Release of phenolic compounds from cork stoppers and its effect on protein-haze

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ABSTRACT

Cork stoppers contain low molecular weight phenols, mainly as ellagic tannin, whose role in the protein stability of white wine has been not elucidated. The release of cork phenols from cork granules, disks and stoppers of different quality classes (A and D) in synthetic wine was investigated as well as its effect on animal gelatin, lysozyme and wine protein. Amounts of cork phenolic compounds up to 115 and 179 μg/cm^2 were released within two weeks for best and worst quality cork disks, respectively, indicating the cork quality can strongly affect the phenolic compounds release. Similar trend was found for cork stoppers even if the concentration of phenolic compounds was lower (68 μg/cm^2). Protein-haze was observed in presence of both animal gelatin and lysozyme (50 mg/l) when the phenol level exceeded 30 and 9 mg/l, respectively, whereas no effect on wine protein was observed. This research suggests that even if the overall amount of phenolic compounds released from cork stoppers is low, protein-haze can be achieved when the bottle is stored horizontally and motionless due to the high phenols concentration close to the cork stopper.

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1. Introduction

Cork stoppers are traditionally employed in corking wine bottle and their technological, microbiological and sensorial properties are affected by the production procedures which include sanitation steps by boiling water or steam and the use of hydrogen peroxide in order to prevent the growth of molds and the occurrence of off-flavors (Rocha, Delgadillo, & Ferrer Correia, 1996; Vlachos, Kampioti, Kornaros, & Lyberatos, 2007; http://www.corkfacts.com/natural-cork/raw-material-and-production-process/). Among the latter 2,4,6-trichloroanisole is the compound the winemakers fear the most, nevertheless cork contains further compounds potentially affecting wine properties. Fernandes et al. (2009) and Fernandes, Sousa, Mateus, Cabral, and de Freitas (2011) showed the presence of mainly polygalloyl groups, either free or glycosylated, in *Quercus suber* cork phenolics, and the ellagic tannin fraction accounts for more than 85% of the overall phenols content (Conde, Cadahía, García-Vallejo, & Fernández de Simón, 1998; Varea,·García-Vallejo, Cadahía, & Fernández de Simón, 2001). Similar hydrolysable tannin occurs in oak wood and it is extracted in barrel wine aging. Oak wood tannin can hardly

affect the astringency of red wine or its bitter taste because of the low amount of low molecular weight tannin released during the aging (Hale, Mccafferty, Larmie, Newton, & Swan, 1999). The washing and lubrication steps carried out for producing the cork stopper as well as the narrow contact surface in the bottle neck area between wine and stopper head barely lead to sensorial changes caused by the release of phenolic compounds from cork stopper in red wine, but the effect of cork phenolic compounds in white wine has not been fully investigated. The binding ability of ellagic tannin to wine proteins can be potentially responsible for haziness, especially when the protein stabilization has not been properly achieved. Chitinases and thaumatin-like proteins (TLP) are the proteins mainly involved in this defect (Waters et al., 2005) also due to their high heat sensitivity since they are irreversibly precipitated by heating at 50–62 °C (Falconer et al., 2010). Moreover, they are precipitated by grape tannin (Esteruelas et al., 2011; Waters et al., 2005), though they are unaffected by low molecular weight phenols of grape (Pocock, Alexander, Hayasaka, Jones, & Waters, 2007). Therefore, the accidental transfer of cork phenols into white wine might favor the protein instability which can be limited by either an effective treatment of stabilization or the capping with screw cap or synthetic stopper. The protein-haze in white wine can be also produced by hen's egg-white lysozyme which is added to wine for preventing the activity of lactic acid bacteria

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(Bartowsky, Costello, Villa, & Henschke, 2004; Gerbaux et al., 1999; Gerbaux, Villa, Monamy, & Bertrand, 1997). In spite of the high solubility of lysozyme in wine its heat sensitivity is well known (Bartowsky et al., 2004) as well as its tannin-binding ability (Gerbaux et al., 1999; Tirelli & De Noni, 2007). Moreover, the lysozyme amount used in wine making approaches or exceeds the chitinase and TLP amounts usually occurring in white wine (100–250 mg/L) before performing the protein stabilization (Le Bourse et al., 2011; Waters et al., 2005). Besides the lysozyme, the animal gelatin is also commonly used for the fining (Manfredini, 1989; Riberau-Gayon, Glories, Maujean, & Dubourdieu, 2006; chap. 5). Residual amounts of gelatin in wine (overfining) can be responsible for wine hazing due to either animal gelatin binding with tannin or interaction with the wine proteins (Marchal & Jeandet, 2009).

