Fining Treatments of White Wines by Means of Polymeric Adjuvants for Their Stabilization against Browning

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Browning and maderization represent important problems for white wine stability. Essentially, this is due to polyphenol oxidation in the wine. The problem has been remedied by adsorption of polyphenol compounds with polymeric adjuvants (chitosans, scleroprotein, and polylactic acid) not used traditionally in wine-making. In particular, some chitosans reduced the polyphenol content and stabilized two Italian white wines (Trebbiano and Albana) to the same extent as did potassium caseinate, an adjuvant normally used in enology. Moreover, chitosans could be reused after a simple regeneration process.

Keywords: *Maderization; polyphenols; chitosans*

INTRODUCTION

Maderization and browning adversely affecting wine color, taste, and aroma (Pallotta and Cantarelli, 1979; Piracci and Tamborra, 1987; Singleton et al., 1984; Simpson, 1982) are problems that are often encountered in the making of white wines. Among the various reactions involved, the most important are oxidations of the polyphenols including catechins, proanthocyanidins, and hydroxycinnamic acids and their derivatives and the formation of oligomers, which cause browning. Polyphenol oxidation may essentially be of enzymic and/or chemical nature. Enzymic oxidation is caused by polyphenol oxidase (Margheri et al., 1980; Cheynier and Ricardo Da Silva, 1991; Gunata and Moutounet, 1988; Singleton, 1987; Baron et al., 1997), which oxidizes mainly mono- and orthodiphenolic substrates (such as hydroxycinnamates and catechins). This type of oxidation chiefly occurs in the must rather than in the wine as polyphenol oxidase is inhibited by ethanol. Chemical oxidation, which involves the same substrates as those of the enzymic one, is the main cause of browning in white wines (Singleton et al., 1978; Somers et al., 1987). The reaction, essentially of an autocatalytic nature, presumably takes place via an oxidative mechanism and a radical coupling through the intermediate semiquinones, leading to C−C, C−O, and O−O bonds (Cilliers and Singleton, 1989, 1990). This causes the formation of oligomers, which are still soluble in wine and of a light yellow color, the shade and intensity of which are correlated to the extent of the system of conjugation of the compounds thus formed (Cilliers and Singleton, 1991). These chemical reactions are extremely slow in wine and may require weeks or months even though metal ions such as Fe^{3+} may catalyze oxidation (Goristein et al., 1985).

If until now, therefore, browning has been considered mainly in relation to the impoverishment of the sensorial characteristics and the reduction of the shelf life of wine, the nutritional aspects of browning should also be borne in mind. Phenol oxidation leads to a reduction of oxidizing ability of wine (Frankel et al., 1995; Simonetti et al., 1997), and a reduction of bioavailability of several essential amino acids such as lysine, tryptophan, histidine, cysteine, and methionine (Felton at al., 1992; Hurrell et al., 1982; Hurrell and Finot, 1984) by formation of oxidized phenol−protein compounds (Kalyanaraman et al., 1987; Wada et al., 1969) in the duodenum tract of the digestive system.

The main technological response against maderization has been to decrease the amount of polyphenols present in white wines by avoiding or reducing the maceration of the grape skins in the must as well as by the adsorption of polyphenols from wine. This latter strategy has been the subject of the present study.

To reduce the maderization phenomena, the enological industry traditionally uses additives such as potassium caseinate, which, for its characteristic of almost immediate flocculation due to the action of acidity, is suitable for white wines, which are poor in electronegative colloids and do not provoke "surcollage" (Bastasin and Ceresa, 1991). The potassium caseinate employed at the end of the fermentation (Cantarelli et al., 1989; Bastasin and Ceresa, 1991) constitutes a very effective additive for polyphenols, and it could cause haze in the wines owing to the formation of protein−polyphenol complexes (Siebert et al., 1996a,b), and so it is usually eliminated by bentonite adsorption; moreover, the potassium caseinate is not reusable or regenerable.

Another protein used is gelatin (Amati, 1976, 1981), which presents problems similar to those of caseine with a lower ability in polyphenol removal. The use of adsorbents should reduce such drawbacks, even if in this case, at least in the Italian legislation, they could be used only as adjuvants of filtering.

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Some adsorbents such as active carbons, polyvininylpolypyrrolidone (PVPP), and bentonite are already used to stabilize wines and fruit juice also from haze-active polyphenols (Siebert and Lynn, 1997a).

The active carbons, at normal doses employed in wines (100−500 mg/L), provoke a leveling in sensorial properties because of their low selectivity. The polymers of polyvinylpolypyrrolidone could be also used, but they are not very regenerable and therefore cause also problems of waste disposal.

Bentonite is another adsorbent much used in enology in white wines to eliminate the protein in suspension, but from apple juice (Silbert and Lynn, 1997a) it induces generally only a modest reduction in the polyphenol content (Triberti and Castino, 1992).

The present study has sought to identify a nonconventional adsorbent for enology that could compete in effectiveness with potassium caseinate in the removal of phenols and in the stabilization against browning and could also be regenerable and reusable.

The study compares several nontoxic (food grade) and inexpensive polymeric adsorbents such as different types of chitosan, a biopolymer byproduct of the fish industry, a scleroprotein byproduct of cattle slaughtering, and a polylactic acid.

