Original article

Protein stabilisation of Chardonnay wine using trisacryl and bentonite: a comparative study

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Summary   The stabilisation of a Chilean Chardonnay wine by SP-Trisacryl-M and bentonite was investigated, evaluating protein, polyphenol and polysaccharide adsorption, turbidity and wine quality. The wine could be stabilised by adding at least 0.3 kg m^{-3} of bentonite or 12 kg m^{-3} of trisacryl, removing 95% and 76% of the wine proteins, respectively. The protein adsorption data for bentonite and trisacryl were fitted using the Freundlich isotherm. The wine protein adsorption isotherm on trisacryl was unfavourable. Protein removal from Chardonnay by trisacryl in a packed column at continuous operation was about 50% during the first 70 bed volumes (BV) of treated wine and decreased progressively until the end of the treatment (100 BV). The adsorbents showed a higher selectivity for proteins than for polyphenols and polysaccharides. A sensorial panel could not detect statistically significant differences between the bentonite and trisacryl treatments of wine at $P \leq 0.05$.

Keywords   Adsorption, Chardonnay wine, isotherm, protein, resin, stabilisation.

Introduction

The presence of haze or sediment in bottled white wines is a visual defect, negatively impacting on product commercialisation. Wine proteins, which become insoluble during wine storage, are the main cause of this quality defect (Bayly & Berg, 1967).

Protein concentration and characteristics in wines vary considerably because of factors such as climate, soil, cultivar, growth conditions and winemaking practices. Total protein concentration depends on the wine type and varies typically from 15 to 230 mg L^{-1} (Ferreira *et al.*, 2001). Pathogenesis-related proteins of the grape berry are particularly stable under winemaking conditions, and therefore pass into the wine, where they can precipitate, causing haze and sediments (Waters *et al.*, 1996). The protein fractions responsible for wine instability are thermally unstable glycoproteins of low molecular weights (12 and 20–30 kDa) and low isoelectric points (pI between 4.1 and 5.8) (Hsu & Heatherbell, 1987; Dawes *et al.*, 1994). Although clarity depends on proteins, other factors such as pH, polysaccharides, ethanol and tannins may also influence haze

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formation in white wines (Lagace & Bisson, 1990; Mesquita *et al.*, 2001). Moreover, some mannoproteins from yeast lees improve wine stability because of their haze-protective activity (Waters *et al.*, 1993; Moine-Ledoux & Dubourdieu, 1999; Dupin *et al.*, 2000).

Bentonite has been used for more than 70 years in oenology as a fining agent, using protein adsorption to stabilise wines. Its success is mainly due to its proved efficacy as well as its application in a low cost, simple batch process that does not require any specialised equipment or knowledge. However, bentonite fining results in a significant wine loss and a negative environmental impact due to the use of diatomaceous earth as a filter aid for bentonite removal. Moreover, bentonite is not a very specific adsorbent, removing both desirable and undesirable compounds. A bentonite dose above 0.8 g L^{-1} affects the wine's organoleptic properties by removing important aroma, flavour and anthocyanin compounds (Lubbers *et al.*, 1996; Ribéreau-Gayon *et al.*, 2000).

To identify an alternative adsorbing material implies screening its capacity to stabilise white wine. Pachova *et al.* (2002) studied protein adsorption onto metal oxides. Zirconium oxide was able to remove the unstable protein fraction of 20–30 kDa and could be used to stabilise white wines with an increased adsorption

capacity after heat regeneration (Pashova *et al.*, 2004a,b). Furthermore, macroporous ion-exchange resins with an acrylic, agarose or porous silica matrix were able to stabilise white wine samples and offered a protein adsorption similar to bentonite (Sarmento *et al.*, 2000, 2001).

The objective of this study was to compare the protein stabilisation of a Chilean Chardonnay wine by trisacryl and bentonite, their influence on wine quality and to evaluate the possibility to use trisacryl for continuous wine stabilisation.