In this paper, the release of phenolic compounds from different cork products (i.e. granules, disks and stoppers) was investigated as well as its effect on the protein-haze formation in model wine solution containing lysozyme or animal gelatin and in white wine before the protein stabilization, since protein-haze has detrimental economical effect on wine value.

2. Materials and methods

2.1. Chemicals and reagents

Methanol, ethanol and ethylenediaminetetraacetic acid (EDTA) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Sodium hydroxide (NaOH) was from Merck (Darmstadt, Germany). Gallic acid standard was bought from Carlo Erba (Rodano, Milano, Italy) and sodium metabisulfite was purchased from J.T. Baker (Deventer, Holland). Animal gelatin (high Bloom degree) and plastic stoppers were purchased from Dal Cin (Concorezzo, Italy). Lysozyme was purchased from Intec Technology International (Verona, Italy). All the chemicals were of analytical grade, at least. HPLC grade water was obtained by a Milli-Q system (Millipore Filter Corp., Bedford, MA, USA).

The synthetic wine solution contained 3.0 g/l tartaric acid, 12% ethanol (v/v) at pH 3.2 adjusted with 12 M NaOH, 150 mg/l EDTA and 100 mg/l sodium metabisulfite.

A concentrated solution of cork phenolics (500 mg/l assessed as described in paragraph 2.9) was obtained through the extraction from cork granules with the synthetic wine solution and it was properly diluted to obtain different concentrations of cork phenolics (from 5 to 50 mg/l) aimed to carry out the protein stability trials.

2.2. Samples description

Ten different commercial cork batches of cork granules, natural cork disks, agglomerated with two disks stoppers and natural cork stoppers were provided by Mureddu Sugheri (Nerviano, Italy) and were made from cork of different origin (Spain, Italy and Portugal) (Table 1). All the cork products were industrially washed by steam or hot water, except the cork granules coded as 3 in Table 1. The surface coating of the stoppers was industrially carried out by paraffin.

2.3. Phenolic compounds extraction from cork granules

Thirty grams of cork granules were soaked in 250 ml of synthetic wine solution and stored motionless 64 h at 25 ± 1 °C. The extraction kinetics were carried out in gas-tight glass containers (250 ml) and the cork granules were completely soaked into the synthetic wine solution by a plastic support. The solution was recovered, filtered throughout filter-paper and the total phenolic compounds concentration was assessed. The extractions were carried out in duplicate.

2.4. Kinetics of phenolic compounds release from cork disks and stoppers

The kinetics of phenolic compounds extraction were carried out by using 20 cork disks per sample belonging to two classes of quality (samples coded as 6 and 7 in Table 1; overall surface: 310 cm^2) and 7 natural cork stoppers (sample coded as 10 in Table 1; overall surface: 326 cm^2). Either cork disks or stoppers were soaked in 150 ml of synthetic wine solution, maintained at 25 °C ± 1 °C in gastight glass containers for two weeks without shaking. Glass containers were withdrawn every 2 or 3 days storage up to 6 samplings. Each phenolic solution was recovered, filtered through filter-paper and the phenolic compounds concentration was assessed before dumping the sample. The amount of released phenolics was expressed as micrograms of gallic acid per square centimeter of cork surface. The extractions were carried out in triplicate.

2.5. Stability of enological gelatin and lysozyme in cork phenolics solution

The stability of animal gelatin and lysozyme was assessed in synthetic wine added with a cork phenolics solution. Fifty milligrams of gelatin per liter or lysozyme at three concentration levels (50–75–100 mg/l) were dissolved in a synthetic wine solution containing increasing concentrations of cork phenolic compounds (from 5 to 50 mg/l) obtained through the dilution of synthetic wine solution containing 500 mg/l phenols. The synthetic wine solution containing either animal gelatin or lysozyme (9 ml) was slowly added with 1 ml of phenolic compounds solution under shaking. The obtained solution was stored at 20 ± 1 °C till the appearance of protein-haze. Each sample was prepared in triplicate.

2.6. Evaluation of protein-haze

The protein precipitation induced by phenols was evaluated in bottle-neck shaped tubes using a Metalomecanica JAV instrument (Egitron, Mozelos, Portugal) (Fig. 1), which simulated the contact of wine with the cork stopper occurring in the bottle neck during the wine aging or storage. The apparatus consisted of a 10 cm long plexiglass tubes having 19 mm inner diameter for 6.5 cm of their length (corked side). The remaining 3.5 cm had a 2 mm wide duct which allowed the locking of the tubes on a plexiglass disk by a gas-tight screw closure. The capacity of each corked tube was 5.5 ml. The instrument hosted three plexiglass disks with 8 tubes each.