For the chitosan our group had begun preliminary studies on commercial wines (Cesarin and Pifferi, 1986; Spagna et al., 1994) subjected already to stabilization treatments with potassium caseinate and bentonite, therefore with a low polyphenol content, for which the wines did not allow a more general extrapolation. Although these previous results were partial, they showed that the chitosan has a high affinity toward polyphenol. This research foresees the employment of adsorbents on two Italian raw wines (Trebbiano and Albana) of widespread consumption in Italy and Europe, with a high polyphenol content and consequently particularly susceptible to the maderization phenomena. To such end, the white wines were prepared specifically for this research, with a particularly elevated content in polyphenols (7−10 times greater with respect to the normal content) and without any treatment. Such a choice originated from the need to highlight and diversify the action of the different adsorbents to establish their real effectiveness against maderization.

In particular, the effects of these adsorbents on (a) the removal of several classes of phenols contained in the wine, such as total polyphenols, monomers (catechins) and oligomers, and acids, and (b) the stabilization against maderization as measured by accelerated tests were studied (Singleton and Kramling, 1976; Fernàndez-Zurbano et al., 1995; Mayen et al., 1997). Moreover, the possibility of regenerating and reusing chitosan was also investigated.

MATERIALS AND METHODS

Materials. Trebbiano and Albana wines (1996 vintage), were produced expressly for this research, after a particularly elevated period (∼80 h at 25−28 °C) of maceration of the grape skins with the must and without any clarification treatments. Both of these wines are produced in the Emilia-Romagna (Italy).

Bentonite (Superbenton, Dal Cin Co.) was used for the pretreatment of the wines. The adjuvants employed in the study included a number of chitosans, namely Chitosom (Quindao Co.), Profloc (Protan Co.), Seacure (Protan), and Kurita (Lion Co.), as well as a scleroprotein from bovine origin, M3 (Inaclo Co.), which is a commercial preparation comprising bovine horn and hoof, and finally Biopol (Grace Co.), a polylactic acid (Sigma Co.). Potassium caseinate (Tillmans) was used as a reference adjuvant. It should be noted that currently only caseinate is authorized for use in wine-making. Notwithstanding, to test the properties of the various adsorbent materials, the same procedure was adopted for all of the polymers. Other reagents were of analytical grade and supplied by Carlo Erba (Milan, Italy).

Stabilization Treatments. Before testing, each sample of wine was clarified with bentonite (1 g/L) by stirring it very slightly for ∼30 min at room temperature. The wine was then separated by filtration. To eliminate impurities, the chitosans were preliminarily ground and sieved at 75−125 μm and washed with 0.1 M NaOH, then with 0.1 M H_2SO_4, and finally with water until a neutral solution was obtained.

M3 was ground and sieved at 75−125 μm and then washed with water, ethanol, acetone, and ethylic ether. The caseinate was allowed to swell in distilled water for 45 min.

Twenty milligrams of the various adsorbents was added to 50 mL of Albana and slightly stirred at 25 °C, with a contact time of up to 2 h, for the kinetic determination. The solutions were analyzed after centrifugation at 2000*g*. Subsequently, 50 mL aliquots of Albana and Trebbiano were treated with 20 or 40 mg of the adsorbents, as described above, for 1 h for the stabilization adsorption studies.

Methods of Analysis. Three replications were analyzed for each sample. Titrable and volatile acidities, alcoholic degree, reducing sugars, and sulfur dioxide were determined according to the official OIV methods of analysis (Office Internationl de la Vigne et du Vin, 1990). Δ indicates the percent removal rate, that is, the ratio between the difference in the analytic values obtained for the treated and nontreated wines and the initial value for the nontreated wine.

Total polyphenols (TPh). The Folin−Ciocalteu assay was used to measure total polyphenols (Singleton, 1974). Concentrations were determined by means of a calibration curve by which the total polyphenols reactive to the Folin−Ciocalteau reagent were expressed as milligrams per liter of gallic acid: polyphenols (TPh, mg/L) = $196.8 \times A \times D$, where A is the absorbance at 750 nm and D the number of dilutions performed.

Flavans Reactive to Vanillin (FRV). The concentration of polyphenols susceptible to reacting with vanillin in a highly acid environment was determined (Margheri et al., 1972). Concentrations were determined by means of a calibration curve similarly plotted with (+)-catechin monohydrate: catechin (g/L) = $(A + 0.0287) \times 0.0260 \times D$, where A is the absorbance at 500 nm and D the number of dilutions made.

Proanthocyanidins (Pr). The method for determining proanthocyanidins is based on their transformation into anthocyanidins, which leads to a shift of color toward the red zone in a warm and acid environment (Margheri et al., 1972). Pr concentration is expressed as the amount of cyanidin chloride, with Pr (mg/L) = $A \times D \times 930/V$, where A is the absorbance at 550 nm, D the initial dilution of the wine, and V the volume of diluted wine.

Spectrophotometric Analysis. Absorbance measured at 320 nm, which corresponds to the amounts of hydroxycinnamoyltartaric esters and hydroxycinnamic acids (TIc), with TIc (g/L) = $A/6.88$, where A is the absorbance of the wine diluted 11 times as measured with solutions of caffeic acid (14−300 mg/L) in a model system of wine, water, and 10% ethanol. Absorbance at 420 and 660 nm corresponded to brown color and turbidity, respectively.

Trichromatic Analysis. Wine color variations before and after treatment with the various adsorbents were evaluated by determining the values for Hunter's parameters ΔL^*, Δa^*, and Δb^* by trichromatic analysis. On the basis of these values, ΔE, defined as $[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$, was first calculated; values greater than at least 2 indicated two colors that can be distinguished by the human eye (Spagna et al., 1994).

Maderization Test (Mad). The accelerated Mads entailed determining the increase in absorbance at 420 nm of wine samples contained in test tubes filled to 50% and placed in an oven at 55 °C for 5 days. These tests, conducted on treated

wines and on wines treated with any type of absorbents, enabled us to calculate the difference of maderization values (ΔMad).

