters *et al.*, 1994). In this context, Dupin *et al.* (2000) proposed that the mechanism of haze protection may be a competition between mannoproteins and wine proteins for unknown wine component(s), the latter being required for the formation of large insoluble aggregates of denatured proteins. Therefore, as the presence of mannoproteins decreases the available amount of these unknown components, the size of the haze particles decreases and thus visible turbidity declines. Consequently, the combined effect of the presence of bentonite in the lees and that of the action of the mannoproteins released by the yeast could improve the protein stability of wine.

The main objective of this work was to study the effect of the use of bentonite at different stages of the vinification process on wine protein stability and to compare the results obtained at two different scales (industrial and pilot) in order to assess how they affect the bentonite dose required to stabilize the wine once the alcoholic fermentation is finished. The effect during wine storage and ageing on protein content and stability is also discussed.

MATERIALS AND METHODS

1. Vinification process

This study has been done with Macabeu grapes (vintage 2011, Cooperativa de Vila-rodona – Centre Vinícola del Penedès (CEVIPE), Tarragona, Spain) at two scales: industrial (alcoholic fermentation in 50.000-L stainless steel tanks) and pilot (alcoholic fermentation in 100-L stainless steel tanks, in triplicate). The protocol used to perform industrial vinification was the usual process used by the winery. The same process was replicated for the pilot scale at the research cellar of Mas dels Frares of URV (Constanti, Tarragona, Spain). The must obtained was treated with 60 mg/L of potassium metabisulfite before clarification.

Fermentation was performed with a special selection of *Saccharomyces cerevisiae* (CEVIPE) inoculated at 20 g/hL for each tank. Nutrients were added as diammonium phosphate (DAP) at two different times during alcoholic fermentation: at the beginning of fermentation and when the fermenting must density was around 1,050 kg/m^3. The acidity of musts was corrected by adding up to 5.1 g/L of tartaric acid at the beginning of the fermentation. This addition was done considering the initial acidity of the musts of each tank at each scale, even though they were produced from grapes of the same origin.

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2. Bentonite treatment

The bentonite treatments consisted in the addition of bentonite (5 g/hL) at four different times during the vinification process (must clarification, beginning, half and end of fermentation). Control samples without bentonite addition were also prepared. Natural sodium bentonite Microcol® Alpha (Laffort, France) was used in all cases.

3. Post-fermentation treatment

Once alcoholic fermentation was complete (≤2 g/L of residual sugars), 40 mg/L of potassium metabisulfite was added to the wines. Then 24 hours later the SO_2 content was checked and corrected to keep the wines at 35 mg/L of free SO_2.

The dry wines were kept on their lees, which also contained bentonite for those treated during fermentation. The contact time for all wines (including control wine) was 90 days after the end of fermentation. Wines were sulfited and preserved with their lees in a controlled atmosphere to prevent oxidation. After that period, the wines were separated from their lees and bottled. The protein content and stability of the wines was checked after 7 months of bottling.

4. Protein content and wine stability

Total protein concentration was measured by the Bradford method using Coomassie brilliant blue reagent and checking the absorbance at 595 nm on a spectrophotometer (Cecil CE2021, England) after 5 min of incubation (Bradford, 1976). The protein content was expressed as mg/L of bovine serum albumin (Sigma, cat. no. A-3803). All analyses were done in triplicate.

Wine protein stability was determined as described by Salazar *et al.* (2007). Briefly, a 20-mL wine sample was filtered through a 0.45-µm (pore size) cellulose nitrate membrane (Whatman, cat. no. 7184009, England), heated for 2 hours at 80 ºC in a bath equipped with a digital control immersion thermostat (Digiterm 100), then incubated for 2 hours at 4 ºC. Turbidity was measured by nephelometry (Turbiquant 1000 IR turbidimeter, Merck KGaA, Germany) and expressed in nephelometric turbidity units (NTU). The difference in turbidity before and after the thermal test (ΔNTU) is proportional to protein instability. The wines were considered stable if this difference did not exceed 2 NTU (Moine-Ledoux & Dubourdieu, 1999). All analyses were carried out in triplicate.

