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Comparative Study of Protein Stabilization in White Wine Using Zirconia and Bentonite: Physicochemical and Wine Sensory Analysis

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A semi-industrial application of the continuous stabilization of white wine protein using a column packed with zirconia was studied and compared to the traditional bentonite treatment using a Macabeu white wine. Physicochemical and wine sensory properties were evaluated using a rating system and triangle tests. Continuous protein stabilization was analyzed in three residence times, and the equivalent of 300 BV of wine was used for both treatments. Wine protein content was reduced by 21%, 40%, and 42% using the continuous process with residence times of 7.5, 15, and 30 min, respectively, and by 61.4% using the bentonite treatment. The wines obtained from the packed column were protein stable up to 25, 75, and 175 BV for residence times of 7.5, 15, and 30 min, respectively. The amount of polyphenol removed was less than 10%, and similar amounts were removed from the wine regardless of residence time, while 20.6% of polyphenol was removed using bentonite. The physicochemical and sensory properties of wine treated with bentonite were similar to those of wine treated with zirconia.

KEYWORDS: Unstable wine protein; wine sensory analysis; adsorption; bentonite; zirconia

INTRODUCTION

One of the major factors that influences the quality of white wine is its protein stability. In the winemaking process, proteins are stabilized because wine proteins are adsorbed by bentonite. However, the selectivity of these discontinuous processes is low and their environmental impact high. This affects the quality of the wine and also means that some products are lost. For all of these reasons, it is desirable to develop new alternatives, which are economically viable and maintain wine quality (*1*).

The stabilization of wine protein by bentonite is a discontinuous process, which takes some considerable time during the preparation and the gravity-settling steps. In winemaking, bentonite must be completely hydrated before it is added to wine, and the dose must be appropriate to prevent harming the wine's organoleptic properties (*2*, *3*).

In previous laboratory-scale studies, we have demonstrated that the continuous stabilization of white wines (Chardonnay and Muscat) is viable by means of a packed column using zirconia as the adsorbent material (*4*, *5*). Furthermore, the environmental impact of this continuous stabilization by zirconia is lower than that of the usual bentonite treatment because the chemical and mechanical resistance of this material (among its other physical properties) enables it to regenerate (*4*−*7*). However, the effect of this new continuous process on wine sensory properties has still not been demonstrated, nor do we know what differences, if any, there are between the results obtained using this process and those obtained from the conventional bentonite method of wine protein stabilization.

Therefore, we were interested in comparing the new continuous stabilization of wine protein using zirconia with the conventional bentonite treatment in terms of its physicochemical and wine sensory properties. We were also interested in determining the operating conditions required to achieve protein-stable wines without affecting wine quality.

MATERIALS AND METHODS

Wine Samples. Samples of Monovarietal Macabeu white wine were obtained from the Mas dels Frares winery (the 2005 vintage, Tarragona, Spain). This wine was produced with must clarified by settling. The fermentation was controlled at 18 °C on an industrial scale, and the wine samples were used immediately after fermentation with no additional treatment.

Physicochemical Properties of the Wine. The physical and chemical properties were determined by an infrared technique using WineScan FT120 Basic (Foss, Denmark). The total protein and polyphenol content was determined by Bradford's and Folin's methods, respectively.

Stabilization of Wine Protein Using Zirconia and Bentonite. Wine protein stability was studied using a Turbiquant 1000IR turbidimeter (Merck KGaA, Germany) and a thermal test described by Moine-Ledoux & Dubourdieu, 1999 (*8*). The difference in turbidity between the initial wine and the wine after the thermal test was proportional to

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protein instability. The wines were considered stable if this difference did not exceed 2 NTU.

The wine proteins were stabilized by means of a continuous process using zirconium oxide packed in a fixed bed column and through a discontinuous process by sodium bentonite (Laffort, France). In both treatments, the volume of the wine treated was 25 L.

The treatment with bentonite included a preliminary test that used a variety of different doses (5, 10, 20, 30, and 40 g/hL) so that the most appropriate dose could be determined.

The continuous adsorption process was carried out in a 165 mm-high packed column with an internal diameter of 40 mm using 100 g of granulated zirconia with a particle size of 1−2 mm (Saint-Gobain, USA). The wine was pumped by up-flow mode through the column using a peristaltic pump (Watson Marlow 101 U/R, UK). The volume of wine treated was equivalent to 300 BV, and three residence times were considered (7.5, 15, and 30 min). After each treatment, the adsorbent material was regenerated at 500 °C for 12 h.