Extraction of the Proanthocyanidins. The proanthocyanidins (Pr) were extracted and isolated from grape seeds, as they contain the most elevated concentration of the whole grape.

Sixty grams of seeds was weighed, then ground in a mortar, and put into a bottle of dark glass with 1.5 L of a acetone/water (1:3) solution for 48 h. After the solution had been filtered, the acetone was evaporated under vacuum at 40 °C. The aqueous solution was purified by ethyl ether/water (3:1) extraction. The residual ether was eliminated under vacuum at 40 °C. The proanthocyanidins was poured into a column containing Shepadex LH-20 (height = 10 cm, diameter = 4 cm) pre-equilibrated with water. The column was washed with 3 L of a methanol/water (1:1) solution at a flow rate of 1.9 mL/min and then eluted with 1 mL of an acetone/water (70:30) solution.

Adsorption Isotherms. Before the regeneration tests of the selected adsorbents were performed, further information on the mechanism interaction between the chitosan and the various polyphenols susceptible to removal was sought.

Catechin was chosen as a representative molecule of flavans reactive to vanillin (FRV), and caffeic acid was chosen for hydroxycinnamic (TIc). Tests were performed on proanthocyanidins extracted from the seeds because of the lack of commercial standard for the class of proanthocyanidins (Pr).

Isotherms were drawn by placing in contact a constant quantity of chitosan with equal volumes of a solution of polyphenol in increasing concentration. For the molecules selected the analyses were made in a model solution model of wine at 10% alcohol, containing 5 g/L of tartaric acid. For the production of the mixture, the water used was deoxygenated by N_2 bubbling and correcting the pH up to 3.1 with 0.1 M NaOH.

Regeneration of Chitosan. Attempts were made to optimize NaOH concentrations and the regeneration time for the chitosan Profloc.

Effect of NaOH Concentration. One hundred and twenty milligrams of Profloc was placed in contact with 150 mL of Albana for 1 h at 25 °C under slight magnetic stirring. After the wine had been removed, the chitosan was washed with an equal volume of water and then subdivided into three fractions of the same weight. Each fraction was brought into contact under stirring with 50 mL of a regenerating solution of NaOH at 0.02, 0.1, and 0.2 M for ~2 h. All fractions were then washed with a solution of 0.0005 M sulfuric acid and water and brought into contact again with 50 mL of Albana for 1 h.

Determination of Regeneration Time. One hundred and sixty milligrams of Profloc was placed in contact with 200 mL of Albana for 1 h at 25 °C under slight magnetic stirring. After the wine had been removed, the chitosan was washed with an equal volume of water and then subdivided into four fractions of the same weight. Each fraction was brought into contact with 50 mL of a regenerating solution of 0.1 M NaOH for 5, 15, 30, or 60 min. All fractions were then washed with a solution of 0.0005 M sulfuric acid and water and brought into contact with 50 mL of Albana for 1 h.

Determination of Regeneration Cycles. A series of regeneration tests were then conducted. Two hundred milligrams of Profloc was placed in contact with 250 mL of Albana for 1 h at 25 °C under magnetic stirring. Wine was separated from chitosan. Two hundred and fifty milliliters of distilled water was added to the Profloc, and the mixture was stirred for 10 min, after which time the washing water was removed and the chitosan was placed in contact with 250 mL of 0.1 M NaOH for 30 min under stirring; the alkaline solution was then removed. Profloc was washed with 250 mL of 0.0005 M sulfuric acid for ~10 min, after which time the solution was removed. The regenerated and washed Profloc was put into contact with another 250 mL of Albana for a new adsorption and regenerating treatment; this operation was repeated 15 times.

Table 1. Characteristics of White Wine Samples (Trebbiano, Albana)

analysis	wine	
	Trebbiano	Albana
pH	2.97	3.08
alcohol (%)	10.9 ± 0.18	12.7 ± 0.22
acidity (g/L)		
titrable (tartaric acid)	10.20 ± 0.02	9.20 ± 0.02
volatile (acetic acid)	0.70 ± 0.05	0.92 ± 0.05
reducing sugars (g/L)	0.85 ± 0.04	0.10 ± 0.005
SO_2 (mg/L)		
free	6.40 ± 0.06	6.40 ± 0.06
total	89.6 ± 0.09	83.2 ± 0.08
total polyphenols (TPh) (g/L)	0.740 ± 0.036	1.76 ± 0.086
flavans (FRV) (g/L)	0.328 ± 0.016	0.688 ± 0.035
proanthocyanidins (Pr) (g/L)	0.882 ± 0.042	2.57 ± 0.119
total hydroxycinnamates (TIc) (g/L)	0.151 ± 0.004	0.400 ± 0.005
absorbance (λ = 420 nm)	0.160 ± 0.004	0.344 ± 0.005
turbidity (λ = 660 nm)	0.120 ± 0.003	0.180 ± 0.003
maderization (Mad)	1.00 ± 0.03	2.70 ± 0.04
trichromatic values		
L^*	97.7	93.6
a^*	−1.5	−1.6
b^*	12.6	25

Effect of Storage on the Regeneration of the Chitosan. The effect of storage on the regeneration of the chitosan, Profloc, already used in wine treatment, was also studied. Two hundred and fifty milliliters of Albana was treated with 200 mg of Profloc, and then the Profloc was subdivided into five fractions of equal weight. One of the fractions was immediately regenerated as described above and placed in contact with 50 mL of Albana. The other fractions were stored for 5, 10, 30, and 45 days, respectively, in an open container away from light and at room temperature; no special precautions were taken during storage. This situation was meant to simulate industrial storage prior to the regeneration treatments. After the established time had elapsed, the Profloc was regenerated according to the standard procedure described above and then placed in contact with 50 mL of Albana.