5. Chemical analysis of musts and wines

Chemical analyses of musts and wines were done following the official methodology of analysis proposed by the International Organization of Vine and Wine (OIV) using infrared spectroscopy (WineScan FT120 Basic, Foss, Denmark). Total and volatile acidity and pH were determined by Fourier Transform Infrared (FTIR) spectroscopy using a Foss and Flexible Foss Integrator Software platform, a liquid flow system and a 0.4-µm calcium fluoride cuvette (Foss, Foss Electric España, S.A.) to generate the FTIR spectra. The calibrations provided with the equipment allowed the immediate analysis of pH and volatile acidity, following the resolution OIV/OENO 390/2010. The samples were automatically thermostated at 20 ºC in the spectrometer before analysis. The IR spectrum was scanned between 2.000 nm and 10.000 nm (NIR and MIR). Samples were analyzed in triplicate.

RESULTS AND DISCUSSION

1. Alcoholic fermentation

Alcoholic fermentation was carried out following the traditional procedure of Cooperativa Vila-rodona for industrial scale and following the same protocol for pilot scale at Mas dels Frares.

The mean values of the analytical characteristics of the musts used in this study are as follows: density 1074 ± 1 kg/m^3, total acidity 3.85 ± 0.22 g tartaric acid/L, pH 3.38 ± 0.01, total SO_2 35 ± 9 mg/L, free SO_2 11 ± 3 mg/L, gluconic acid 0.31 ± 0.05 g/L, and expected alcoholic content 10.3 ± 0.1 % v/v.

Figure 1 shows the fermentation kinetics of all experiments. No scale effect was observed. These results are in agreement with Aguera and Sablayrolles (2005), who described that pilot-scale fermentations in 100-L tanks are similar to industrial fermentations. Regarding the effect of the bentonite treatment, it cannot be observed a significant effect, since it could be affected by the presence of bentonite in suspension (Ferrando *et al.*, 1998; Casalta *et al.*, 2010). However, all initial musts have been clarified by static decantation, and the level of suspended solids was similar. The small variations observed were mainly due to the fluctuations of the fermentation temperature.

The fermentation times were around 15 days in all cases, and the fermentation temperatures were maintained in the range of 15 – 21 °C (as shown in Figure 1).

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The analytical parameters of the dry wines are shown in Table 1. The mean values of the wines were: alcohol content of 10.44 ± 0.38 % v/v in pilot scale and 10.68 ± 0.34 % v/v in industrial scale, total acidity of 4.9 ± 0.5 g/L in pilot scale and 4.0 ± 0.4 g/L in industrial scale, volatile acidity of 0.43 ± 0.12 g/L in pilot scale and 0.24 ± 0.06 g/L in industrial scale, residual sugar content of 2.5 ± 0.1 g/L in pilot scale and 2.2 ± 0.2 g/L in industrial scale, and pH of 3.17 ± 0.06 in pilot scale and 3.13 ± 0.09 in industrial scale. The differences found for total acidity between the pilot- and industrial-scale wines are probably due to different rates of heat transfer into the whole mass, leading to different extents of potassium bitartrate precipitation. One possible explanation for the higher volatile acidity values detected in the pilot-scale wines would be their lower volume/surface ratio, resulting in an increased contact with the air oxygen and therefore a higher acetic acid production.

2. Protein concentration and wine stability

The results of the total protein concentration measured in wines at the end of fermentation, after 1 and 3 months of ageing on lees and after 7 months of bottling are shown in Table 2.

At both the industrial and pilot scales, the wines treated with bentonite during fermentation had lower total protein concentration as compared to both the control wines and the wines obtained from bentonite-clarified must, which were also the most unstable, as determined by the heat test (highest ΔNTU); see Table 2). All wines were slightly unstable, in spite of the low protein concentration typical of Macabeu wines, whose usual destination is sparkling base wine

Table 1 - Chemical analysis of the wines produced with addition of 5 g/hL of bentonite at different times of winemaking. Results obtained at the industrial and pilot scale are compared.

