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Riboflavin removal by commercial bentonites and charcoals in white and red wines

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PAPER

Abstract

Riboflavin (RF) represents one of the primary molecules undergoing photodegradation in wine, and its excited form acts as an intermediate in light-induced oxidation reactions responsible for the light-struck fault. A recent study has revealed bentonites (BENs) and charcoals (CHAs) as the most promising fining agents for removal of RF in model wine. This work explored their potential on both white and red wines, where polyphenols could interfere in the fining agent–RF interaction. A total of 11 BENs and 11 CHAs were compared. BENs exhibited a limited capacity, while decoloring carbons confirmed a great attitude for removal of RF in white wine, even at low dosages. Nevertheless, efficiency of CHAs shows a sensible reduction in red wine.

Keywords: bentonite, charcoal, red wine, riboflavin removal, white wine

Introduction

Shelf life of wine assumes a complicated meaning because of high diversity in both wine styles and typical characters searched by consumers. The quality of final product depends on both winemaking practices (fermentation and post-fermentation fining treatments) and storage in bottles. Maturation of wine should be strictly controlled to avoid undesirable chemical reactions, with particular attention to temperature and light conditions during transportation to the final seller and its often long storage. The post-fermentation is particularly critical for red wine because it commonly requires more time for maturation. Exposure to light could have two independent effects on wine: first, it could activate oxidative reactions, and second, it could cause heat-induced damages on direct exposure to sunlight (Jackson, 2011). In order to limit light-induced oxidation, the best protection is represented by storage in dark conditions and use of ultraviolet (UV)-masking bottles. It is known that green glass bottles can filter a larger spectrum of UV-Visible (VIS) wavelengths than uncolored bottles, thus reducing the rate of photodegradation reactions (Grant-Preece *et al.*, 2017). However, this technological requirement is in contrast with the consumer preference for uncolored bottles, which is rapidly growing along with market for rosè wines. Riboflavin (RF) assumes a particular relevance in wine's shelf life because of its implication in intermolecular photoreduction. In fact, RF is one of the principal responsible for the development of light-struck taste, a wine fault characterized by 'cooked-cabbage' aroma. RF is thermostable at the temperature of winemaking process, but it is highly photosensitive and can easily undergo photochemical degradation (Sheraz *et al.*, 2014). In aqueous solutions, RF is implicated in photosensitization reaction that acts following two mechanisms. The type 1 pathway results in the formation of two charged free radicals, RF and a

target molecule, through hydrogen- or electron-transfer reactions between the excited triplet state of RF ($^3RF_1$) and the substrate. When this substrate is methionine (Met), it leads to the formation of methional. While RF radical is involved in a recycling reaction, methional is unstable, and thus readily decomposes to methanethiol (MeSH) and acrolein. In addition, two molecules of methional can combine into dimethyl disulfide (DMDS). The type 2 process uses the energy transferred from $^3RF_1$ to oxygen to form singlet oxygen, which can then react with multiple biological substrates. In light-struck reactions, it oxidizes methionine sulfur, generating methionine sulfoxide (Silva *et al.*, 2019). MeSH and DMDS are the main compounds responsible for the 'cooked-cabbage' aroma of light-struck reaction; they have a perception threshold of 2–10 μg/L and 20–45 μg/L, respectively (Fracassetti *et al.*, 2019). In a recent study, Fracassetti and colleagues (2019) explored the relationship between Met and RF and verified that Met degradation could be avoided if RF concentration remains below 50 μg/L. In a different study, the same authors compared different fining agents with the aim to determine the clarification practice that could be the most efficient one for removal or lowering of RF (Fracassetti *et al.*, 2017). This study compared several fining agents, namely polyvinylpolypyrrolidone (PVPP), bentonite (BEN), zeolite, silica, kaolin, albumin, and charcoal (CHA), using different concentrations in model wine, and identified BEN and CHA as the most promising agents. However, the efficacy of fining agents seems strictly dependent on the media composition, as demonstrated by comparison of BEN, CHA, and zeolite performance in both model wine and a real Chardonnay wine (Fracassetti *et al.*, 2017). In this case, the CHA removal efficacy was lower in real wine at all the tested dosages. From the applicative point of view, it would be interesting to understand whether different categories of BENs and CHAs could be used successfully in real wines. Besides white wines, which are commonly preferred for RF studies because of being more subjected to light-struck fault, red wines represent an interesting case of study because, as reported by Lagunes *et al.* (2017), their extracts could act as photosensitizer if exposed to light, generating $^1O_2$, which in turn is able to oxidize other compounds. Moreover, polyphenols make more complex the removal of RF because of the high affinity of phenols to CHAs (Lisanti *et al.*, 2017). A fine balance between the quenching and the photosensitizing nature of red wine polyphenols is of particular interest for monitoring the removal of RF in this kind of wine. In the present study, different commercial BENs and CHAs, provided by different suppliers, were compared in two wines to explore deeply their ability to remove RF. In particular, it was verified whether this ability is correlated to other fining properties, such as protein removal and decolorizing (DEC) capacity.

Materials and methods

Chemicals and reagents

Methanol, *trifluoroacetic acid (TFA)*, tartaric acid, acetic acid, and sodium acetate were purchased from Sigma-Aldrich (Milano, Italy). Enocyanin powder (Enocianina GSE12 UC) was provided by EVER S.R.L. (Pramaggiore, Italy). Water of HPLC grade was obtained from Milli-Q system (Millipore Filter, Bedford, MA, USA).

Bentonites and charcoals

Eleven BENs, two calcic (CAL), one sodic–calcic (SOD-CAL), and eight sodic (SOD), and 11 CHAs, four deodorizing (DEO) and seven DEC, were provided by different commercial suppliers.