RESULTS AND DISCUSSION

The tests for the removal of the various phenolic species were conducted on wines (Trebbiano and Albana) prepared with a prolonged contact between must and marc during wine-making and without any clarification treatment, so as to allow a very high polyphenol content (Table 1). It was in fact observed that maderization was high in both wines, particularly in Albana. This characteristic makes these wines particularly suitable for comparing the polyphenol removal and stabilization against maderization properties of the various new adjuvants tested with those of potassium caseinate, the adsorbent most widely used in wine-making. The crude wine samples, however, exhibited a high degree of haze, a factor that was liable to adversely affect adsorption. Pretreatment with bentonite was therefore performed to reduce turbidity (by ~80%), causing in the process only a slight adsorption of the total polyphenols (~5−10%). Bentonite is usually capable of adsorbing haze active protein, whereas it has very little effect on free and haze active polyphenols (Siebert and Knudson, 1989; Siebert and Lynn, 1997a−c).

To employ the potassium caseinate and the other adjuvants under the most appropriate conditions, the removal kinetics of the various classes of polyphenols, total polyphenols (TPh), flavans reactive to vanillin (FRV), proanthocyanidins (Pr), and total hydroxycinnamics (TIc), was studied using the wine with the

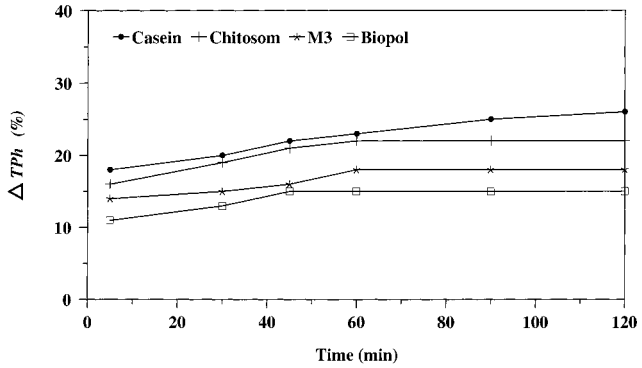

Figure 1. Total polyphenol (ΔTPh) removal rates in Albana wine with various adjuvants.

highest polyphenol content, that is Albana. Figure 1 shows the adsorption kinetics only of the TPh on different adjuvants, as the other classes of polyphenols follow the same pattern. All of the adjuvants tested exhibited considerable adsorption capacities at reduced contact times. A slight continuous increase in the adsorption of the TPh was observed only with potassium caseinate. After 60 min of contact time, all of the other adjuvants were in a state of equilibrium. This contact time was therefore adopted for all of subsequent adsorption tests. The maximum concentration of the adsorbents employed for the stabilization adsorption studies was equivalent to that of caseinate generally used in wine-making of these kinds of wine.

Potassium caseinate is a water-soluble salt, which, in the acidity and composition conditions typical of wine, precipitates in the colloidal form of floccules (isoelectric point of casein = 4.6). Following on the aggregation of the colloids, the precipitate thus formed is capable of taking the polyphenols into its protein structure and/or adsorbing them by setting up hydrogen bonds between its functional groups (−COOH, $-NH_2$, −OH, etc.) and the hydroxyl groups of the polyphenols (Siebert et al., 1996a). Moreover, the presence of aromatic groups both in the casein and in the polyphenol may favor the formation of $\pi-\pi$ type bonds as well.

Let us now take a look at the behavior of the various adjuvants with respect to that of potassium caseinate (Figures 2 and 3).

The property of the chitosans (Chitosom, Profloc, Kurita, and Seacure) to remove various classes of polyphenols (Figure 2) is largely dependent on the polymer concentration employed. The removal values for the three main classes of polyphenols (TPh, FRV, and Pr) (Figure 2A−C) at the lowest concentration of the chitosans (0.4 g/L) were almost always less than those of potassium caseinate, whereas at the higher concentration (0.8 g/L) the removal values, in particular for Chitosom and Profloc, were close to those of the casein. The ΔTIc values for chitosans were on average greater than those for caseinate (Figure 2D). In general, among the chitosans, Chitosom was seen to remove a little more than Profloc, whereas, except in a few cases, Kurita and Seacure were found to remove less.

With regard to the stability assays (Figure 3), as was expected, maderization (Mad) was seen to be reduced much more by those chitosans (Chitosom and Profloc) that on average remove more polyphenols. The ΔMad values for Chitosom and Profloc were in fact practically equal to those for the caseinate except in the case of Chitosom used at a concentration of 0.8 g/L in Albana. The ΔMad values obtained with Kurita and Seacure were instead always less. In addition to the removal of TPh, FRV, and Pr, these findings may also be accounted for by the considerable capacity of chitosan to adsorb TIcs, which seems to play a major role in wine oxidation (Singleton et al., 1978; Cilliers and Singleton, 1990).

Chitosan is essentially a polyglucosoamine deriving from the deacetylation of chitin (*N*-acetyl-2-amino-2-deoxy-β-D-glucopyranosidase). At the pH of wine, TIc acids are partially present in a dissociated form and may therefore be adsorbed also by means of interactions of an electrostatic nature by the protonate aminic groups of the biopolymer. Compared to that of caseinate, upon flocculating in the wine the surface of chitosan is limited. Nevertheless, upon its swelling, which in wine is quite marked (with a 7−8-fold increase in weight), the surface area of chitosan does increase. This phenomenon may partially account for the considerable ability of chitosan to remove polyphenols. Why the various chitosans behave differently, however, is not easy to explain. For instance, this different behavior does not appear to be associated with the degree of deacetylation, the values of which are fairly similar (76−83%).