Dosing time	Scale	Alcohol content (% vol)	Volatile acidity (g/L)	Total acidity (g H_2SO_4/L)	pH	Residual sugars (g/L)
Must clarification	Industrial	11.25 ± 0.01	0.26 ± 0.02	3.81 ± 0.02	3.13 ± 0.02	2.39 ± 0.05
	Pilot	11.05 ± 0.06	0.35 ± 0.02	4.94 ± 0.03	3.15 ± 0.01	2.67 ± 0.14
Beginning fermentation	Industrial	10.62 ± 0.02	0.22 ± 0.02	3.49 ± 0.02	3.28 ± 0.02	2.05 ± 0.15
	Pilot	10.33 ± 0.05	0.61 ± 0.04	4.09 ± 0.01	3.31 ± 0.03	2.41 ± 0.05
Half fermentation	Industrial	10.57 ± 0.02	0.18 ± 0.03	4.38 ± 0.02	3.05 ± 0.03	2.27 ± 0.04
	Pilot	10.43 ± 0.03	0.52 ± 0.05	5.17 ± 0.05	3.13 ± 0.04	2.44 ± 0.08
End fermentation	Industrial	10.62 ± 0.02	0.33 ± 0.02	4.38 ± 0.04	3.05 ± 0.00	2.44 ± 0.06
	Pilot	10.40 ± 0.02	0.33 ± 0.03	5.09 ± 0.01	3.15 ± 0.02	2.35 ± 0.03
Control	Industrial	10.32 ± 0.01	0.21 ± 0.01	3.78 ± 0.01	3.12 ± 0.01	2.08 ± 0.02
	Pilot	10.00 ± 0.03	0.36 ± 0.06	5.13 ± 0.10	3.11 ± 0.03	2.55 ± 0.12

Table 2. Evolution of protein concentration (mg/L) in wines produced with addition of 5 g/hL of bentonite at different times of winemaking (* indicates unstable wine). Results obtained at the industrial and pilot scale are compared.

Dosing time	Scale	End Fermentation (Day 0)	Turbidity ΔNTU	1 Month Later (Day 30)	3 Months Later (Day 90)	7 Months after bottling
Must clarification	Pilot	40.2 ± 0.2*	6.5 ± 2.0	34.4 ± 0.3	32.2 ± 0.5	30.2 ± 0.2
	Industrial	42.4 ± 0.3*	9.5 ± 1.6	36.8 ± 0.2	37.0 ± 0.4	29.7 ± 0.0
Beginning fermentation	Pilot	36.5 ± 0.5*	2.4 ± 0.0	35.3 ± 0.6	33.1 ± 0.1	29.5 ± 0.2
	Industrial	39.5 ± 0.5*	4.8 ± 1.9	38.6 ± 0.7	37.2 ± 0.6	31.6 ± 0.1
Half fermentation	Pilot	36.6 ± 0.5*	6.1 ± 2.1	34.5 ± 0.3	30.7 ± 1.2	30.3 ± 0.1
	Industrial	36.6 ± 0.4*	2.3 ± 0.1	34.7 ± 0.3	35.6 ± 0.7	31.1 ± 0.2
End fermentation	Pilot	37.0 ± 0.7*	3.8 ± 0.9	35.9 ± 1.0	32.4 ± 0.1	30.2 ± 0.3
	Industrial	37.1 ± 0.5*	3.7 ± 0.0	34.5 ± 0.2	31.3 ± 0.3	29.0 ± 0.1
Control	Pilot	40.9 ± 0.2*	5.9 ± 0.3	35.0 ± 0.6	31.4 ± 0.4	29.5 ± 0.3
	Industrial	44.1 ± 0.0*	8.9 ± 0.1	38.2 ± 0.2	36.2 ± 0.9	28.9 ± 0.0

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production (Salazar *et al.*, 2006). After fermentation, the protein concentrations of the wines decreased with time in all cases, with a reduction in the first month of around 5 % and 13 % in the wines treated during fermentation and the wines obtained with control and clarified must, respectively. However, the protein concentrations were similar in all cases, ranging from about 34 to 39 mg/L. All wines were stable (ΔNTU<2, mean of all wines 1.3 ± 0.3) after 1 month without any additional treatment, even in the case of the control wines, where no bentonite was applied throughout the process. This decrease has been also observed during the post-fermentation period in Manzoni Bianco wine (Vincenzi *et al.*, 2011). This fact may be partially explained by protein insolubilization and precipitation, although the activity of proteolytic enzymes released from yeast after the end of the fermentation cannot be excluded.

