Protein stabilisation of white wines using zirconium dioxide enclosed in a metallic cage

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Abstract

Backgrounds and Aims: White wines are stabilised by removing the heat unstable proteins through adsorption by bentonite. Bentonite fining is not an efficient wine processing step and can also remove other wine components. Alternative absorbents are thus sought; zirconium dioxide (zirconia) is recognised as a promising candidate. The aim of this work was to assess the viability of zirconia treatments to stabilise white wines, with particular attention on process development.

Methods and Results: Effective treatment was achieved by enclosing zirconia pellets into a metallic cage submerged in the wine. With this method, the wine could be treated with the adsorbent for the time required for protein stabilisation, and then removed without further manipulation. Zirconia treatments of three unstable wines partially or fully stabilised them without detectable modifications of their physicochemical parameters and colours, apart from the removal of metals and some acids, particularly when wines were treated for long times and with high dosages of the adsorbent. A simple and inexpensive zirconia regeneration method was also developed.

Conclusions: The zirconia application to wine was very effective in removing proteins, and the proposed regeneration procedure could facilitate the uptake and development of zirconia-based solutions for the wine industry.

Significance of the Study: This study confirmed the effectiveness of zirconia in removing wine proteins and demonstrated that the proposed method of application has the potential to become a viable alternative to bentonite.

Abbreviations

CIELAB Commission Internationale de l'Eclairage Lab transmission values L* a* b*; **MW** molecular weight; **NTU** nephelometric turbidity unit; **PR** pathogenesis related; **TCA** trichloroacetic acid; **TL protein** thaumatin-like protein

Keywords: *zirconium dioxide*, *protein*, *wine*, *haze*, *Sauvignon Blanc*, *Riesling*, *Chardonnay*

Introduction

The presence of residual proteins from grapes in finished white wines is undesirable because of their key role in causing haze during wine storage (Bayly and Berg 1967). Since the 1930s, bentonite (a clay cation exchanger) has been used widely in oenology as a fining agent, to stabilise wines by protein adsorption (Saywell 1934). Bentonite is still extensively used because of its established efficacy as well as its low cost. However, bentonite fining has some drawbacks such as a significant wine volume loss (3–10%) because of poor settling (Waters et al. 2005). Other bentonite-associated costs include waste disposal, occupational health and safety issues, and interference with increasingly common membrane-based winemaking technologies (Waters et al. 2005, Salazar et al. 2007). Moreover, bentonite is not a specific adsorbent and may reduce both undesirable and desirable compounds such as aroma, flavour and anthocyanin compounds (Miller et al. 1985, Voilley et al. 1990, Lubbers et al. 1993, Ribéreau-Gayon et al. 2000). For these reasons, alternative procedures for protein removal from white wine have been extensively investigated, including other adsorbents (Sarmento et al. 2000, Cabello-Pasini et al. 2005, Vincenzi et al. 2005, de Bruijn et al. 2009a,b), use of immobilised tannic acid (Weetall et al. 1984) or proanthocyanidins (Powers et al. 1988), ultrafiltration (Hsu et al. 1987), and proteases (Waters et al. 1992), but no alternative has proven sufficiently cost-effective to date.

One promising solution is represented by the adsorption of unstable proteins on the surface of zirconium oxide (Pachova et al. 2002, Pashova et al. 2004a,b, Salazar et al. 2006, 2007). Zirconium dioxide, a metal oxide commonly known as zirconia, is a material characterised by low corrosion potential, low thermal conductivity, hardness, and high thermal and mechanical resistances (Piconi and Maccauro 1999, Liu et al. 2005, Manicone et al. 2007). Because of its features, it has many applications such as a catalyst or support material, refractory material, ceramic material and biomechanical support in medical implants (Stichert and Schuth 1998, Piconi and Maccauro 1999, Rovira-Bru et al. 2001, Chevalier 2006, Mallick et al. 2006). Despite very promising results obtained with both batch addition and continuous systems for wine stabilisation treatments (Pachova et al. 2002, Pashova et al. 2004a,b, Salazar et al. 2006, 2007), fining with zirconia has not been developed into a commercial process or used commercially, and its ability to be a bentonite substitute for wines with high protein concentrations has not been established.

The aim of this work was to confirm the ability of zirconium dioxide to protein stabilise white wines, to test the impact of this

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treatment on wine quality and to develop an alternative method of its application that would be feasible as common winemaking practice.

Materials and methods

Wine samples

Three unfined wines (Chardonnay, Riesling and Semillon) from the 2007 vintage from Adelaide Hills (South Australia) were used in preliminary experiments undertaken in 2009. A further three unfined wines (Chardonnay, Riesling and Sauvignon Blanc) from the 2009 vintage from South Eastern Australia were used in the larger scale experiments in 2009. All wines were donated by commercial producers, were made using standard winemaking practices and on a commercial scale, and were stored below 10°C before the experiments were undertaken.