Wine selection

The wines were selected from different wine samples for their medium–high RF content. One white wine (Glera base wine, harvest 2018, produced by School of Oenology Cerletti, Conegliano (TV), Italy), with a content of 123.9 μg/L of RF, was chosen for BEN trials. This wine showed 9.6% alcohol and 6.5 g/L of titratable acidity. One white wine (Glera base wine) and another red wine (Wildbacher), both produced by Collalto winery (Susegana (TV), Italy), with 104.0 μg/L and 138.6 μg/L of RF, respectively, were chosen for CHA treatments. Glera wine (harvest 2018) showed 11.6% alcohol and 6.2 g/L of titratable acidity, while Wildbacher (harvest 2018) showed 12.5% alcohol and 5.1 g/L of titratable acidity.

Bentonite's protein adsorption trial

Deproteinization capability of BENs was evaluated according to modified Oeno 441-2011 Resolution (The International Organization of Vine and Wine [OIV], 2011). Trial solution was prepared using bovine serum albumin protein (BSA) 500 mg/L instead of ovalbumin 5 g/L, and the protein was dissolved into model wine (5 g/L tartaric acid, 12% v/v ethanol, pH = 3), whereas in the OIV method, the ethanol was absent. Eight BEN dosages (namely 10, 20, 30, 40, 50, 60, 70, 80 g/hL) were tested in order to determinate BEN adsorption curves. Samples were shaken and maintained in dark at 25°C for 30 min before performing protein quantification using a Pierce BCA Protein Assay Kit (Fischer Scientific Italia, Rodano (MI), Italy). After an incubation of 30 min at 37°C, samples were read using Microplate Reader (Euroclone) and data were elaborated by Software Manta and quantified over a calibration curve of BSA between 12.5 mg/L and 1000 mg/L.

Charcoal's decolorization power in enocyanin solution

Decolorizing power (DP) of a commercial CHA was evaluated applying the method reported in OIV 7/2007 (OIV, 2007) with little modifications. The enocyanin solution was prepared adding 4.5 g/L of enocyanin, 7 g/L of tartaric acid, 4 g/L of acetic acid, and 7 g/L of sodium acetate. The solution was stirred to allow complete dissolution and centrifuged for 10 min at 14,000 g. Supernatant was recovered and its absorbance was read in quartz cuvettes (2-mm path length) at three different wavelengths, namely 420, 520, 620 nm, using spectrophotometer ULTROSPEC 2100pro (Amersham Bioscience Europe GmbH, Cologno Monzese (MI), Italy). The color intensity (CI) was calculated as the sum of the three absorbance values standardized to a length path of 10 mm. Each CHA was added to 100 mL of enocyanin solution at a final concentration of 1 g/L. Samples were stirred for 30 min; after 10 min, they were collected into Eppendorf tubes and centrifuged for 10 min at 14,000 g to remove CHAs. Supernatant CI was measured as described for enocyanin solution. Finally, DP was expressed as percentage using the following equation:

$$DP = 100 \times \frac{CI1 - CI2}{CI1}, \quad (1)$$

where CI1 is the enocyanin solution color intensity and CI2 is its color intensity after CHA treatment. The specific DP of each CHA was calculated by three independent replications.

Bentonites and charcoals riboflavin removal in wine

Four BEN dosages were chosen for removal of RF in white wine (Glera wine with 123.9 µg/L of RF), namely 20, 40, 60, and 80 g/hL; the experiment was performed twice in 50-mL Falcon tubes. Tubes were vigorously shaken to allow dispersion of BEN and maintained at 25°C for 10 min before centrifugation at 14,000 g for 10 min. Removal assay was repeated in two independent tests. For CHAs, five dosages were selected for both red and white wines (Wildbacher and Glera, with 138.6 µg/L and 104 µg/L of RF, respectively), namely 10, 5, 2, 1, and 0.5 g/hL. The test was repeated for three times. Samples were shaken and kept in dark for 24 h. At the end of time contact, samples were mixed and centrifuged for 10 min at 14,000 g in order to assure removal of CHA. The supernatant obtained was used for both RF quantification and color intensity determination. For the latter analysis, control CI (CC) and samples CI (CS) were used in Eq. (1) in place of CI1 and CI2, respectively. Color intensity was calculated as 420-nm absorbance value for the white wine and sum of the 420, 520, and 620 nm absorbance values for the red wine.

Quantification of riboflavin in high-performance liquid chromatography (HPLC)

A Nexera HPLC system (Shimadzu) equipped with RF 20-A XS fluorescence detector was used. Filtered samples (20 µL) were separated in a Kinetex C18 (5 µm, 100 Å, 150 × 4.6 mm Phenomenex). Eluting solvents were as follows: (A) Milli-Q water and 0.1% of TFA v/v and (B) gradient-grade methanol and 0.1% of TFA v/v. The gradient program was 0–2 min, 30% B; 2–10 min, 30–60% B; 10–11 min, 60–100% B; 11–14 min, 100% B; 14–15 min, 100–30% B; and 15–18 min, 30% B. The flow rate was set to 0.6 mL/min and the column temperature was kept at 37°C. The RF was detected by fluorescence using 452 and 516 nm as excitation and emission wavelengths, respectively. RF was quantified using the external standard method. Data were acquired and processed with LabSolutions version 5.93.