The capacity of M3 to adsorb the three classes of polyphenols, TPh, FRV, and Pr (Figure 2A−C) was always much less than that of the chitosans Chitosom and Profloc and of the caseinate. The amount of TIcs removed by M3 (Figure 2D) was, however, slightly greater than that removed by caseinate. M3 is a scleroprotein essentially of the alpha kind with a high degree of crystallinity and a reduced surface area (Kirk and Othmer, 1964).

The main interactions of M3 with the polyphenols could be ascribable to the setting up of hydrogen bonds between amino acid residues of scleroprotein and the oxyhydrilic groups of polyphenols, whereas, to a limited extent, basic amino acids such as lysine and arginine, which are protonated at the pH of wine, could contribute to adsorption of acid phenols with electrostatic interactions.

M3 reduces Mad (Figure 3) to a lesser extent than caseinate, probably because it absorbs fewer polyphenols.

For all three main classes of polyphenols, namely TPh, FRV, and Pr, Biopol's removal ability (Figure 2A−C) was less than that of caseinate and of the chitosans Chitosom and Profloc. The amount of TIcs removed by Biopol (Figure 2D) was, however, slightly greater than that reported for caseinate and in some cases close to that for the chitosans. Biopol, being a polylactic acid, has a carbonylic carbon every three atoms of the main chain, and it can provide a large number of sites suitable for the setting up of hydrogen bonds with the polyphenols. The fact that polyphenols were poorly removed would seem to explain the values reported for ΔMad (Figure 3), which were always less than for caseinate, in particular in Trebbiano.

The Hunter method was used to determine color variations using the three spatial coordinates L^*, a^*, and b^* (CIE space), where the vertical axis, L^*, represents brightness, which ranges from white (0) to black (100), and axes a^* and b^* represent the opposing colors, green-red and blue-yellow. The differences in these parameters (Δ) between the nontreated wine samples and those treated with the various adjuvants were