Theory

Adsorption is the accumulation of material at the solid–liquid inter-phase due to a mass transfer process. Equilibrium data of the amount of material adsorbed per unit mass of sorbent (q_e) and the solute concentration remaining in solution (C_e) at a constant temperature, commonly known as adsorption isotherms, are basic requirements for the design of adsorption systems. Several non-linear models have been used to describe this sorption equilibrium.

The Langmuir equation (Langmuir, 1918), which is valid for monolayer adsorption onto a completely homogeneous surface with a finite number of identical sites with negligible interaction between adsorbed molecules, is given by

$$q_e = \frac{q^m \cdot K_L \cdot C_e}{1 + K_L \cdot C_e} \quad (1)$$

where q^m is the maximum adsorption capacity of the monomolecular layer and the Langmuir constant K_L is related to the adsorption energy, which is assumed to be constant. Values of K_L indicate the type of adsorption isotherm, which is irreversible for $K_L \rightarrow \infty$; favourable for $0 < K_L < \infty$; linear for $K_L = 0$; and unfavourable for $-1/C_0 < K_L < 0$, where C_0 is the initial solute concentration.

The Freundlich model (Freundlich, 1906) assumes an infinite number of surface sites for adsorption with a heterogeneous energy distribution of the active sites. The Freundlich equation has the following form:

$$q_e = K_F \cdot C_e^{1/n} \quad (2)$$

where the Freundlich constant K_F and the Freundlich exponent n are related to the adsorption capacity and intensity, respectively. Values of $n > 1$ indicate a favourable adsorption isotherm; $n = 1$ linear adsorption isotherm; and $0 < n < 1$ an unfavourable adsorption isotherm.

The Koble–Corrigan model assumes a homogeneous surface with a constant adsorption energy and adsorption to be a co-operative process due to adsorbate–adsorbate interactions (Koble & Corrigan, 1952). This model is a combination of the Langmuir and Freundlich models and is given by

$$q_e = \frac{q^m \cdot K_{KC} \cdot C_e^{\alpha}}{1 + K_{KC} \cdot C_e^{\alpha}} \quad (3)$$

where K_{KC} is a thermodynamic equilibrium constant, q^m is the total number of active centres and the exponent α indicates the type of adsorption. This model is valid if $\alpha > 0$, having an unfavourable adsorption isotherm for $0 < \alpha < 1$ and a favourable adsorption isotherm for $\alpha > 1$.

The following equation has been proposed by Redlich & Peterson (1959) to improve the fit by the Langmuir and Freundlich equations:

$$q_e = \frac{A_{RP} \cdot C_e}{1 + B_{RP} \cdot C_e^{\gamma}} \quad (4)$$

The Redlich–Peterson isotherm, which contains the parameters A_{RP}, B_{RP} and γ, has features of both Langmuir and Freundlich isotherms.

Assuming an exponential relationship for both the adsorption rate and the desorption rate with the fraction of surface covered by adsorbate, the adsorption equilibrium can be expressed according to the Temkin isotherm (Brunauer *et al.*, 1942)

$$q_e = A_T \cdot Ln(B_T \cdot C_e) \quad (5)$$

where A_T and B_T represent the isotherm parameters.

Resin may adsorb both undesired compounds (i.e. unstable proteins) and desired wine compounds (such as aromas, polyphenols and polysaccharides, amongst others) from the wine. Thus, selectivity of resin is another parameter of interest in separation processes and is defined as:

$$\beta_{ij} = \frac{y_i/x_i}{y_j/x_j} \quad (6)$$

where y_i and y_j are the mass fractions of desired compound i and undesired compound j on the resin; and x_i and x_j are the mass fractions of desired compound i and undesired compound j in the wine. For $\beta_{ij} = 1$, no separation has been achieved.

Materials and methods

Wines

A Chardonnay wine from the Bio-Bio Valley, Chile (vintage 2005) was obtained after fermentation and prior to any protein removal or stabilisation treatment from a local winery. Prior to stabilisation at laboratory scale, suspended solids were separated from the wine by centrifugation at 9.000 *g* for 10 min (Damon IEC HN-SII, Needham, MA, USA).