Statistical analyses

R software (R version 3.0.1) was used for statistical analysis. Differences were evaluated by one-way ANOVA, Welch's ANOVA, and Kruskal–Wallis H test depending on data distribution. *Post hoc* analyses Tukey HSD test and Games–Howell test were used for ANOVA and Welch's ANOVA, respectively, while Dunn test with Holm correction was chosen as Kruskal–Wallis *post hoc* test. Correlations were tested using Pearson's correlation test. Statistical significance was attributed with $P < 0.05$ or a Confidence Interval of 0.95.

Results and discussion

Bentonite's deproteinization capacity

Bentonites are mainly used to remove proteins and avoid unpleasant haze in white wines. Selected BENs have been characterized for determining their performance in protein removal using modified Oeno 11/2003 (OIV, 2011) protocol. For this purpose BSA was considered more suitable than egg albumin because of its isoelectric point and absence of glycosylation, which make BSA more similar to wine proteins (Sarmento *et al.*, 2000). In addition, 12% ethanol was added to the test solution, as BEN is generally used in wine and it has been demonstrated that ethanol can influence the swelling of BEN, modifying its protein removal ability (Achaerandio *et al.*, 2001). Moreover, the quantity of protein used was 500 mg/L, more close to the real protein content in unstable white

Table 1. Summary of selected bentonites. For each bentonite, de-proteinization curve slope and related R^2 values are reported.

Sample ID	Category	Slope	R^2
BENT1	SOD	7.97	0.99
BENT2	SOD	7.17	0.99
BENT3	CAL	4.11	1.00
BENT4	CAL	2.44	0.94
BENT5	SOD-CAL	2.61	0.94
BENT6	SOD	6.75	0.96
BENT7	SOD	5.34	0.97
BENT8	SOD	5.30	0.97
BENT9	SOD	7.00	0.99
BENT10	SOD	4.99	1.00
BENT11	SOD	7.14	0.99

wines (Marangon *et al.*, 2011) than the 5-g/L of ovalbumin used in the original OIV method.

Removal curve slopes of 11 BENs belonging to CAL, SOD, and SOD-CAL categories were used to compare efficiency of BENs, the highest slope value corresponded to the best performing BEN. Data revealed a positive linear relationship between BEN dosage and quantity of protein removed in this range of concentration. Only in two cases, namely BENT4 and BENT5, R^2 was lower than 0.95 (Table 1) because of the lower absorbing capacity of these BENs. Statistical analyses revealed a significant difference between categories ($F_{(2,8)}$ = 10.5, P < 0.05) and *post hoc* test-grouped SOD-CAL with CAL being the reason of their low deproteinization capacity (Figure 1).

This trend is in accordance with Jönsson *et al.*'s (2009) findings, which demonstrated that SOD bentonites are characterized by about 10 times major swelling capability than CAL ones. The nature of cations arranged between

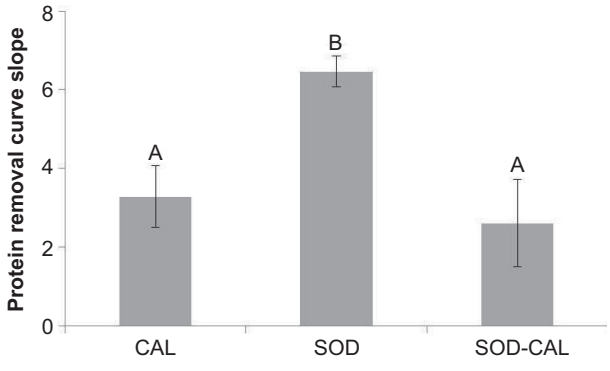

Figure 1. Slope index of protein removal curves. Least-square mean values and standard errors are presented. Different capital letters identify significantly different groups (P < 0.05) according to Tukey test.

montmorillonite lamellae strongly affects physical properties of BEN, resulting in a great difference in protein-binding ability. Sodium guarantees a major distance between BEN layers enhancing protein entrance and a higher availability of binding surfaces.

Bentonite's effect on removal of riboflavin

Attitude of BEN to removal of RF was recently studied in comparison to other fining agents (Fracassetti *et al.*, 2017). BEN was identified as one of the most useful fining agents. In fact, in the study conducted by Fracassetti *et al.* (2017), all the tested BENs (six, all from the same supplier) were able to remove about 60% of original RF in an RF-enriched model wine (350 µg/L of RF). For the screening presented in this work, BENs were chosen with the aim to explore a major variability of commercial products, and therefore 11 BENs furnished from seven different suppliers were selected. In Glera wine, BENs showed a limited removal of RF even at the highest dosage (on average, 28% of reduction at 80 g/hL) in accordance with the data reported by Fracassetti and colleagues (2017) when testing a calcic BEN at 100 g/hL in Chardonnay wine. Interestingly, the treatment with SOD-CAL BEN (BENT5) at 80 g/hL led to a sensible reduction, corresponding to about 60%, of RF (Fig. 2). Considering that white wines present a mean RF content of 115 µg/L (Cataldi *et al.*, 2002), this is the only BEN treatment that assured to decrease RF content below 50 µg/L, which is considered the threshold for light-struck taste risk (Fracassetti *et al.*, 2019). Nevertheless, several cases of white wine in which concentration of RF overcame 151 µg/L have been reported (Ournac, 1968; Pilcher, 1996), and it should be taken into account that high dosages of BEN could lead to severe side effects, that is, wine aroma depletion (Lambri *et al.*, 2012; Lira *et al.*, 2015).