Materials

The zirconia used was originally in pellet form (Saint-Gobain NorPro, Staw, Ohio, USA) and was donated by Prof Francisco López. Zirconia pellets were small disks with a diameter of 3 mm and a thickness of 1 mm, a pore size of 6.2 nm, a surface area of 108.5 m^2/g and with tetragonal morphology (Salazar 2007). Zirconia powder was obtained by grinding the pellets with a mortar and pestle. The bentonite used was a sodium-calcium bentonite, Nacalit (Erbslöh, Geisenheim, Germany). Bentonite stock was prepared at 50 g/L in water, at least 24 h before use. The material used to prepare the zirconia bags was Miracloth (Calbiochem, Los Angeles, California, USA).

Protein High-Performance Liquid Chromatography (HPLC)

Protein concentration and composition was determined by reverse-phase HPLC with a Vydac 2.1 × 250 mm C8 column (208TP52 Grace Davison Discovery Sciences, Baulkham Hills, New South Wales, Australia) on an Agilent Technologies 1200 system (Santa Clara, California, USA) according to the method of Marangon et al. (2009) with modifications as suggested by Van Sluyter et al. (2009). Injection volumes were 25 µL. From the 210 nm chromatogram, protein identity was assigned by comparison with retention times of purified grape pathogenesis related (PR) proteins (Marangon et al. 2009, Van Sluyter et al. 2009) as follows: peaks with a retention time between 12 to 16 min were assigned to the thaumatin-like (TL) protein class, whereas peaks eluted from 24 to 28 min were classified as chitinases.

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

SDS-PAGE was performed with NuPage 4–12% Bis-tris, 1.5-mm thick, 15-well gels (Invitrogen, Mt Waverley, Victoria, Australia) and a XCell SureLock Mini Cell (Invitrogen) following the manufacturer's instructions. Approximately 50 mg of $Na_2S_2O_5$ were added to the top reservoir prior to running to prevent cysteine oxidation. Samples were prepared by precipitating proteins from 100 µL of wine with two volumes of cold trichloroacetic acid (TCA) (10%) in acetone. After incubating overnight at −20°C, samples were centrifuged (13 000 × *g*, 15 min, 0°C) and the pellet washed with 80% acetone to remove the TCA. After a second wash with 80% acetone (13 000 × *g*, 15 min, 0°C) pellets were dissolved in 40 µL of loading buffer (Invitrogen NuPage recipe) with 5% (v/v) 2-mercaptoethanol and boiled for 5 min. Precision Plus Protein unstained standards were from Bio-Rad (Bio-Rad Laboratories Pty.Ltd, Regents Park, New South Wales, Australia). Proteins were stained with Pierce Imperial Protein Stain (Quantum Scientific, Sydney, New South Wales, Australia) according to the manufacturer's microwave instructions with an extended incubation in the stain to increase sensitivity.

Protein content determination

Protein content was determined by EZQ® protein quantitation kit (Invitrogen) following the manufacturer's instructions. The calibration curve was built using serial dilution from 0 to 250 mg/L of thaumatin from *Thaumatococcus daniellii* (Sigma-Aldrich, Castle Hill, New South Wales, Australia). Fluorescence measurements were taken using excitation/emission settings of 485/590 nm with a SpectraMax M2 microplate reader (Molecular Devices, Sunnyvale, California, USA).

Heat test

Wines were heated at 80°C for 2 h and cooled in ice for 2 h. After equilibration at ambient temperature, the haze was measured by calculating the difference in the absorbance values at 540 nm (Waters et al. 1992) or in a nephelometric turbidity unit (NTU) by means of a nephelometer (Pocock and Rankine 1973) between the heated and unheated samples.

Analytical methods

Alcohol, specific gravity, pH, titratable acidity, glucose/fructose and volatile acidity analysis were performed by the Commercial Service of The Australian Wine Research Institute using a Foss WineScan FT 120 as described by the manufacturer (Foss, Hillerød, Denmark). Free and total SO_2 were measured by the aspiration method (Rankine and Pocock 1970).

Organic acids by HPLC

The concentration of organic acids (citric, tartaric, malic, succinic and lactic) was determined by HPLC using an Aminex column (HPX-87H, 300 × 7.8 mm, Bio-Rad) fitted on an Agilent 1200 series quaternary pump LC (Agilent Technologies). The column was eluted at 65°C in isocratic mode with 5 mM H_2SO_4 at a flow rate of 0.5 mL/min. Samples were diluted 5-fold to a final content of 1.25% (v/v) isopropanol and 1 mM sulphuric acid, and centrifuged at 13 000 × *g* for 15 min at 4°C. The supernatant was held in an Agilent 1200 series High Performance temperature-controlled auto-sampler at 4°C prior to injection. Injection volumes were 10 µL. Organic acids were detected by absorbance at 210 nm by an Agilent 1200 series diode-array Detector.

Colour analyses

Wine colour was assessed by the tristimulus method CIELAB as described by Kwiatkowski et al. (2007).

Metal analysis

Metal contents in wines were determined by inductively coupled plasma – optical emission spectrometry performed by the Waite Analytical Services (Glen Osmond, South Australia, Australia http://www.adelaide.edu.au/was/), School of Agriculture, Food and Wine, University of Adelaide.