A white wine model solution was prepared with 13.5% (v/v) ethanol and 5.0 g L^{-1} of potassium tartrate buffer in distilled water. The solution had a final pH of 3.35. Bovine serum albumin (BSA; Merck, Darmstadt, Germany), ovalbumin (Merck) or haemoglobin (Sigma-Aldrich, St. Louis, MO, USA) (25 mg L^{-1}) was used as a model protein.

Chemicals

All reagents were of analytical grade and supplied by Merck; if not, it will be specified otherwise. The following adsorbents were used in this study: bentonite, Volclay, KWK Food Grade (American Colloid, Arlington Heights, IL, USA) having a particle size less than 59 μm and a cation exchange capacity between 800 and 1000 μeq g^{-1} and SP-Trisacryl-M (Sigma-Aldrich) with a cation exchange capacity between 190 and 250 μeq mL^{-1} and a particle size of 40–80 μm. Bentonite was prepared in distilled water as 5% (w/v) slurry. This slurry was prepared at least 48 h prior to use to allow for hydration. Trisacryl was washed in triplicate (500 mL distilled water per 100 g resin), followed by filtration (Whatman no. 541).

The following reagents were used in wine analyses: Coomassie brilliant blue reagent and BSA (Calbiochem, La Jolla, CA, USA) in protein analysis; Folin–Ciocalteu reagent, anhydrous sodium carbonate and gallic acid (Sigma-Aldrich) in polyphenol analysis; *o*-hydroxydiphenyl (Aldrich, St. Louis, MO, USA), phenol, sodium hydroxide, sodium tetraborate, sulphuric acid, hydrochloric acid, ethanol, galacturonic acid and galactose, in polysaccharide analysis.

The following reagents were used in the determination of physicochemical wine properties: silver nitrate, nitric acid, potassium chloride, barium chloride, hydrochloric acid, phenolphthalein, ethanol, sodium hydroxide, sulphuric acid, starch, sodium chloride, tartaric acid, iodine, potassium iodide, sodium thiosulfate, sodium bicarbonate, Luff reagent and Carrez reagent.

Stabilisation treatments

After pre-treatment, different amounts of adsorbents were added to 100 mL of wine in Erlenmeyer flasks of 250 mL. These samples were sealed and placed in a water bath for 48 h at 19 °C and shaking (100 r.p.m.). After centrifuging at 9.000 *g* for 10 min, protein, polyphenol and polysaccharide concentrations and turbidity in the supernatant were determined. Each analysis was performed in duplicate. The amount of solute adsorbed per unit mass in equilibrium state was calculated from a mass balance as:

$$q_e = \frac{V \cdot (C_0 - C_e)}{M} \qquad (7)$$

where C_0 and C_e are the initial and equilibrium concentrations (kg m^{-3}), V is the volume of the solution (m^3) and M is the mass of the adsorbent (kg).

Then continuous stabilisation process was carried out in the Spectra/Chrom LC column (H × ID = 8 × 5 cm) with adjustable plunger packed with 178 g of trisacryl. Wine or white wine model solution (16 L) was pumped downwards through the column using the Masterflex L/S peristaltic pump (Model 77250-62) at a flow rate of 24.5 mL min^{-1}. After each treatment, the adsorbent material was washed with 2 L of distilled water, and then regenerated with 2 L of 1 M HCl, followed by 11 L of distilled water.

Analytical methods

Total soluble protein concentration in the aliquots was evaluated by adding Coomassie brilliant blue reagent and reading absorbance at 595 nm (Bradford, 1976). BSA was used as a standard to assess the protein concentration. All measurements were performed in duplicate.

The Folin–Ciocalteu assay was used to measure total polyphenol content, using the Folin–Ciocalteu reagent and reading absorbance at 750 nm (Singleton & Rossi, 1965). Concentrations were expressed as equivalents of gallic acid. All measurements were performed in duplicate.