Physical Properties of Zirconia. The surface properties of zirconia were studied with Brunauer−Emmett−Teller (BET) model adsorption with liquid N_2 using a surface analyzer (Micromeritics ASAP 2000, USA) and assuming a cross-sectional area of 0.162 nm^2 for nitrogen. Before the adsorption measurements were taken, the samples were outgassed under a vacuum of 0.001 mbar at 120 °C. The morphology of the material was studied by X-ray diffraction (XRD) using a nickel-filtered Cu $K\alpha_1$ radiation ($\lambda = 1.5418$ Å) in the 2θ range 10−70° through a SIEMENS D5000 diffractometer.

Cold Stabilization and Microfiltration of the Wine. Once the wine protein stabilization treatments had concluded, samples of the wine were taken to evaluate its tartaric stability using conductivity measurements at a temperature near 0 °C and Boulton's test (*9*) using a Crison CM35 conductimeter (Crison Instruments, Barcelona, Spain).

All wines were treated with 4 g/L of potassium hydrogen tartrate (KHT) and stored for 2−3 weeks at 5 °C until tartaric stability was achieved. Finally, all of the wines were filtered by tangential microfiltration using a ceramic membrane of zirconium oxide with a pore size of 0.45 μm (Tami, France). Filtration was effected at room temperature with a transmembrane pressure of 150 kPa and a tangential velocity of 2 m/s.

Wine Sensory Analysis. Macabeu wine treated with zirconia and bentonite was subjected to sensory evaluation by means of a triangle test in accordance with ISO 4120:2004 (*10*). Additionally, both nontreated and treated wine was tested using rating system tests according to Office International de la Vigne et du Vin. Resolution ENO 2/94. OIV standard for the international wine competitions (*11*). Both tests were performed in a wine tasting room in compliance with standard NF V09-105 AFNOR (*12*). The triangle test was conducted by 17 untrained wine tasters involved in enology research, and the rating system test was conducted by a panel of six experienced wine tasters.

The main objective of the triangle test was to determine any significant overall differences between wine samples treated with zirconia and those treated with bentonite. To this end, we prepared four individual sensory triangle tests of the treated wine. These individual triangle tests were carried out using random formation and placement.

The sensory rating system test was carried out on nontreated and treated wines to confirm the results of the first test and to determine which wine protein stabilization treatment rated higher among experienced tasters. The wine samples were placed in random formation, and the tasters performed the sensory test in no predetermined order.

RESULTS AND DISCUSSION

Physical Properties of Zirconia. The results found using the BET method showed that zirconia has a BET surface area of 164 m^2/g, that its average pore diameter is 44 nm, and that it is mainly a mesoporous material. According to X-ray diffraction, the zirconia has tetragonal morphology. The material was crushed into small 1−2 mm particles to increase its contact surface area during the continuous adsorption process of the wine protein.

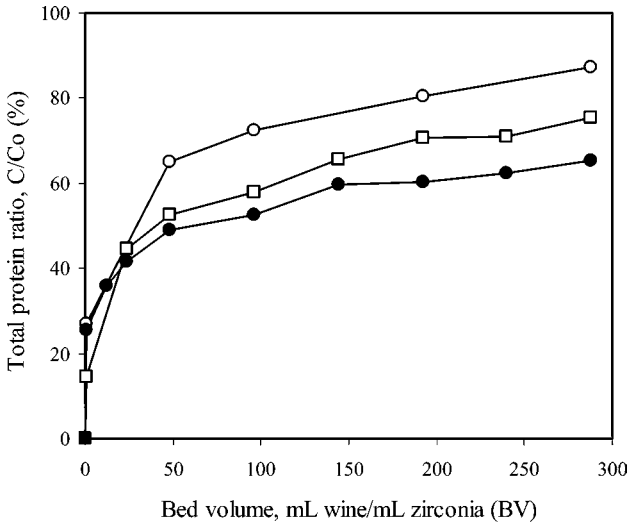

Figure 1. Total protein adsorbed for different residence times during continuous wine protein stabilization (○, residence time of 7.5 min; □, residence time of 15 min; ●, residence time of 30 min).

Stabilization of Wine Protein Using Zirconia and Bentonite. In this study, the wines treated presented a low total protein concentration of 17.92 ± 0.87 mg/L BSA. However, all of the samples were unstable in terms of protein, with a turbidity of 11.94 ± 1.86 NTU.

We tested five different doses of bentonite in the Macabeu wine (5, 10, 20, 30, and 40 g/hL) in an attempt to determine the minimum dose that stabilized the wine protein. It is important to use a minimum dose so that the quality of the wines studied is changed also minimally. The best results were obtained with a dose of 20 g/hL.