Experimental design for large scale experiments

Zirconia pellets were applied to wine samples in a stainless steel, 4 cm diameter tea infuser ball. Each of the three wines was treated with the infuser cages containing four doses of zirconia in pellet form (0, 5, 10, 25 g/L). The experiment was conducted at 18°C. Stirring with a magnetic bar (at 140 rpm) was applied throughout the experiment. A polypropylene container of 450 mL volume (Sarstedt, Nümbrecht, Germany) previously

tested for its relative non-permeability to oxygen for the duration of the experiment was filled with 430 mL of wine. The system was sealed with a high density polyethylene screw cap (Sarstedt) and Parafilm. Two syringe needles were inserted into the lid to allow both the sampling and maintenance of N_2 ullage (by means of a balloon). Each experiment was performed in triplicate, and analyses for each replicate were performed in triplicate except where indicated.

Regeneration experiment

Zirconia was regenerated by washing the pellets (enclosed in the metallic cage) twice with 3 M NaOH at 50°C for 2 h and then with 5% citric acid at room temperature for 30 min. The pellets in the cage were then rinsed three times in distilled water before use. An aliquot (100 mL) of 2009 Chardonnay was treated for 24 h with 25 g/L of zirconia pellets under the same conditions as described above. At the end of each treatment the pellets were regenerated and the procedure repeated.

Sensory assessment

An informal tasting was carried out to provide an indication of differences in aroma and flavour among the treatments and to highlight possible faults from the treatment. Six Australian Wine Research Institute (AWRI) tasters with extensive experience in wine sensory assessment, independently and in silence, assessed the 2009 Riesling and Chardonnay wines including untreated, bentonite-treated and zirconia-treated samples of each variety. Samples (30 mL) were poured in constant order across tasters, in coded International Organisation for Standardisation (ISO) tasting glasses. The tasters were asked to write free-choice notes about the wines' appearance, aroma and flavour, and also to indicate any perceived taints or faults. After the wines were tasted, a discussion was held.

Results and discussion

Preliminary experiments

Several exploratory experiments were undertaken to identify the most appropriate conditions to use to assess the efficiency and utility of this adsorbent for wine. Firstly, the protein removal efficiency of pellet and powder forms of zirconia was assessed by adding them (at 10 g/L) to a 2007 Semillon wine (preliminary results, data not shown). Results confirmed previous observations (Salazar 2007), with faster protein adsorption shown by the powder, especially in the first minutes of the treatment. Protein adsorption by the pellet had not reached a plateau even after 350 h. The slower protein adsorption shown by the pellet could be related to its protein binding sites being less accessible than those of the powder. This might be because of a different surface area and/or pore size of the two materials. Similar results were obtained with a 2007 unfined Riesling wine (data not shown). In the absence of stirring, no protein removal was observed even after 50 h (data not shown), indicating that circulation of wine was essential for the correct functioning of the adsorbent within a reasonable time period.

The addition of adsorbents to wine without physical restrictions has the disadvantage of subsequent removal of the adsorbents, usually by filtration or centrifugation, with the associated costs of doing so. We therefore assessed the performance of both the pellet and powder forms immobilised within a structure that allowed fluid exchange. The first experiments were undertaken by trapping both the powder and pellet forms of zirconia into a spherical 'bag' made from Miracloth and extending contact times for more than 2 days. The final protein removal of the two forms was more similar under these circumstances than under addition of the absorbent without physical restrictions (Table 1); indeed, the residual protein content was reduced by 42% by the powder and by 56% by the pellet. This might seem inconsistent with our preliminary results in which the powder worked better than the pellet, but it is possible that the longer contact time between the wine and the adsorbents meant that both forms reached the maximal binding capacity by the end of the contact period. Enclosing the adsorbent in a bag also has the advantage of easy recovery of wine through simply removing the bag rather than having to centrifuge, rack or filter the wine.

Another key parameter to investigate was the possible effect of temperature on adsorption. Therefore, an experiment was undertaken with pellets in bags at 2°C and 18°C and a shorter contact time of 18 h. Wines were more stable (Figure 1) and more protein was removed (data not shown) after treatment at 18°C than at 2°C for all treatments except the highest addition. It is possible that heat stabilisation could be achieved at 2°C with longer contact time and that the delay is because of mixing and access to the protein binding sites of the adsorbent.

We also examined whether the shape of the structure enclosing the pellets affected adsorption (Figure 2a). Over the 18-h test period, wine stabilisation (Figure 2b) occurred slightly more rapidly in a long narrow ('sausage-like') bag compared with a spherical shape, even though they contained the same amount of zirconia. This is likely because the geometry of the 'sausage' is allowing easier access of wine to all the pellets relative to a sphere. However, with contact times of 18 h, the differences in haze between the treatments disappeared. It is noteworthy that wine haze (Figure 2b) decreased more quickly when a metallic cage was used, probably because of the combined effect of having a mass of pellet through which the wine passes slowly, but a higher turnover of wine through the bigger pores of the cage compared with those of the fabric bag.

Overall, these results suggest that parameters that influence accessibility and contact between wine and the pellet are important because they drive protein adsorption. These preliminary results were used to design the operational conditions for a larger scale experiment carried out in the second part of this work.