Polysaccharide content of wine samples was examined by two colorimetric methods using *o*-hydroxydiphenyl reagent for the analysis of the acid polysaccharide fraction and phenol reagent for the total polysaccharide level (Segarra *et al.*, 1995). The results were expressed as equivalents of galacturonic acid and galactose, respectively. All measurements were performed in duplicate.

Physicochemical properties, such as pH, total acidity, volumetric alcohol degree, density, total dry extract, chloride and sulphate content of wine samples, were measured according to EU methods (Comisión de las Comunidades Europeas, 1990). Other wine properties, like volatile acidity, reducing sugar, free and total sulphur content, and chromatic characteristics, were measured according to Chilean methods (Bordeu & Scarpa, 2000). Wine colour variations were evaluated by trichromatic analysis using the Hunterlab Color Quest colorimeter. The analyses were performed in triplicate.

Heat test

The wine's protein stability was determined by heating samples in sealed bottles at 80 °C for 2 h in a water bath, followed by cooling at 4 °C for 2 h. After allowing the samples to reach room temperature, their turbidity was measured with a LaMotte 2020 turbidimeter. Difference in wine turbidity before and after heat

treatment is proportional to its protein instability (Pachova *et al.*, 2002).

Sensorial analysis

Sensorial wine analysis was performed by a panel of sixteen semi-expert tasters using the duo–trio test (Anzaldúa-Morales, 1994). This test is used to determine sensory difference between two wines. This method involves the simultaneous presentation of two numerically coded samples and one sample coded R (reference). The panellists were asked to select the sample perceived as different from the reference wine. Moreover, differences of wine attributes corresponding to sight, smell and taste between coded samples were identified. A global assessment of each type of wine was carried out.

Data analyses

The parameters of adsorption isotherms were fitted according to the Langmuir, Freundlich, Koble–Corrigan, Redlich–Peterson and Temkin models by non-linear least-square regression analysis using the statistical software SPSS, version 8.0. In order to determine the best-fit model and its validity, the average percentage error between the experimental and predicted values ($q_{e, i, exp}$ and $q_{e, i, cal}$) was calculated according to

$$e = \frac{\sum_{i=1}^{N} \left| \frac{q_{e,i,\exp} - q_{e,i,\mathrm{cal}}}{q_{e,i,\exp}} \right|}{N} \cdot 100 \qquad (8)$$

The correlation between protein concentration and wine turbidity was calculated by the linear regression correlation coefficient R using the software Statgraphics Plus, version 5.0. The effect of adsorption on the physicochemical properties of wine was determined by one-way ANOVA at $P \leq 0.05$. Sensory data were evaluated according to the critical values at a significance level of 5% (Roessler *et al.*, 1978).

Results and discussion

The adsorption capacity of the resins for wine proteins varies with adsorbent type and dose. Bentonite showed a high affinity for wine proteins with a complete adsorption at 0.4 kg m^{-3}, whereas trisacryl was able to remove 76% of the proteins at 12 kg m^{-3}. Adsorption of wine proteins by both bentonite and trisacryl is primarily due to cation exchange. Most of the proteins have an overall positive charge at the wine's pH because of their isoelectric points, competing with other cations like potassium, calcium, magnesium, hydrogen, most amino acids and some peptides for the negatively charged, active sites of the adsorbent material. After cation exchange, they cluster together and precipitate out of the wine (Blade & Boulton, 1988).