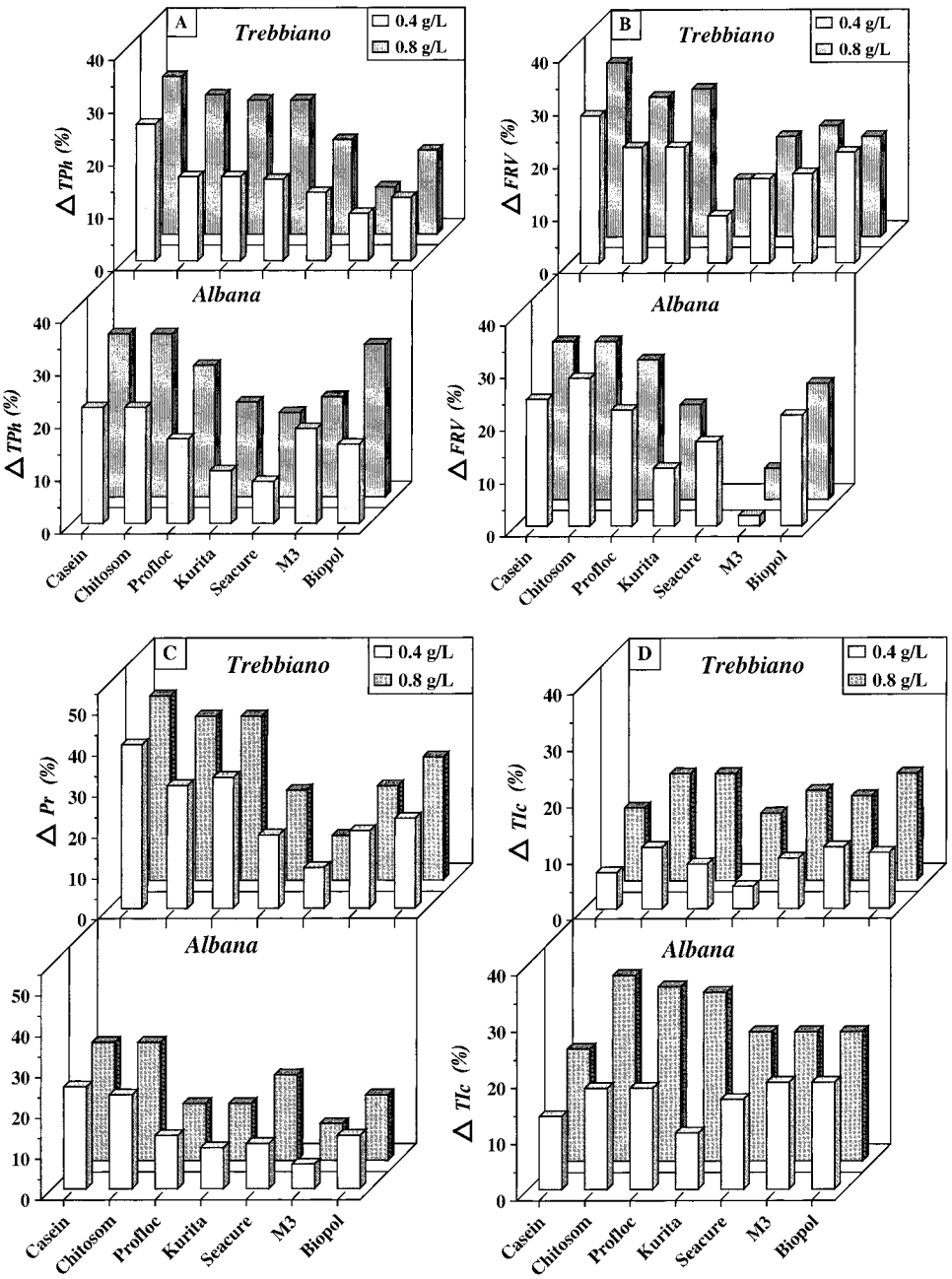

Figure 2. Removal of total polyphenols (ΔTPh, A), flavans reactive to vanillin (ΔFRV, B), proanthocyanidins (ΔPr, C), and total hydroxycinnamates (ΔTIc, D) in Trebbiano and Albana wines with various adjuvants at concentrations of 0.4 and 0.8 g/L. The results are the mean of three analyses (±10% with a 95% confidence interval).

considered. In both wines, variations in a^* following treatment with the various adjuvants were slight ($\Delta a^* = \pm 0.4$), while those in L^* were a little higher ($\Delta L^* = \pm 2$), with the greatest values being reported for Albana and for the adjuvant caseinates Chitosom and Biopol. The increases in the values of b^* were higher (up to 13) and always considerably greater than the values of a^* and L^* for the same treatment.

This means that in all cases examined, variations in the color of the wine, ΔE^*, which is the geometric mean of ΔL^*, Δa^*, and Δb^*, depend almost exclusively on Δb^*; this indicates a general reduction in the yellow color of the treated wines. The variations in color, ΔE^*, shown in Figure 4 were almost always >3 and therefore perceivable by the human eye. The greater values of ΔE^* were invariably found for the Albana wine and for the adjuvant caseinate.

Among the chitosans, the greatest variations in the values of ΔE^* were reported for Chitosom, followed by Profloc, Seacure, and Kurita. These findings on average seem to closely follow the same trend as that for the removal of the polyphenols.

In both wines, the ΔE^* values for M3 were quite similar, being almost equal in Trebbiano to those for the chitosans Kurita and Seacure (which adsorbed less) and, in Albana, always less for all chitosans.

Variations in the values of ΔE^* for Biopol were always marked in both wines; in the case of Trebbiano, they were greater than those reported for all of the chitosans, whereas in Albana they were only greater than those for the chitosans, which removed fewer polyphenols (Kurita and Seacure).

The adsorbents to be reused must first be regenerated. For instance, the chitosan Chitosom is an excellent

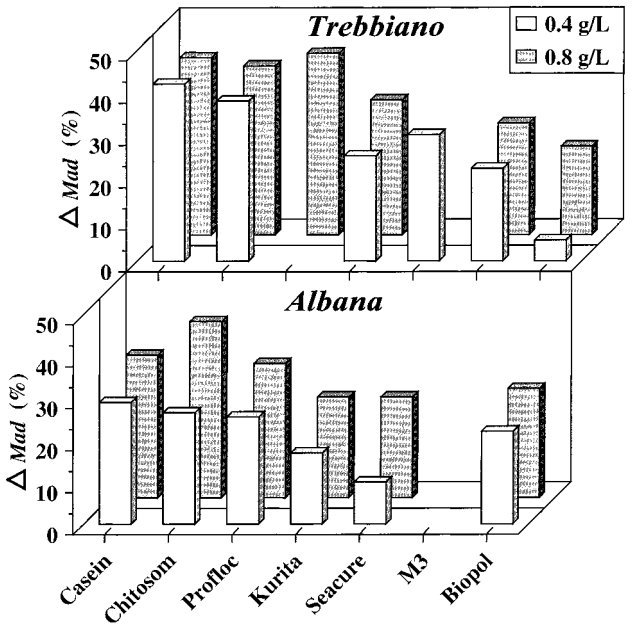

Figure 3. Reduction of maderization (ΔMad) in Trebbiano and Albana wines treated with various adjuvants at concentrations of 0.4 and 0.8 g/L. The results are the mean of three analyses (±5% with a 95% confidence interval).

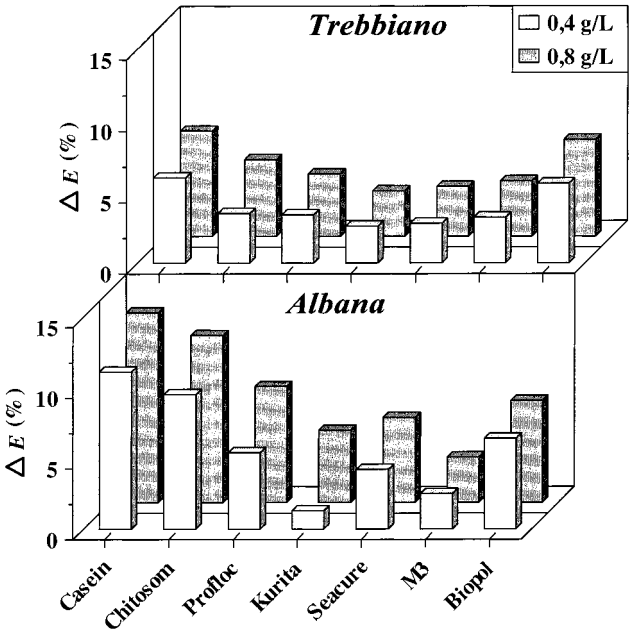

Figure 4. Variations of color (Δ*E**) in Trebbiano and Albana wines treated with various adjuvants at concentrations of 0.4 and 0.8 g/L.

adsorbent, yet already after its first use, its adsorption capacity toward the various classes of polyphenols is considerably reduced, diminishing by ∼40% in the case of TPh, FRV, and TIc and by as much as 50% in the case of Pr. Unlike polyphenols, chitosan is not soluble in an alkaline environment; thus, it appears to lend itself to being easily regenerated at an alkaline pH.