Large scale experiment

Protein removal and heat stability. Three unfined wines from the 2009 vintage (Riesling, Chardonnay and Sauvignon Blanc) were treated with zirconia pellets enclosed in tea infusers at three different rates (see Figure 2a, shape 3). The wines were chosen because of their relatively high total protein concentration, heat instability and bentonite requirement (Table 2). To our knowledge, zirconia has never been used in wines with a protein concentration higher than 31 mg/L (Pashova et al. 2004a, Salazar 2007), that is values several fold lower than the wines under investigation in this study.

There were clear effects of dosage of zirconia and contact time on protein removal as had been observed in the preliminary experiments. The increased removal of protein with increasing dose was particularly evident at the maximal contact time for each of the wines (Figure 3). The kinetics of protein removal seemed to be wine-dependent. In fact, protein removal reached a plateau in Riesling after 48 h and in Sauvignon Blanc after only 6 h, whereas it did not appear to do so in the Chardonnay wine during the 72 h of treatment even though this latter wine contained the lowest initial total protein concentration. While the wines were similar in most of the standard compositional parameters (Table 3) it is possible that differences in colloidal components such as polysaccharides (not measured) or the proportions of the types of wine proteins present influenced the adsorption

Table 1. Effect of 65 h of contact time (with stirring) of a Semillon 2007 unfined wine treated with 10 g/L of pellet/powder enclosed in a bag made of Miracloth.

Sample	Change in turbidity after heat test (in mAU at 540 nm)	Protein content (in mg/L of thaumatin)
Semillon wine	60	102.0
Semillon wine treated with pellet	21	44.8
Semillon wine treated with powder	30	58.4

Heat test net values lower than 20 mAU indicate stability.

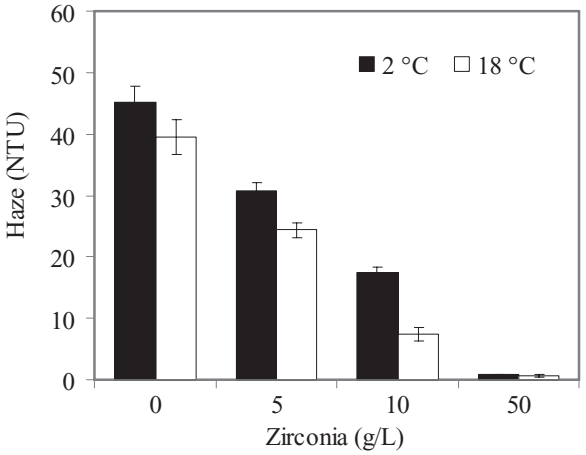

Figure 1. Effect of temperature and zirconia dose on the haze potential of a 2007 Riesling wine after 18 h of treatment with stirring. (NTU, nephelometric turbidity unit.)

Table 2. Parameters involved in wine heat instability.

	Chardonnay	Riesling	Sauvignon blanc
Initial protein content (mg/L)	100.6	195.6	138.2
Bentonite requirement (g/L)	0.9	1.6	1.7
Haze potential (NTU)†	53.4	46.3	27.4

† Change in turbidity (in NTU) following a heat test. (NTU, nephelometric turbidity unit.)

kinetics. The Chardonnay wine contained the lowest percentage of chitinases in comparison with the total proteins in the control and Sauvignon Blanc the highest (measured by HPLC, Figure 4a), yet the Sauvignon Blanc wine reached maximum protein removal (plateau) the quickest and Chardonnay the slowest (Figure 3). These results could indicate a higher affinity of zirconia towards TL proteins than chitinases. This was explored by determining protein composition of the wines by HPLC and SDS-PAGE. There was no clear evidence of preferential removal of TL proteins from the HPLC chromatogram (Figure 4a). The SDS-PAGE profiles of the wines (see data for Sauvignon Blanc in Figure 4b) confirmed that protein removal was general rather than preferential. The 20–40 mg/L of protein still found in wines

Table 3. Physicochemical parameters of wines after treatment with zirconia.