Statistical analysis evidenced a significant difference among BENs only at 60 g/hL, and BENT5 confirmed to be the most efficient BEN. In two cases, BENT5 and BENT6, a statistically significant effect of dosage was registered (Figure 2). As also reported by Fracassetti and colleagues (2017), no evidence of different responses was observed between CAL and SOD. The statistical analysis revealed no significant correlation (r = -0.33, P = 0.328) between the percentage of removed RF at the highest BEN dosage and the protein removal curve slope, reinforcing the idea that protein and RF are linked to BEN surfaces through different mechanisms. BENs are known to link molecules by means of three different mechanisms: the first involves dipole bindings, the second is based on hydrogen bonding through the water bridge mechanism, and the third is based on Van der Waals forces (Luckham and Rossi, 1999). Probably, the chemical structure of RF, characterized by low polarity, induces the

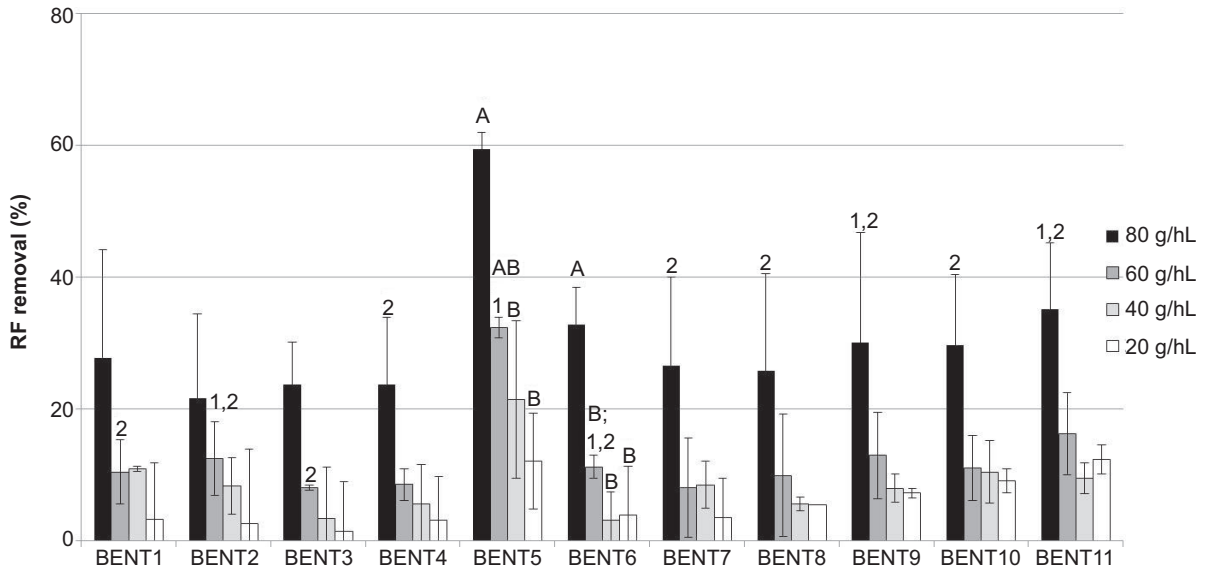

Figure 2. Riboflavin removal by bentonites. Mean values and standard deviation of two replicates are presented. Statistical differences between doses of bentonite are expressed by capital letters, while differences between bentonites at the same dosage are expressed by numbers (Tukey test, $P < 0.05$).

establishment of low polar bonds belonging to two latter types (Kasimova *et al.*, 2019).

Charcoal's decolorizing capacity

Active CHAs are commonly used in oenology to reduce organoleptic fault because of phenolic off-odors (Lisanti *et al.*, 2017) as well as fining agents to correct color intensity of white wines obtained by the vinification of red grapes. Their application depends on physical properties of CHA, in particular on the pore size, which strongly affects CHA permeability through molecular size exclusion. In fact, decolorizing CHAs are characterized by 20–500 Å macropores, while deodorizing CHAs show a dominance of small pores (Yahya *et al.*, 2015). Resolution Oeno 7/2007 (OIV, 2007) categorizes CHAs into the two groups based on the percentage of enocyanin removed from a model wine (decolorizing power). In particular, CHAs are assigned to DEC group if they are able to remove more than 40% of initial enocyanin, while they are recognized as DEO if the removed enocyanin is less than 40%. The decolorizing power of 11 selected CHAs was analyzed.

For all CHAs, decolorizing power test confirmed the category assignment declared in the technical datasheets. Four CHAs belonged to DEO, namely CHA3, CHA4, CHA8, and CHA11, while six CHAs were clearly assigned to DEC (CHA1, CHA5, CHA6, CHA7, CHA9, and CHA10; Figure 3). The ambiguous case of CHA2

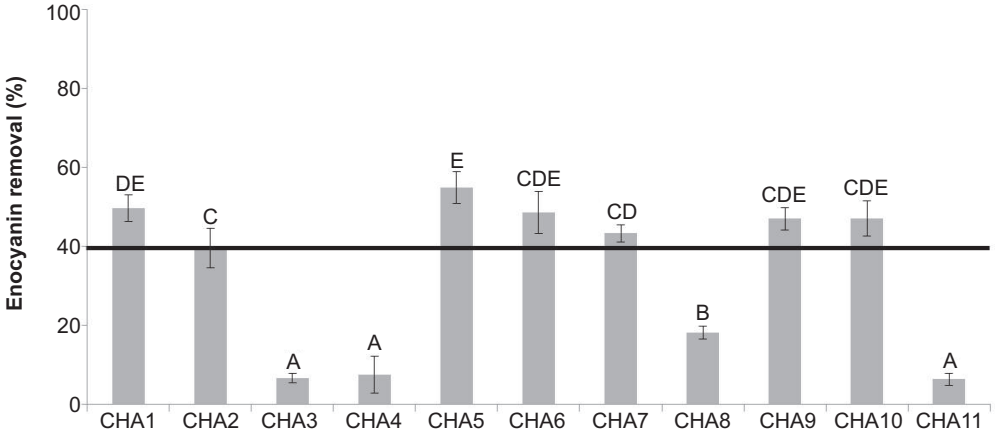

Figure 3. Percentage values of enocyanin removal. Mean values and standard deviations are presented. Each test was repeated for three times. Different capital letters identify significantly different groups ($P < 0.05$) according to *post hoc* Tukey test. Line corresponds to OIV threshold.