Of the chitosans tested, Chitosom gave the best results in terms of polyphenol removal and stabilization, but it is today prepared only in laboratory scale. With regard to suitability for regeneration in view of possible future industrial applications, it was decided to use Profloc, as this chitosan is more easily available on the market and its removal and stabilization rates are comparable to those of Chitosom.

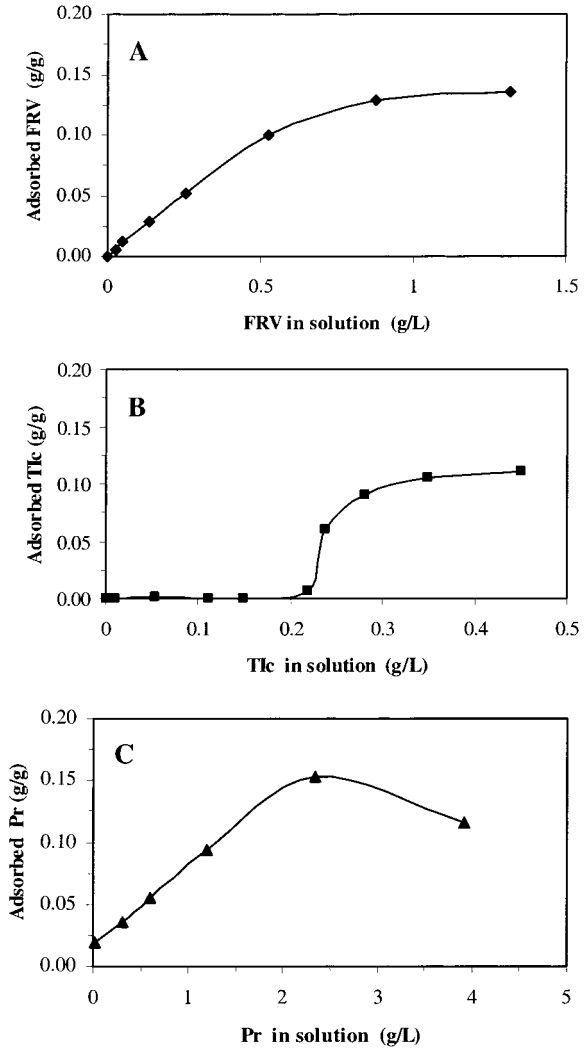

Figure 5. Adsorption isotherms (at 25 °C) of various classes of polyphenols: FRV (A), TIc (B), and Pr (C) with chitosan (Profloc) in a model wine solution.

Adsorption isotherms with the classification proposed from Giles et al. (1960) could complete the explanations of some results on adsorption mechanisms between the various classes of polyphenols and the chitosan Profloc.

The adsorption isotherm of the FRV (Figure 5A) on chitosan is the common Langumir type (L), which indicates the formation of a monolayer (at ∼0.14 g/g) (Giles et al., 1974a,b). The catechin molecules could be adsorbed prevalently by the setting up of hydrogen bonds with the hydroxyl groups of the chitosan.

Instead, the adsorption of TIc on chitosan (Figure 5B) follows a characteristic trend of an S curve (with a plateau at ∼0.11 g/g), which indicates a typical effect of "cooperative adsorption"; in this case further adsorption is favored by the presence of molecules already adsorbed on the support (Giles et al., 1974a,b). This phenomenon could be explained by considering that beyond the attracting forces solute−adsorbent, forces can be set up between the molecules of solute that, being stronger, increase the number of the sites and the adsorption enthalpy. A coherent mechanism with this trend (Giles et al., 1960) could foresee (a) a perpendicular orientation of the caffeic acid molecules adsorbed on the surface, which could be induced by a marked

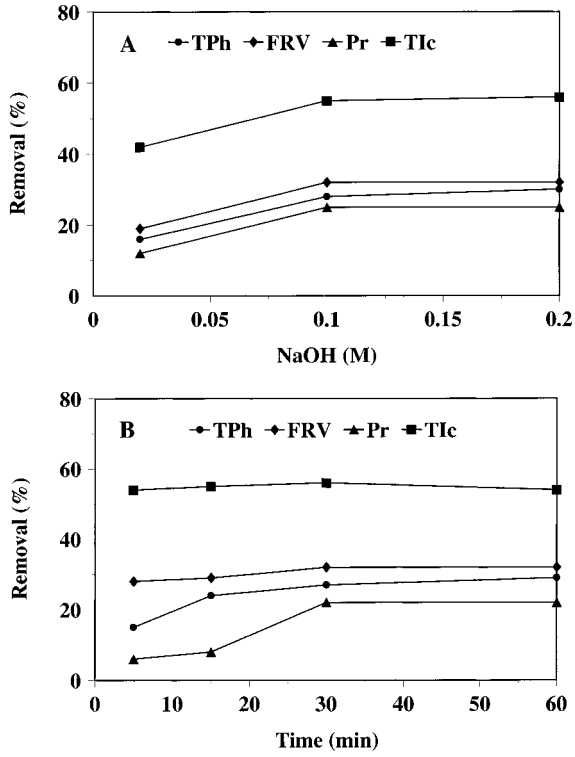

Figure 6. Removal of various classes of polyphenols (ΔTPh, ΔFRV, ΔPr, and ΔTIc) with chitosan (Profloc) in Albana as a function of NaOH molarity [(A) contact time, 60 min; temperature, 25 °C] and as a function of regeneration time [(B) 0.1 M NaOH; temperature, 25 °C].