Parameter†	Chardonnay (72 h treatment)					Riesling (192 h treatment)					Sauvignon Blanc (72 h treatment)				
	Untreated wine	Control	5 g/L Zirconia	10 g/L Zirconia	25 g/L Zirconia	Untreated wine	Control	5 g/L Zirconia	10 g/L Zirconia	25 g/L Zirconia	Untreated wine	Control	5 g/L Zirconia	10 g/L Zirconia	25 g/L Zirconia
Alcohol (% v/v)	12.6	12.6	12.5	12.6	12.5	11.2	11.2	11.2	11.2	11.1	12.4	12.6	12.5	12.6	12.5
Specific gravity	0.9912	0.9912	0.9912	0.9909	0.9906	0.9933	0.9932	0.9931	0.993	0.9927	0.9909	0.9909	0.9908	0.9906	0.9903
pH	3.27	3.24	3.26	3.27	3.33	3.08	3.08	3.12	3.15	3.21	3.22	3.22	3.25	3.26	3.33
Titratable acid pH 8.2 (g/L)	6.4	6.4	6.2	6.0	5.4	6.8	6.7	6.6	6.3	5.9	6.0	6.0	5.8	5.6	5.0
Titratable acid pH 7.0 (g/L)	6.0	6.2	5.9	5.7	5.2	6.4	6.4	6.3	6.0	5.5	5.5	5.5	5.3	5.1	4.6
Glucose + fructose (g/L)	0.6	0.6	0.6	0.6	0.7	<0.27	0.4	<0.27	<0.27	0.4	0.4	0.6	0.5	0.5	0.6
Volatile acidity as acetic acid (g/L)	0.30	0.32	0.33	0.31	0.31	0.24	0.25	0.21	0.23	0.23	0.22	0.23	0.21	0.22	0.2
Sulphur dioxide (free) (mg/L)	47	42	41	40	34	21	20	19	18	16	<4	4	4	4	4
Sulphur dioxide (total) (mg/L)	145	139	137	136	129	73	76	75	72	71	41	42	42	42	40
Bentonite requirement (g/L)	0.9	0.9	0.6	0.3	0	1.6	1.6	0.9	0.5	0	1.7	1.7	1.2	0.8	0.2
Haze (NTU)	54.7 ± 0.4	53.4 ± 0.5	33.2 ± 0.8	20.5 ± 1.0	2.1 ± 0.2	52.2 ± 0.2	46.2 ± 0.9	32.7 ± 0.7	18.5 ± 0.8	1.8 ± 0.4	27.4 ± 0.4	24.0 ± 1.0	22.8 ± 0.8	21.0 ± 0.6	11.4 ± 0.3

† Data from single determinations except for haze that was done in triplicate. (NTU, nephelometric turbidity unit)

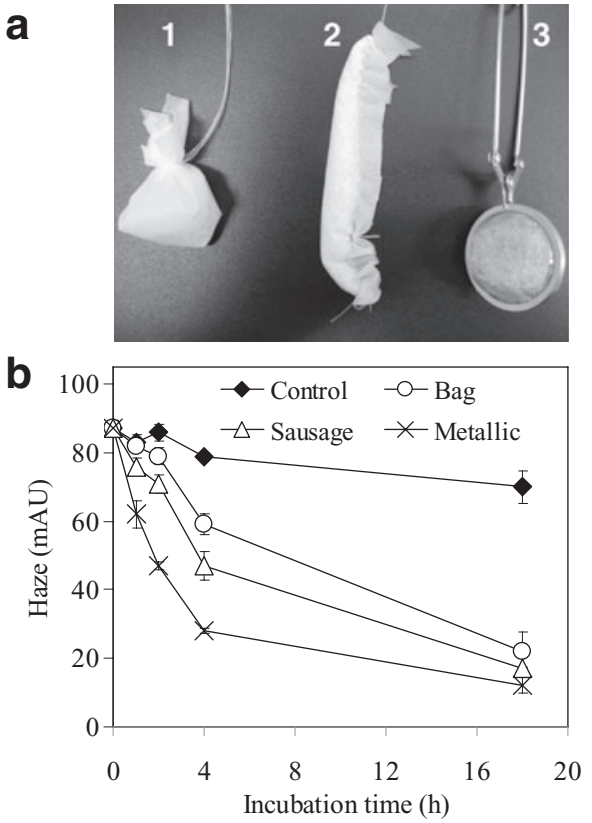

Figure 2. (a) Three bag shapes used in the experiments: (1) Miracloth spherical bag; (2) Miracloth long/narrow bag ('sausage'); and (3) metallic cage. Each bag contains the same amount of zirconia pellets. (b) Effect of bag shape and contact time on the haze potential of a 2007 Riesling wine. Wines were held at 18°C and stirred during the experiment. Each point is the average of at least three replicates ($n = 3$).

at the end of the contact time mainly had a molecular weight of 22–25 kDa, consistent with these proteins being both TL proteins and chitinases (Marangon et al. 2009, Van Sluyter et al. 2009).

A reduction in protein levels is usually accompanied by a reduction in haze potential and thus an increase in wine stability (Mesquita et al. 2001), but there have been exceptions to this observed by others (Bayly and Berg 1967, Pashova et al. 2004a). Therefore, the heat stability of wines after treatments was further assessed by subjecting the wine to a heat test and measuring changes in turbidity, while the bentonite required to stabilise the wines after the zirconia treatment was also determined (Table 3). Two of the three wines were fully stabilised with 25 g/L of zirconia. These wines contained residual protein concentrations below 21 mg/L whereas the Sauvignon Blanc wine that did not achieve stability still contained 43 mg/L protein (Figure 3). Despite not achieving stability, the 25 g/L treatment of the Sauvignon Blanc wine resulted in a decrease in the amount of bentonite required for stability from 1.7 g/L to 0.2 g/L (Table 3). These results confirm that protein removal from wines and the resulting increase in stability and decrease in bentonite requirement was proportional to the quantity of pellets used. The results also further confirmed the non-specific adsorption of proteins by this material.