Taking into account the validity of model parameters and comparing the values of average percentage error, the Freundlich isotherm fits the protein adsorption data for bentonite and trisacryl better than the other sorption equilibrium models. These adsorbent materials should have a heterogeneous surface with adsorption energy that changes in time and space during sorption, without a limited number of active sites due to the low initial protein concentration of 19.9 ± 5.0 mg L^{-1} of the Chardonnay wine. The adsorption isotherm of the protein–bentonite system (Fig. 1a) is convex in shape (favourable; Freundlich exponent *n* is greater than 1) and has a slight inflection point due to adsorbate–adsorbate interactions causing an ordering effect of the adsorbed protein molecules on the surface (Pachova *et al.*, 2002), where this type of adsorption with an increased adsorption at low solute concentration is of greatest practical interest. A sharp front of saturated adsorbent will move in time through a continuously operating packed column. However, continuous operation of bentonite within a percolation system is not possible because of bentonite's physical properties (e.g. swelling, particle size and distribution). The adsorption isotherm of wine proteins on trisacryl is unfavourable (Freundlich exponent *n* ranges from 0 to 1) (Fig. 1b). The curve is concave with a low adsorption at low solute concentration. Herein a broad front of saturated adsorbent will move through the continuously operating
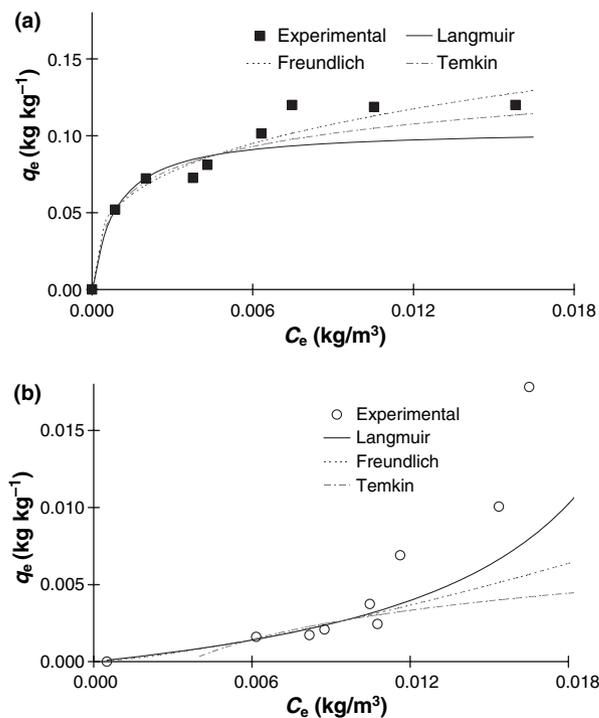
Figure 1 Adsorption isotherms of wine proteins on bentonite (a) and trisacryl (b).
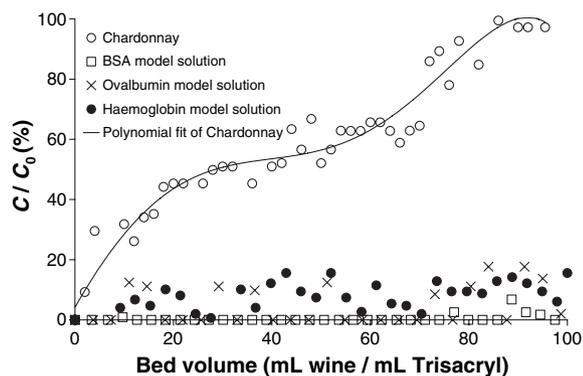
Figure 2 Breakthrough curves of proteins of Chardonnay and white wine model solutions.
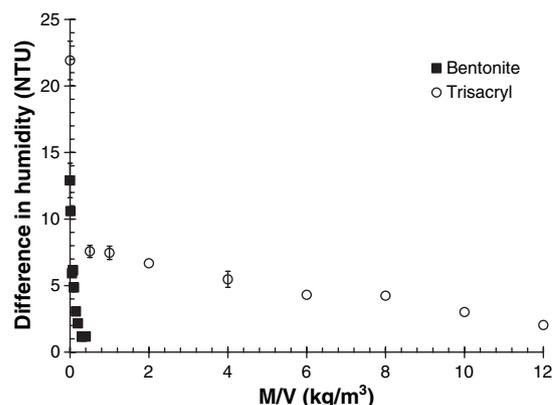
Figure 3 Effect of the dose of adsorbent on protein stability in wine.