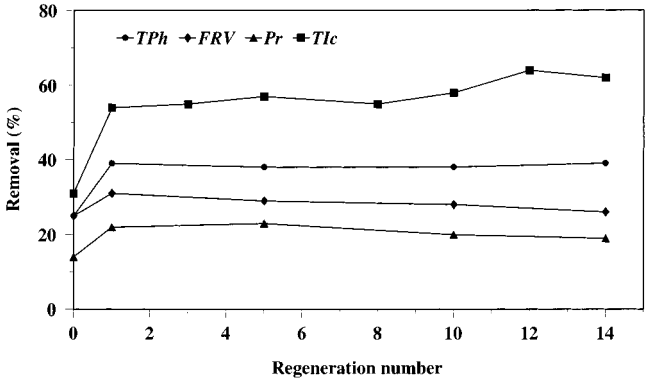

Figure 7. Removal of various classes of polyphenols (ΔTPh, ΔFRV, ΔPr, and ΔTIc) with Profloc in Albana as a function of the number of regeneration treatments (0.1 M NaOH; contact time, 30 min; temperature, 25 °C).

localization of the electrostatic interactions between the acid group with the protonate amine group of the chitosan, (b) the setting up of side by side association, by $\pi-\pi$ bonds among the rings of the adsorbed molecules, that help to hold them to the surface, and (c) a competition for the chitosan by tartaric acid (contained in the wine model solution), which allows the caffeic acid absorption only at a greater concentration.

The Pr isotherm (Figure 5C) was of the H type ("high affinity"; Giles et al., 1974a,b).

These curves could be considered a particular case of the *L* curves, in which the first part is practically vertical, because of the elevated affinity of the solute with the adsorbent. The solutes that show this trend are generally large units, like the Pr, oligomers of catechin and epicatechin, with a number of monomers that goes from two to eight. The presence of a maximum (at ~0.16 g/g) could be due to the tendency of the Pr to aggregate; it therefore appears that with an increase in Pr concentration, a point is reached at which the solute−solute van der Waals overcome solute−support interactions, so that some solutes are desorbed from the surface (Giles et al., 1974a).

A study was conducted to determine the optimal conditions for Profloc regeneration. For this, to best preserve a good adsorption capacity of chitosan, its regeneration requires the use of NaOH at a molarity of at least 0.1 (Figure 6A). The kinetic tests conducted (Figure 6B) indicated that regeneration requires on average no less than 30 min for Pr, whereas for FRV and TIc a minimum contact time is already sufficient to obtain maximum removal values. Among the various classes of polyphenols, the Prs are those with greater molecular dimensions, so they present diffusion problems toward the chitosan structure (steric hindrance). From a practical point of view these tests are essential, and with a view to the possible recovery of the adsorbed polyphenols, it is important that the regeneration conditions be as neutral as possible so as to minimize the oxidative degradation of the polyphenols, which is favored at alkaline pH values.

In the consecutive regeneration treatments (Figure 7) it was noted that the removal of all the classes of polyphenols examined increased after the first treatment. Moreover, at least until the 14th regeneration treatment, the amount of the various polyphenol classes removed did not vary significantly. As the chitosans used for the regeneration tests were pretreated with a procedure similar to that of the regeneration treatment, it may be assumed that the increase in the initial removal rate was not ascribable to the elimination of some chemical species present as an impurity in the chitosan or to its structural modification, but rather to the action of the phenolic species bound to the polymer. Therefore, the molecules of the adsorbed polyphenols are not totally removed during regeneration, but rather transformed into stable species. A reaction may be assumed to take place between the aminic group of the chitosan and the carbonylic group of the quinones originating from the phenolic molecules oxidized in the alkaline regeneration environment. This reaction is rather evident already after the first regeneration treatment as evidenced by the brown color acquired by the regenerated chitosan.

In an industrial application of the treatment with chitosan and subsequent regeneration, it is unlikely that chitosan will be immediately regenerated after adsorption as in the laboratory. In view of this, storage tests were carried out showing that chitosan can safely be stored for up to 45 days, a time which is long enough for industrial applications, without any variations in the adsorption rate for any of the phenolic species examined.

CONCLUSIONS

Several polymeric adjuvants (chitosans, scleroprotein, and polylactic acid) were studied as alternatives to potassium caseinate for the stabilization of white wines against browning. In particular, two chitosans, Chitosom and Profloc, were shown to effectively remove the polyphenols (polyphenols reactive to Folin−Ciocalteu, flavans reactive to vanillin, and proanthocyanidins) contained in two white wines (Trebbiano and Albana) with a high polyphenol content and to have a stabilization capacity comparable to that of potassium caseinate.

Furthermore, the removal values of the chitosans for the total hydroxycinnamates acids in the wine were seen to be much higher. The results for the other polymers tested were generally poorer than those for caseinate.

The possibility of regenerating the chitosan and of reusing it for at least 14 cycles was also demonstrated. The perfect regeneration of the chitosan used as an adsorbent was also found to be possible even after storage under industrial conditions for relatively long periods. The chitosan's low cost and its regeneration and reuse possibility, with consequent reduced environmental impact, are all factors that make this biopolymer particularly interesting for industrial applications in wine-making as compared to caseinate.

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