Physico-chemical and metal analysis. Investigations by others have suggested that zirconia treatments have small effects on the chemical and sensorial properties of wine (Salazar et al. 2006). However, these studies relate to contact times

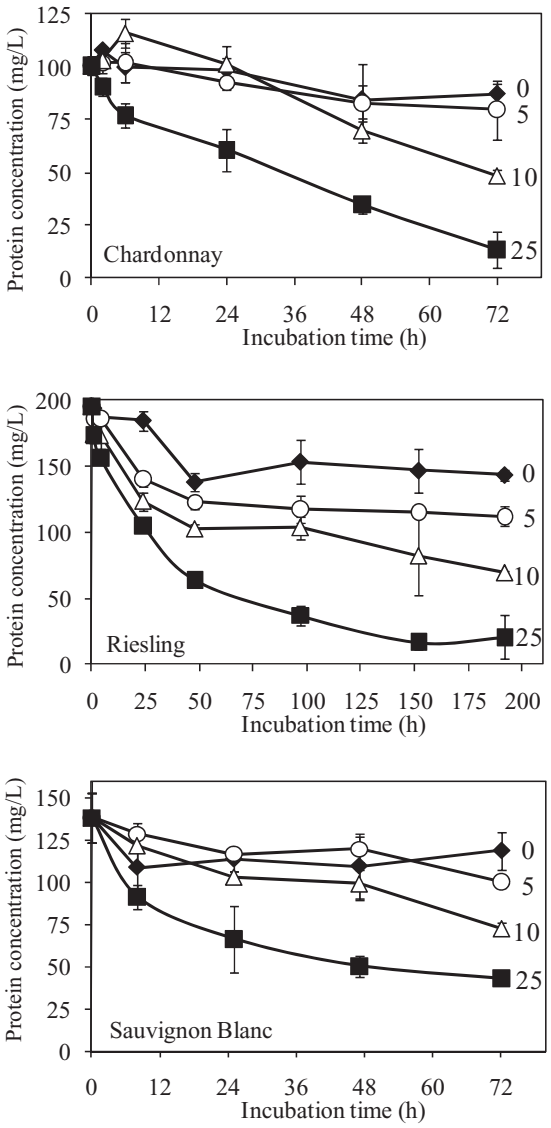

Figure 3. The effect of zirconia dose and contact time on the protein concentration of three wines. Protein removal measured by EZQ kit. Zirconia addition rates: C, 0 g/L; 5, 5 g/L; 10, 10 g/L; 25, 25 g/L. Wines were held at 18°C and stirred at 140 rpm during the experiment. Each point is the average of at least three replicates ($n = 3$). Note: incubation times and initial protein contents differ between the three wines.

between wine and zirconia that were shorter than those under investigation here. Hence, common wine compositional parameters were determined to check if other wine components were removed along with protein. In addition, metal analysis was also undertaken to determine if the zirconia released metals into the wine, even though it was high purity grade and was washed with distilled water and rinsed with wine before each experiment. The data show that only wine acidity (Table 3) and levels of Cu, Fe and Al (Table 4) were affected, and in a dose-dependant manner. In particular, a slight increase in pH and decrease in total acidity were noted, in a trend that was consistent with the increasing dosage of zirconia used.

HPLC was used to quantify organic acids in the three wines treated with increasing dosages of zirconia (Table 5). Results suggest that the reduction in total acidity (see Table 3), was probably attributable to the diminution of three acids: citric, tartaric and malic, while no differences were noticed in the levels of succinic and lactic acids. It is likely that the drop in total

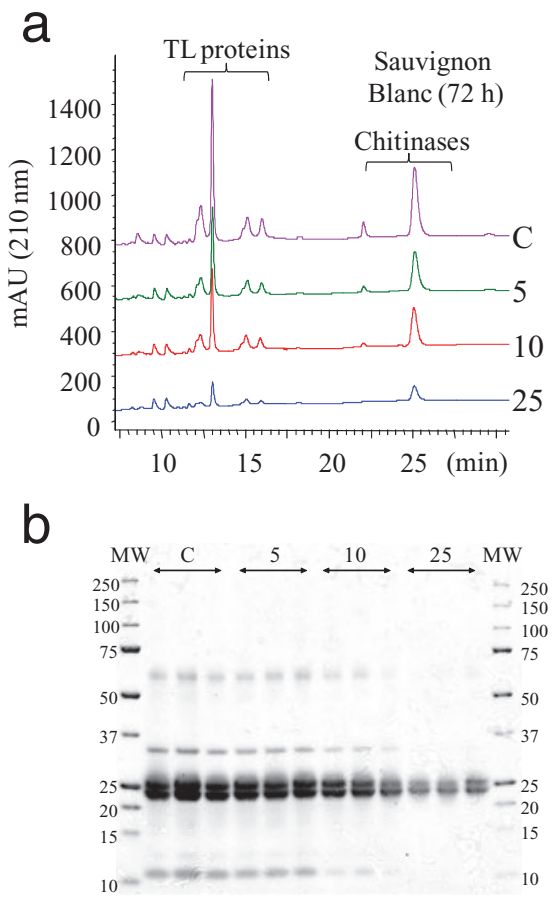

Figure 4. (a) The effect of zirconia dose on the protein composition and concentration of Sauvignon Blanc shown by reverse phase (C8) high-performance liquid chromatography chromatogram. The contact time is given in the figures. Wines were held at 18°C and stirred during the experiment. (b) The effect of zirconia dose on the protein composition of Sauvignon Blanc wine after 72 h of zirconia treatment shown by NuPAGE. Proteins from 100 µL of wine were loaded per lane. The three replicates made for each of the treatments are shown. Wines were held at 18°C and stirred during the experiment. Zirconia addition rates: C, 0 g/L; 5, 5 g/L; 10, 10 g/L; 25, 25 g/L. (MW, molecular weight in kDa.)