that reduced 40% of enocyanin content was ascribed to DEC in accordance with the statistical analysis, which grouped CHA2 with CHA6, CHA7, CHA9, and CHA10. Statistical analysis evidenced significant differences between samples ($F_{(10, 22)}$ = 108.8, P < 0.01) and allowed to differentiate decolorizing power ability even inside the two categories. For example, different from the other DEO, CHA8 revealed a singularly higher decolorizing power, which confirmed the specific supplier declaration of presence of high mesopores. Among DEC, CHA5 achieved the highest percentage of subtraction (almost 55%; Figure 3).

Charcoal's riboflavin removal in white wine

In a recent study, CHA was recognized as the best fining agent for removal of RF (Fracassetti *et al*., 2017). Nevertheless, CHA should be carefully used in order to avoid undesirable effects on wine, such as depletion of color and flavor. Therefore, in this work five dosages of low-range CHA (between 0.5 g/hL and 10 g/hL) have been used for comparison. In a preliminary test performed with different contact periods, CHAs showed the best vitamin removal property after 24 h of contact (Figure SI-1); therefore, the CHA samples are compared after 24 h. In white wine, the results evidenced that DEC have a better performance than DEO at all tested doses, achieving about 90% of RF removal (Figure SI-2). As reported for other absorption kinetics, RF showed a positive but nonlinear trend of reduction with respect to CHA concentration increment (Ribéreau-Gayon *et al*., 2006). Differences between decolorizing and deodorizing CHAs were statistically significant at all doses, and drop in doses corresponded to a progressive decrease in differences between average of categories (Figure SI-2). As indicated by Fracassetti *et al*. (2017), in real wine even the highest CHA concentration didn't allow the complete removal of RF. In fact, Fracassetti *et al*. (2017) reported that after 24 h of contact a large-pore CHA was able to remove 100% RF at 5 and 10 g/hL in a model wine, while it reached only 58% and 71% of RF removal in Chardonnay wine. When compared with Fracassetti *et al*. (2017), the decolorizing CHAs studied in the present work showed higher RF removal capacity in real wine. This phenomenon probably depends on differences in wine compositions because of grape varieties and winemaking processes.

Recently, it has been demonstrated that decreasing the final RF concentration below 50 µg/L drastically reduced the risk of light-struck development (Fracassetti *et al*., 2019), and therefore this concentration was chosen as a threshold for the evaluation of efficacy of CHAs. DEC assured sufficient removal of RF at 10 g/hL and 5 g/hL (residual RF concentration of 13.58 ± 5.24 µg/L and 31.25 ± 9.01 µg/L, respectively). On the other hand, DEO permitted to achieve the threshold only after treatments with the highest doses and only with two CHAs (CHA4 and CHA8 with a residual RF of 39.61 ± 2.80 µg/L and 34.95 ± 2.49 µg/L, respectively; Figs 4a and 4b). The percentage of RF reduction in samples treated with DEC varied between 85% and 94%, with the only exception of CHA2, which is the worst color removal DEC. Among DECs, CHA5 evidenced the best performance, reducing the RF value from 104 µg/L to 5.71 µg/L. It could be observed that CHAs demonstrated interesting variation in their efficiency depending on the dosage. CHA5 showed a reduction of only 10%, passing from 10 g/hL to 5 g/hL, other two CHAs, namely CHA1 and CHA10, showed a reduction of about 15%, while CHA6, CHA7, and CHA9 showed a marked reduction (higher than 20%). CHA2 lost 18% of its removal ability passing from 10 g/hL to 5 g/hL. This resulted in a greater difference registered among DEC at 5 g/hL, which showed an RF removal varying between 63.2% and 88.6%, corresponding to 41–15 µg/L of residual RF (Figure 4a).

DEO could be divided into two subcategories. CHA4 and CHA8 achieved a maximum RF reduction of about 65%. Differently, CHA3 and CHA11 evidenced a very low RF removal, without exceeding 25% even at 10 g/hL, leaving a residual vitamin concentration of 82.78 ± 5.63 µg/L and 79.15 ± 0.21 µg/L, respectively (Figure 4b) in the investigated white wine. Differences in RF adsorption could be attributed to specific surface availability between the two categories as well as within DEO. It is well known that the main difference between DEC and DEO lies in pore size (Yahya *et al*., 2015). Even if mesopores and micropores seem to be the main contributors in CHA's removal power, by extending the surface area, macropores could represent an indispensable way for large molecules to achieve internal surfaces. Recently, it has been demonstrated that reduction in large-size pores affects CHA affinity toward methylene green, a cationic dye showing high similarity to RF molecule structure (Tran *et al*., 2017). Most likely, pore configuration could also affect RF permeability and consequently its removal, confirming the previous finding of having close relationship between pore size and RF permeability (Kisler *et al*., 2001). Color intensity of white wine was evaluated after treating with 10 g/hL of CHA in order to quantify color depletion (CD) at 420 nm. Color depletion significantly differed between CHA categories ($F_{(1,31)}$ = 40.42, P < 0.05; data not shown); on average, DEO removed 5%, while DEC removed 8% of color. Pearson's correlation analysis between percentage of RF removal and color depletion at 10 g/hL revealed a positively significant correlation (r = 0.77, P < 0.05), which indicated that RF and flavan-3-ols/flavonoids have similar interaction with CHA. Concerning this, Gogoi and colleagues (2010) suggested that the catechin sequestration by activated carbon depended on external