Figure 1 shows the effect of residence times on the amount of protein adsorbed during the continuous protein stabilization of Macabeu wine. The data show that for the first accumulated 25 BV there is no difference in the amount of protein adsorbed. However, above these bed volumes, the total protein adsorbed depends considerably on the residence time, and the protein is reduced by 21%, 40%, and 42% for residence times of 7.5, 15, and 30 min, respectively, and by 61.4% using the bentonite treatment.

The results of the wines treated with zirconia shown in **Tables 1**and **2** are of the accumulated 300 BV.

The total polyphenol content in the nontreated wines was 219 ± 11 mg/L gallic acid, and the amount of polyphenol adsorbed through the treatment with zirconia was less than 10% and similar for the three residence times and 20.6% in the bentonite treatment (**Table 1**). Therefore, according to these results, zirconia has greater selectivity for wine protein adsorption than polyphenol compounds, as reported by Salazar et al., 2006 (*6*).

Zirconia adsorbed a smaller amount of polyphenols from Macabeu wine than the bentonite treatment. Therefore, stabilization of wine protein using zirconia results in stable wines and does not greatly affect their polyphenol content (**Table 1**).

The wine obtained at the exit of packed column can be considered protein stable up to 25, 75, and 175 BV for residence times of 7.5, 15, and 30 min, respectively (**Figure 2**). However, when we analyzed the accumulated volume of the wine (equivalent to 300 BV), the turbidity values were lower and in some cases just slightly higher than 2 NTU for residence times of 15 and 30 min. Therefore, these wines are practically stable.

It should also be pointed out that, from an economic perspective, industrial-scale processes should consider a residence time of 15 min, as wine protein stabilities were similar

Table 1. Total Protein and Polyphenol Content in the Wine after Protein Stabilization and Bottling[a]

	total protein (mg/L BSA)		total polyphenol (mg/L gallic acid)		protein stabilization (ΔNTU)	
wine condition	AA	AB	AA	AB	AA	AB
zirconia/ residence time						
7.5 min	13.73 ± 0.18	11.08 ± 0.11	206 ± 1	203 ± 2	4.50 ± 0.46	0.95 ± 0.12
15 min	11.28 ± 0.21	10.31 ± 0.11	198 ± 1	195 ± 1	2.28 ± 0.31	0.65 ± 0.18
30 min	10.03 ± 0.33	9.14 ± 0.06	201 ± 1	197 ± 1	1.10 ± 0.33	0.49 ± 0.11
bentonite (20 g/hL)	8.45 ± 0.07	7.58 ± 0.22	174 ± 2	170 ± 2	0.20 ± 0.10	0.16 ± 0.05

[a] All of the values are presented as means and standard deviation of at least two independent experiments. AA, wine after protein stabilization treatment; AB, wine after being bottled (wine cold stabilized, filtered, and bottled).

Table 2. Physicochemical Properties of Nontreated and Bottled Wines[a]

		zirconia residence time (min)			
parameter	nontreated wine	7.5	15	30	bentonite dose (20 g/hL)
pH	3.22 ± 0.07	3.17 ± 0.00	3.18 ± 0.01	3.19 ± 0.01	3.17 ± 0.01
total acidity, g/L tartaric acid	3.77 ± 0.02	3.53 ± 0.01	3.56 ± 0.04	3.49 ± 0.00	3.52 ± 0.01
volatile acidity, g/L acetic acid	0.26 ± 0.02	0.19 ± 0.01	0.18 ± 0.01	0.20 ± 0.00	0.19 ± 0.01
total dry extract, g/L	16.10 ± 1.39	16.20 ± 0.14	16.15 ± 0.42	15.70 ± 0.28	15.78 ± 0.13
sugar reducers, g/L	1.33 ± 0.25	2.03 ± 0.05	1.82 ± 0.04	1.96 ± 0.21	2.12 ± 0.11
glycerol, g/L	7.06 ± 0.75	6.35 ± 0.07	6.48 ± 0.10	6.35 ± 0.07	6.30 ± 0.00
gluconic acid, g/L	0.36 ± 0.03	0.38 ± 0.00	0.38 ± 0.01	0.37 ± 0.00	0.38 ± 0.01
malic acid, g/L	1.01 ± 0.13	0.98 ± 0.01	0.95 ± 0.02	0.94 ± 0.01	0.94 ± 0.01
tartaric acid, g/L	3.32 ± 1.14	1.89 ± 0.00	1.89 ± 0.04	1.96 ± 0.01	1.92 ± 0.05
absorbance at 420 nm	0.060 ± 0.004	0.064 ± 0.001	0.062 ± 0.002	0.042 ± 0.000	0.041 ± 0.003

[a] All of the values are presented as means and standard deviation of at least two independent experiments and correspond to results of nontreated and bottled wines.