Figure 4 Turbidity behaviour of Chardonnay wine treated by trisacryl.

packed column of trisacryl and results in a less efficient use of the packed bed. Indeed the breakthrough curve of wine proteins (Fig. 2) agrees with the unfavourable adsorption isotherm for trisacryl. Protein removal by trisacryl is about 50% during the first 70 bed volumes (BV), followed by a breakthrough of wine proteins between 70 and 85 BV, saturating the adsorbent material completely after 85 BV of Chardonnay (Fig. 2). However, white wine model solutions show increased protein retention by trisacryl because of a lack of natural wine compounds to compete for the active sites of trisacryl (Fig. 2). As the isoelectric point of the model wine proteins is above the pH of the model wine solution, trisacryl was able to remove almost all proteins by cation exchange. According to Sarmento *et al.* (1999) diffusion of proteins within non-swelling ion-exchange resins is greatly diffusion controlled having a characteristic diffusion time of about 20 s that is less than the residence time of about 6.4 min in our case. However, protein stability of Chardonnay wine might be improved, increasing residence time (Salazar *et al.*, 2006).

Wines are considered to be stable if the difference in turbidity does not exceed 2 NTU (Moine-Ledoux & Dubourdieu, 1999). According to this criterion, Chardonnay wine could be stabilised by the addition of at least 0.3 kg m^{-3} of bentonite or 12 kg m^{-3} of trisacryl (Fig. 3). Furthermore, a linear relationship between the decline of turbidity difference and the reduction in protein concentration by cation exchange adsorbents has been found with a correlation coefficient R of 0.979 and 0.953 for bentonite and trisacryl, respectively. Thus the removal of positively charged wine proteins improves wine's protein stability. Indeed the continuous protein stabilisation of Chardonnay wine shows a similar trend, that is, the increase of protein concentration in the wine fractions coincides with the increase of their turbidity with a correlation coefficient R of 0.910 (Figs 2 and 4).
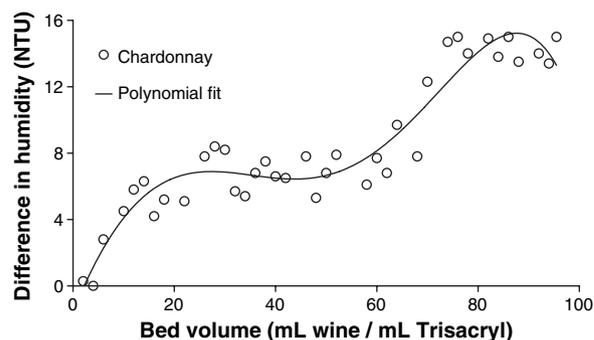
Both the adsorbents show an improved selectivity for proteins. Bentonite's high selectivity for proteins agrees with previous results obtained with Pinot Noir wine (Salazar *et al.*, 2007). Table 1 indicates a large effect produced by the dose of bentonite on selectivity of polyphenols–proteins and polysaccharides–proteins, which would probably affect wine quality. Furthermore, trisacryl is a very selective adsorbing material for wine proteins combined with a poor adsorption of wine polyphenols (Table 1). Minimum polyphenol adsorption onto trisacryl due to its low selectivity for polyphenols should be favourable to the quality of continuously stabilised wine (Fig. 5). At high adsorbent dose, trisacryl presents an increased selectivity for wine proteins up to polysaccharides (Table 1). Waters *et al.* (1993) purified a macromolecule made up of a polysaccharide component (96%) and a protein component (4%) that may protect wines from protein haze formation and contributes to wine stability. Thus, adsorbent's high affinity for wine polysaccharides may negatively affect wine stability.