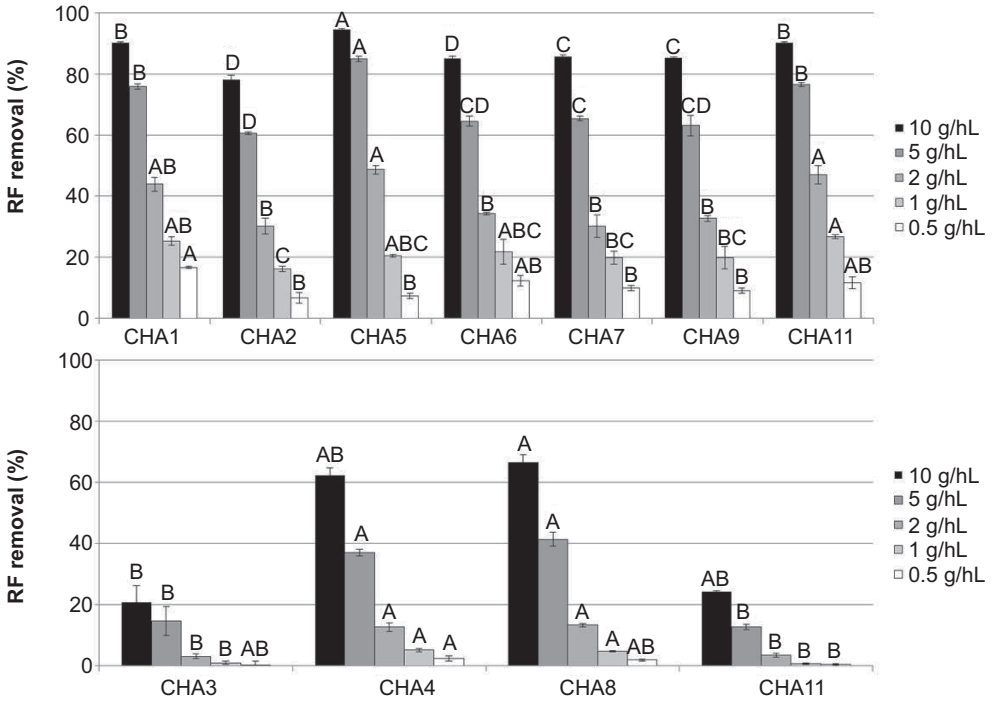

Figure 4. Removal of riboflavin in white wine. (a) Wine treated with decolorizing CHAs. Different capital letters identify statistically significant differences within carbon according to *post hoc* Games–Howell test (CI = 0.95, dosage 10 g/hL) and Tukey test (*P* > 0.05, other doses). (b) Wine treated with deodorizing CHAs. Different capital letters identify statistically significant differences within carbon according to *post hoc* Dunn test (CI = 0.95, dose: 10 g/hL and 0.5 g/hL) and Tukey test (*P* > 0.05, other doses). Mean values and standard deviations of three replications are expressed.

physicochemical parameters, for example, pH and competing compounds present in the solution that could interfere in the solute–sorbent association. This linkage was supposed to occur as an equilibrium between the hydrogen bonding (less important in the water environment because of high number of water hydrogen bonds) and the π-electron interaction between phenol ring and carbon backbone, which directly determines the bond strength.

Charcoal's RF removal in red wine

Quantity of RF in wine directly depends on grape variety and winemaking process (Cataldi *et al.*, 2002). On average, red wines revealed higher vitamin content with respect to both white and rosè wines. Although the light-struck phenomenon is not relevant in red wines, it has been demonstrated that RF-mediated oxidation could play a role in degradation of anthocyanins (Kim *et al.*, 2010). Moreover, CHAs are more often used in fining of red wine (Lisanti *et al.*, 2008). Therefore, CHA's ability to remove RF in red wine is of particular interest. As before, RF quantification was performed after 24 h of treatment, samples were kept in dark condition, and five dosages were tested. As before, DEC resulted in a higher RF removal capacity than DEO at all selected concentrations (Figure SI-3). The best performance was shown by DEC at 10 g/hL with a significant difference from DEO ($F_{(1,32)}$ = 53.82, $P < 0.05$). In general, CHAs evidenced a reduced ability of RF removal in red wine in comparison to white wine; in fact, DEC registered less than 25% of RF removal at the highest concentration. This effect could depend on other wine components which interfere in the RF–CHA interaction. These compounds likely belong to phenolics, which have been studied for their high affinity to CHA (Dąbrowski *et al.*, 2005). Deodorizing CHAs revealed no RF removal capacity in red wine; on the contrary, they seem to display a slight RF protection, as the final RF content in the treated samples was slightly higher than in the control (Figure SI-3). It is widely reported that spontaneous degradation of RF occurs during the first few hours of opening of bottle (Mattivi *et al.*, 2000) and that this phenomenon is accelerated in organic solvents (Sheraz *et al.*, 2014). Even by keeping the samples in the dark, a slight RF degradation occurred during the treatment; however, the presence of DEO seemed to prevent this degradation, as the RF content was greater at higher CHA concentrations. It can be assumed that the same RF degradation occurred in all the samples; although in DEC-treated samples the RF removal masked this effect, in DEO-treated samples the removal power was too low.