Wine ageing on lees produces an enrichment of mannoproteins, contributing to a major stability and probably the maintenance of wine quality after bottling (Rowe *et al.*, 2010). As a matter of fact, all the wines showed a further slight improvement of protein stability after 3 months of ageing on lees, showing more stable protein (i. e., lower ΔNTU values) after 3 months of ageing (ΔNTU<2, mean of all wines 1.1 ± 0.2) compared to after 1 month, as determined by the heat test. A slight decrease in protein content and complete protein stability (ΔNTU=0) was also observed in all wines after 7 months of bottling.

CONCLUSION

This study shows that the fermenter size (industrial and pilot scale) has no significant effect on the kinetics of alcoholic fermentation, and therefore, from a practical point of view, pilot-scale fermentations satisfactorily reproduce industrial-scale fermentations.

The wines treated with bentonite during the fermentation process present a lower protein concentration. Nevertheless, in wines with low protein concentrations, as those made from Macabeu grapes, and under the vinification conditions used here, a greater and more extensive ageing on lees could be recommended as an alternative to additional treatment with bentonite, as demonstrated by the complete protein stability achieved in the bentonite-free wines.

Further work is however required to confirm the results reported here for these wines

Acknowledgements : Eugenio Lira is grateful for the financial support provided by the Universitat Rovira i Virgili (predoctoral fellowship).

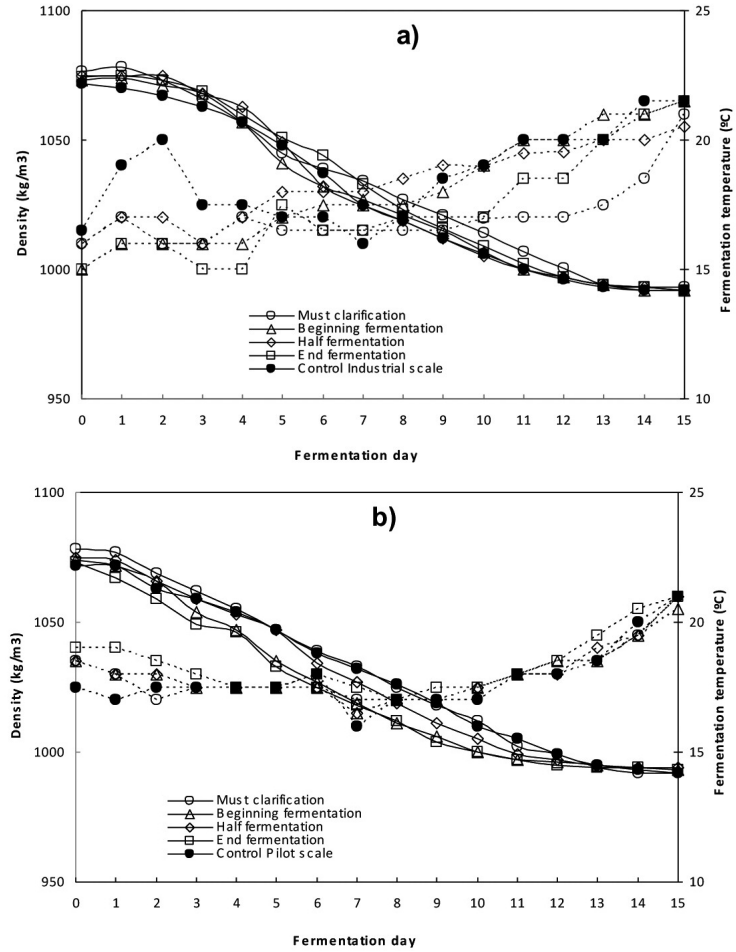

Figure 1. Evolution of must density and fermentation temperature during alcoholic fermentation at industrial scale (a) and pilot scale (b). 5 g/hL of bentonite were added at the winemaking steps indicated in the figure.

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