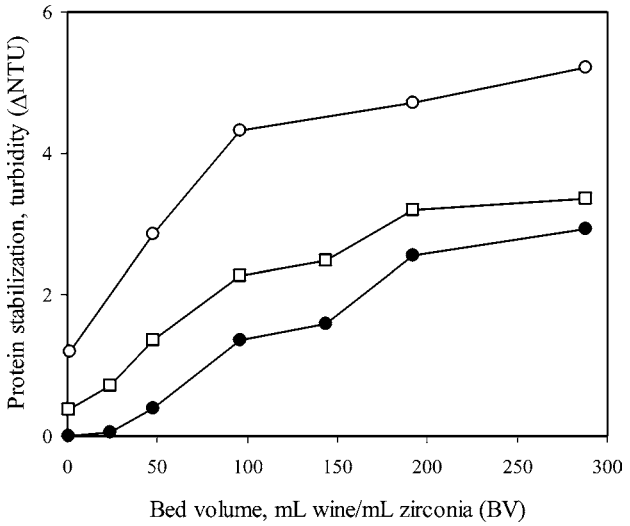

Figure 2. Wine protein stability for different residence times during the continuous wine protein stabilization (○, residence time of 7.5 min; □, residence time of 15 min; ●, residence time of 30 min).

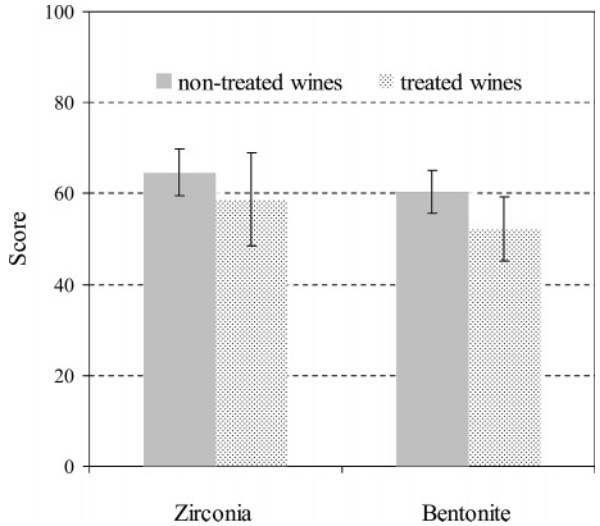

Figure 3. Results of the sensory rating system test for the wines (a) before treatment with zirconia (retention time 15 min) and bentonite, and (b) after treatment (protein and cold stabilized, and microfiltrated) and bottling.

at residence times of 15 and 30 min. Therefore, a residence time of 15 min may be sufficient for the protein stabilization of 300 BV of Macabeu wine on an industrial scale using zirconia. Furthermore, a subsequent clarification by crossflow microfiltration may improve wine protein stabilization, as is shown in **Table 1** and in accordance with that reported by Salazar et al., 2006 (*6*).

The amount of zirconia required to treat considerable volumes of wine during the continuous stabilization of wine protein

indicates that this process may be promising for use on an industrial scale.

Table 2 shows that the physicochemical properties of Macabeu wine were not greatly affected by either treatment, in agreement with previous results from other wines (*4*−*6*). The one exception was the absorbance at 420 nm in both wines treated with the continuous process for a residence time of 30 min and those treated with bentonite.

Wine Sensory Analysis. The untrained wine tasters were not able to distinguish significant differences between the wines treated with bentonite and those treated with zirconia using a triangle test ($p < 0.005$).

Figure 3 illustrates the results obtained from the sensory rating system test. The nontreated wines showed better scores than the wines treated with either bentonite or zirconia. This could be attributable to a loss of some aroma compounds in the wine, occurring along with the protein stabilization, tartaric stabilization, or wine microfiltration processes.

In conclusion, there were no significant sensory differences between the wines treated with zirconia and those treated with bentonite. However, the wines stabilized with zirconia were scored slightly better than wines treated with bentonite according to the results of the rating system test.

ABBREVIATIONS

BV, bed volume (volumetric ratio between the wine treated and the zirconia used at process time determined); NTU, nephelometric turbidity units; ΔNTU, difference of nephelometric turbidity units between the wine before and after thermal test applied.

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