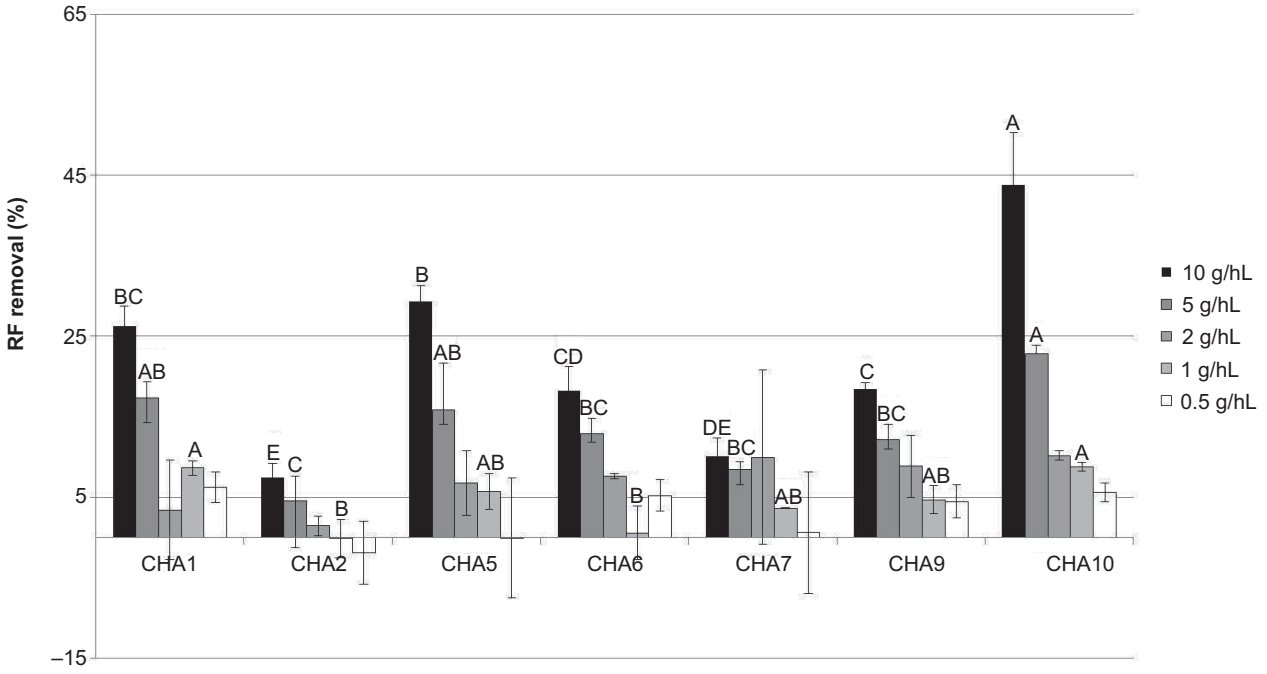

Figure 5. Riboflavin removal of decolorizing CHAs in red wine. Different capital letters identify statistically significant differences within DEC according to *post hoc* Tukey test ($P > 0.05$) at the dosages of 10 g/hL, 5 g/hL, and 1 g/hL. Mean values and standard deviations of three replications are expressed.

Even in DEO-treated samples, differences between categories were statistically significant at all dosages.

The analyses of individual CHAs belonging to DEC group revealed strong differences in their behaviors. In particular, CHA10 was identified as the best one for RF removal. In fact, it is the only one that reduced the RF value by 43% at 10 g/hL (corresponding to a final concentration of 77.96 ± 9.11 µg/L in treated wine; Figure 5). Considering the initial RF concentration of 138 µg/L, it means that about 60 µg/L was removed, similar to the depletion recorded in white wine at 5 g/hL. Two CHAs, namely CHA1 and CHA5, exhibited limited ability, reaching a little more than 25% of removal (Figure 5). In all other cases, the RF sequestering was very low. Color depletion at 10 g/hL significantly differed between CHA categories ($F_{(1,31)} = 85.19$, $P < 0.05$); DEO removed on average 2%, while DEC removed 9% of color intensity. The correlation between percentage of removed RF and color depletion at 10 g/hL highlighted a positively significant correlation ($r = 0.88$, $P < 0.05$). This suggests that the interaction between RF and polyphenols with CHA is characterized by a similar binding mechanism. In a dynamic equilibrium, this mechanism depends on the ionic strength (and pH) of the solvent that probably involved electron donor–acceptor interactions between the aromatic phenolic ring and the surface oxygens, dispersion effect between the aromatic phenolic ring and the π electrons of the graphitic structure, and, if ions are present, then electrostatic attraction and repulsion (Dąbrowski *et al.*, 2005).

Conclusion

This work explored the potential of commercial BENs and active CHAs in lowering of RF in real wines. BENs showed a reduced ability to sequester RF which appeared independent from their shied cation nature, and thus from their swelling properties. Among 11 different commercial BENs, only a sodic-calcic BEN (BENT5) revealed an interesting higher ability in RF removal. This work represents the first study in which a sodic-calcic BEN has been tested for this purpose. The future studies would define whether SOD-CAL matrices could play a role as RF removal agents. On the other side, activated CHAs confirmed their high attitude to remove RF, which for the first time was tested in both white and red wines. A great difference between deodorizing and decolorizing CHA was registered in both wines, which probably depends on CHA porosity. Additionally, in comparison to white wine, CHA RF removal was dramatically reduced in red wine. On average, decolorizing CHAs revealed 87% of RF removal in Glera wine to 22% in Wildbacher wine. This phenomenon has been attributed to complex interactions between CHA and wine phenols. Phenolic acids,

flavan-3-ols, and flavonoids could establish ʌ-electron interactions between phenolic ring and the active binding sites of CHA, and therefore could represent direct RF competitors. This phenomenon could also explain different RF removal efficiency of CHA fining in real wines (present work) compared to a model wine (Fracassetti *et al.*, 2017). To date, it is not clear whether the phenolic compounds belonging to different classes have the same effects on CHA–RF interaction.