acidity is mainly because of the diminished content of tartaric acid, even if, in terms of percentage, it is the citric acid that is removed most.

Wine colour differences because of the treatment were determined by CIELAB. The values ΔE^*_{ab}, a measure of differences in colour between samples was calculated for pairs of treatments (Table 6). Pairs with values of ΔE^*_{ab} greater than 1 are likely to be detected as different by the human eye (Kwiatkowski et al. 2007). The treatments with increasing dosages of zirconia modified the colour in a way that seems proportional to the dosage utilised, but none of the treated wines could potentially be detected as different from the respective controls by the human eye (Table 6).

Altogether, these results demonstrate that long contact times with high doses can lower the acidity of the wine and affect the metal ion composition. Preliminary results indicate that shorter treatments would result in less drastic acidity diminutions (not shown).

Sensory assessment. An informal sensory evaluation of zirconia-treated Riesling and Chardonnay wines was conducted to assess whether the zirconia treatment had any sensory effect.

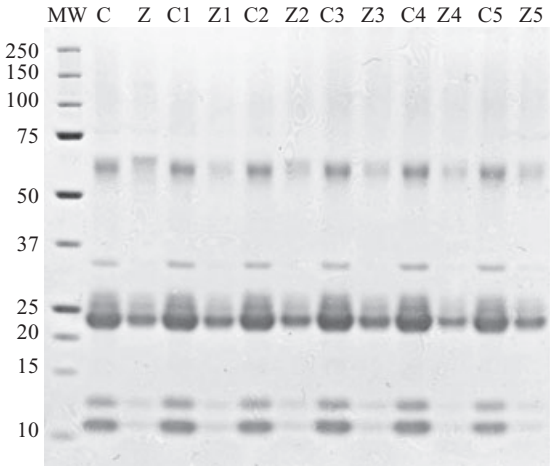

Figure 5. The effect of zirconia regeneration on the protein content of treated wines (Z) versus controls (C), as shown by sodium dodecyl sulfate polyacrylamide gel electrophoresis. The numbers refer to the number of regenerations the pellets had undergone. (MW, molecular weight in kDa.)

Previous studies (Salazar et al. 2006, Salazar 2007) have indicated that the treatment had no influence on the sensory properties of the treated wine, but the contact times between wine and zirconia were shorter than those under investigation here.

For both wines several tasters commented on lower perceived acidity for the zirconia-treated samples, consistent with the approximately 1 g/L difference in titratable acidity between the control and zirconia treated wines.

For the Chardonnay wine, the judges were not able to easily discriminate among the wines. There was some indication of a low-level rubbery, sulfide-related aroma for this sample. The Riesling wine treated with zirconia exhibited a stronger sulfide character, described as cabbagey or rubbery. The sulfide-related aroma does not appear to be because of zirconia itself adding a taint to the wine as analysis of model wines left in contact with zirconia showed an absence of thiol and other sulfur compounds commonly associated with sulfide off-flavour in wines (data not shown). The development of these sulfide characters in wines is often associated with reductive conditions (Kwiatkowski et al. 2007, Lopes et al. 2009) and it is possible that the removal of copper and iron (see Table 5) by the treatment and subsequent storage of the wine in anaerobic conditions could have resulted in an environment in which these characters were more likely to develop.

Zirconia regeneration. One particularly interesting characteristic of zirconia as an adsorbent is its ability to be regenerated. It was previously reported that regeneration could be achieved by heating at 500°C for 12 h (Salazar et al. 2007), but this type of procedure is unlikely to be commercially applicable. We therefore assessed the ability of cleaning products commonly available in most cellars and wineries, such as citric acid and NaOH, to regenerate the material.

Zirconia pellets enclosed in tea infusers were repeatedly exposed to wine and then regenerated by treatment with NaOH and citric acid. Results showed that the pellets maintained their ability to remove protein for at least 11 regenerations. Protein composition in treated wines for pellets regenerated six times is shown in Figure 5. The ability of the zirconia to be regenerated was confirmed by the heat stability of wines treated with zirconia that had undergone up to 11 regenerations without a