Comparing the physical and chemical properties of Chardonnay wine without and with adsorption

Table 1 Selectivity of bentonite and trisacryl for polyphenols/proteins and polysaccharides/proteins in wine

Dose (kg m^{-3})	Selectivity for polyphenols/proteins	Selectivity for polysaccharides/proteins
Bentonite		
0.01	0.304	22.12
0.05	0.062	0.318
0.075	0.026	0.156
0.10	0.016	0.107
0.15	0.037	0.015
0.175	0.031	0.136
0.20	0.011	0.026
0.30	0.005	0.003
Trisacryl		
0.5	0.001	0.157
1	0.008	0.052
2	0.027	0.520
4	0.015	0.689
6	0.020	0.814
8	0.014	0.143
10	0.003	0.014
12	0.005	0.033

Table 2 Physical and chemical properties of Chardonnay wine

Property	Wine without treatment	Wine treated by 12 g L^{-1} of trisacryl
Conductivity (μS cm^{-1})[a]	1315 ± 1	1210 ± 1
Density (g L^{-1})	992 ± 1	992 ± 1
Intensity (A$_{420}$)	0.085 ± 0.005	0.065 ± 0.005
Alcohol (% vol)	13.5 ± 0.3	13.5 ± 0.3
Chlorides (mg L^{-1})[a]	94.1 ± 2.1	84.6 ± 0.0
pH	3.35 ± 0.01	3.34 ± 0.01
Reducing sugars (g L^{-1})[a]	2.23 ± 0.02	2.07 ± 0.00
SO$_2$ free (mg L^{-1})	38.4 ± 6.6	36.8 ± 2.5
SO$_2$ total (mg L^{-1})	55.0 ± 0.0	55.7 ± 0.6
Sulphates (g L^{-1})	0.632 ± 0.031	0.629 ± 0.032
Total dry extract (g L^{-1})	42.8 ± 0.5	34.7 ± 3.4
Total acidity (g L^{-1})[a]	5.36 ± 0.02	5.06 ± 0.02
Volatile acidity (g L^{-1})	0.273 ± 0.013	0.342 ± 0.038

[a]Indicates a statistically significant difference ($P \leq 0.05$).

Figure 5 Breakthrough curve of polyphenols of Chardonnay wine.

The members of the sensorial panel were not able to detect a statistically significant difference between wine treated by bentonite and trisacryl at $P \leq 0.05$. Nevertheless, 67% of the judges identified wine treated by trisacryl as different in appearance and taste, while 71% of the judges were able to mark trisacryl-treated wine as different in aroma. No difference in colour between both the wines could be distinguished. Wine treated by bentonite had an increased reflectance of light between 500 and 610 nm (results not shown), indicating an increased amount of green–yellow colour compounds compared with trisacryl-treated wine. Hunter's parameters of bentonite-treated wine ($L^* = 1.48$; $a^* = -0.17$; $b^* = 0.41$) and trisacryl-treated wine ($L^* = 1.36$; $a^* = 0.01$; $b^* = 0.14$) again showed a decrease of green and yellow components of trisacryl-treated wine. However, $[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$ was 0.35 here and the colours of both the wines could not be distinguished by the human eye (Spagna *et al.*, 1996).

Conclusions

Both bentonite and trisacryl were able to stabilise Chardonnay through batch adsorption of wine proteins. However, Chardonnay treated continuously by trisacryl was only stable during the first 5 BV of wine. Cation exchange was the main separation mechanism during the wine stabilisation. Macroporous resins can improve wine stability by separating positively charged proteins. Trisacryl-treated wine presented only minor changes of physicochemical properties and sensorial characteristics. The Freundlich model was chosen to correlate the experimental equilibrium data for protein adsorption. However, the isotherm's concave shape for wine protein adsorption on trisacryl results in a less efficient use of the continuously operating packed bed.

treatment by 12 g L^{-1} of trisacryl, statistically significant decreases in total acidity, reducing sugars, conductivity and chlorides were found due to adsorption (Table 2). The ionisable sulphate groups of trisacryl transfer hydrogen ions into the wine, and simultaneously trap positively charged ions (such as proteins, amino acids, amines and metal ions) within the resin, possibly affecting wine quality and stability. At the same time, an electrical double layer of ionic species will be formed around the resin particles. The small difference between the pH values of wine with and without trisacryl treatment is due to the wine's high buffer capacity. On the contrary, sorption of carboxylic acids and chloride anions within the electrical double layer may explain the decline of total acidity and conductivity after trisacryl treatment.

Acknowledgments

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