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Supplementary information

Removal of Riboflavin by Commercial Bentonites and Charcoals in White and Red Wines

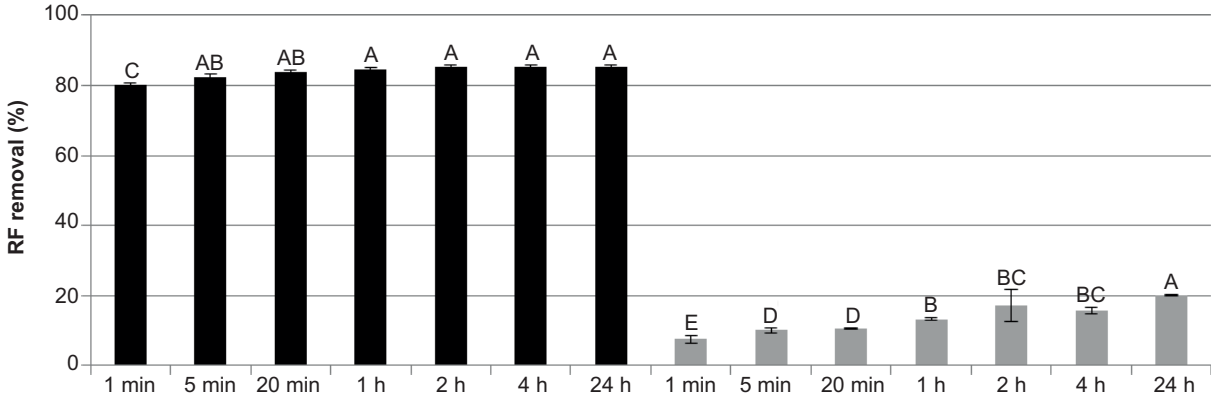

Figure SI-1. Trend of removal of riboflavin with CHA5 and CHA3 in white wine. Mean values and standard deviations are expressed. Each test was repeated for three times. Black: decolorizing CHA (CHA5), and gray: deodorizing CHA (CHA3). Different capital letters identify statistically significant differences within category according to Tukey test (*P* < 0.05).

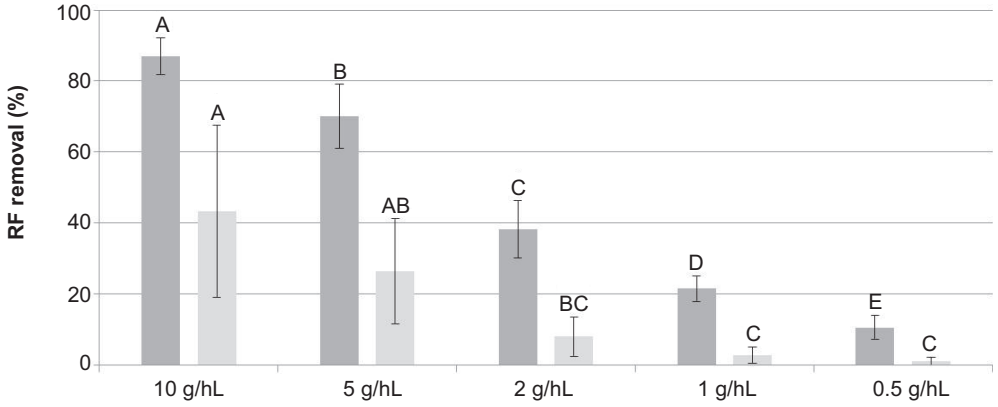

Figure SI-2. Comparison of removal of riboflavin with CHAs in white wine. Mean values and standard deviations are expressed. Each test was repeated for three times. Gray: decolorizing CHA, and light gray: deodorizing CHA. Different capital letters identify statistical significant differences within categories according to *post hoc* Dunn's test (CI = 0.95). CI: Color intensity.

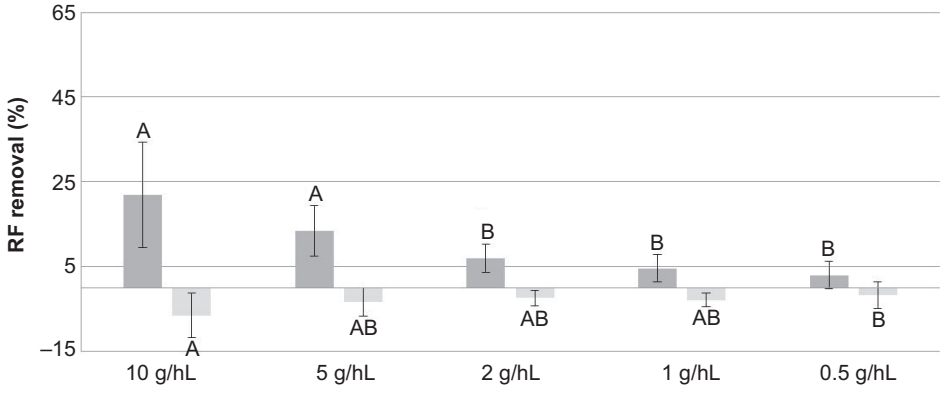

Figure SI-3. Comparison of removal of riboflavin with CHA in red wine. Mean values and standard deviations are expressed. Each test was repeated for three times. Gray: decolorizing CHA, and light gray: deodorizing CHA. Different capital letters identify statistically significant differences within category according to Dunn's test (CI = 0.95) and Tukey test (P < 0.05) respectively. CI: Color intensity.