Table 4. Metal concentration of wines† after treatment with zirconia

Metal (mg/L)‡	Chardonnay 72 h				Riesling 192 h				Sauvignon Blanc 72 h			
	Control	5 g/L Zirconia	10 g/L Zirconia	25 g/L Zirconia	Control	5 g/L Zirconia	10 g/L Zirconia	25 g/L Zirconia	Control	5 g/L Zirconia	10 g/L Zirconia	25 g/L Zirconia
Fe	0.58	0.58	0.08	0.03	1.20	0.52	0.15	0.55	0.34	0.13	0.11	0.04
Mn	0.73	0.73	0.64	0.55	1.60	1.64	1.60	1.30	0.50	0.45	0.43	0.36
B	6.00	5.97	5.89	5.40	4.90	4.94	4.86	4.40	2.52	2.45	2.42	2.28
Cu	0.11	0.11	0.07	0.03	0.12	0.10	0.16	<0.03	0.08	0.06	0.05	<0.03
Zn	0.51	0.51	0.48	0.42	1.30	1.27	1.31	1.00	1.16	1.08	1.02	0.85
Ca	60.00	59.86	61.14	60.00	89.00	91.02	85.96	87.00	61.97	63.20	61.58	61.03
Mg	107.00	107.32	108.83	105.00	95.00	96.51	93.23	93.00	94.65	95.69	96.04	94.43
Na	68.00	67.80	76.32	84.00	41.00	42.57	40.63	47.00	21.15	24.59	26.30	37.96
K	400.00	400.00	400.00	390.00	410.00	420.00	430.00	400.00	490.00	500.00	500.00	490.00
P	114.00	113.65	69.71	32.00	135.00	109.03	133.95	32.00	157.24	132.05	107.42	53.60
S	84.00	84.07	83.94	79.00	111.00	112.15	115.71	105.00	59.86	58.04	57.19	55.08
Al	0.08	0.08	0.03	0.03	0.09	0.04	0.09	0.03	0.06	0.03	0.02	0.01
Cr	<0.04	<0.04	<0.04	<0.04	0.08	0.05	<0.04	0.09	<0.04	<0.04	<0.04	<0.04

† Data from single determinations. ‡Mo, Co, Ni, Ti, Cd, Pb and Se were not included in the table because their contents were below the detection limit (respectively 0.06, 0.06, 0.07, 0.009, 0.04, 0.2, 0.6 mg/L).

Table 5. Organic acid concentration† by HPLC of wines after treatment with zirconia.

	Zirconia (g/L)	Citric (g/L)	Tartaric (g/L)	Malic (g/L)	Succinic (g/L)	Lactic (g/L)	Total Acidity (g/L)
Chardonnay after 72 h	0	0.19	2.92	2.32	2.07	0.14	7.65
	5	0.19	2.96	2.39	2.14	0.14	7.83
	10	0.15	2.82	2.31	2.10	0.14	7.53
	25	0.13	2.61	2.23	2.11	0.15	7.24
Riesling after 197 h	0	0.11	4.17	1.46	2.43	0.25	8.43
	5	0.11	4.08	1.45	2.42	0.25	8.30
	10	0.10	3.96	1.35	2.34	0.24	7.99
	25	0.09	3.71	1.36	2.41	0.25	7.82
Sauvignon Blanc after 72 h	0	0.20	3.98	1.40	2.26	0.27	8.11
	5	0.19	3.66	1.35	2.11	0.24	7.55
	10	0.19	3.41	1.38	2.17	0.24	7.39
	25	0.17	3.03	1.32	2.18	0.25	6.95

† Data from single determinations.

Table 6. Mean CIELAB ΔE^*_{ab} values for wines treated with increasing dosages of zirconia.

Treatments	ΔE^*_{ab}†		
	Chardonnay	Riesling	Sauvignon Blanc
Control vs 5 g/L zirconia	0.3	0.3	0.3
Control vs 10 g/L zirconia	0.5	0.7	0.4
Control vs 25 g/L zirconia	0.7	0.9	0.8

† $\Delta E_{ab}^* = \sqrt{\left[(L1-L2)^2 + (a1-a2)^2 + (b1-b2)^2\right]}$.

noticeable decrease in efficiency (the average NTU value of 11 regenerations was 10.5 and the value of the last regeneration was 9.1 NTU). It is possible that the pellets may have continued to perform after further regeneration, because no evidence of reduced protein absorption capacity was detected up to 11 regenerations. However, small physical losses of the material could eventually result in reduced performance.

Conclusions

Zirconia was confirmed to be an excellent candidate for protein adsorption from wines. The proposed modification of its application to wine (pellets enclosed in a metallic cage) represents a step forward in the adoption of this material commercially. Other immobilisation possibilities may be more suitable to larger scale wine production than the method used here, and this should be further explored. This study has also demonstrated that regeneration of the material can be relatively simple. Its ability to reduce acidity and metal ion concentration may be an additional advantage for wine production and could also be potentially exploited by other industry sectors.

One of the main drawbacks of the zirconia treatment seems to be the necessity of stirring and relatively high dosage levels. The easy regeneration process somewhat reduces the negative aspects of the requirements for high doses, because the material can be reused many times. However, the physical properties of

the zirconia used in this study were not optimised. It is possible that, if optimised, the addition rates and contact times could be significantly reduced. This issue of the need for stirring could be overcome by treating the wines when agitation occurs for other reasons, such as during fermentation. Preliminary results indicate this solution to be both feasible and promising.

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