2.7. Formation of protein-haze with animal gelatin and lysozyme

The bottle-neck shaped tubes were employed to assess the formation of protein-haze with either animal gelatin or lysozyme in synthetic wine solution. Twelve tubes were corked with class A agglomerated stoppers (sample coded as 8 in Table 1) and 12 with class D agglomerated stoppers (sample coded as 9 in Table 1). Six tubes of each cork class were filled up with the synthetic wine solution and 6 with 50 mg/l of animal gelatin dissolved in the synthetic wine solution. Class A natural cork stoppers (sample coded as 10 in Table 1) were used for the trial with lysozyme (50 mg/l) dissolved in synthetic wine solution; this protein was added in 12 tubes, while the remaining 12 were filled up with only the synthetic wine solution. The equipment was tilted 45° in order to mimic the bottle storage position and to ensure the stopper head

Table 1
Description of the cork products; commercial classes are in brackets.

Code	Product	Diameter (mm)	Length (mm)	Particle size (mm)	Origin	Washing	Surface coating
1	Granules	–	–	0.5–1.5	Italy	Steam	No
2	Granules	–	–	1	Spain	Steam	No
3	Granules	–	–	2–4	Spain	No	No
4	Granules	–	–	2–4	Spain	Steam	No
5	Granules	–	–	2–3	Portugal	Steam	No
6	Disk (A)	26	6	–	Spain	Steam	No
7	Disk (D)	26	6	–	Italy	Water	No
8	Agglomerated + 2 disks stopper (A)	23.5	43	–	Spain	Steam	Yes
9	Agglomerated + 2 disks stopper (D)	23.5	43	–	Spain	Water	Yes
10	Natural stopper (A)	26	44	–	Italy	Steam	Yes

Fig. 1. Metalomecanica JAV instrument with the screwed bottle-neck shaped tubes. The cork stoppers and the synthetic wine solution are visible.

was completely in contact with the synthetic wine solution, then it was stored at room temperature until the appearance of haze. The samples were withdrawn when the haze was observed in half of the tubes containing protein. The content of phenolic compounds was assessed in the protein-free samples, whereas the turbidity was monitored in the protein-containing samples.

2.8. Formation of protein-haze in white wine

The protein-haze formation was tested in white wine produced from Verdicchio bianco grape produce under rational industrial conditions in the vintage 2014. The wine was collected from a cellar in the northern Italy (Brescia area) before the protein stabilization. It was centrifuged at 5000 × g for 10 min at 10 °C (Sorvall centrifuge, Thermo, Waltham, MA) and stored at 5 °C overnight.

It was transferred into 16 bottle-neck shaped tubes 8 of those were corked with natural cork stoppers (sample 10 in Table 1) and 8 with plastic stoppers (Dal Cin) using a semi-automatic corking machine. Further 8 bottle-neck shaped tubes containing the synthetic wine solution were corked with natural cork stoppers in order to assess the release of phenolic compounds. The equipment was tilted 45° and stored at room temperature until the appearance of haze. All the samples were withdrawn when the haze was observed in half of the bottle-neck shaped tubes containing the white wine.

The presence of unstable wine proteins was assessed by a heat stability test (Pocock & Rankine, 1973). Twenty milliliters of wine was incubated in sealed glass tubes at 80 °C for 30 min either with or without addition of cork phenolics (10 mg/l). The haze was spectrophotometrically assessed and compared with an untreated sample. Moreover, the protein-haze formation was evaluated in white wine spiked with increasing concentrations (from 5 to 50 mg/l) of phenolic compounds and the wine samples were kept at 20 ± 1 °C. The haze was measured after 4 days. Each sample was prepared in triplicate as well as an unspiked white wine sample (control).

The protein content of Verdicchio bianco white wine in the bottle-neck shaped tubes was assessed by the Bradford method (Bradford, 1976) using an enzymatic kit (Biorad, Hercules, CA, USA).

2.9. Quantification of phenolic compounds and turbidity

The concentration of phenolic compounds was assessed spectrophotometrically at 270 nm using gallic acid as external standard and the results were expressed as μg of gallic acid per liter. The calibration curve was obtained in triplicate by spiking the synthetic wine solution with known amounts of gallic acid (10–200 mg/l). Five milliliters of phenolic compounds extract was centrifuged at 15.000 × g for 10 min at 15 °C (Hettich Centrifuge Mikro 220R, Buckinghamshire, England) and the sample was filtered with 0.22 μm pore size PVDF membrane (Millipore Filter Corp., Bedford, MA, USA). The sample was diluted 1:10 (v/v) with the synthetic wine solution and the absorbance was measured. The spectrophotometric data acquisition and processing were performed by PerkinElmer's UV WinLab Software (Massachusetts, USA).

The turbidity was measured by a spectrophotometric absorbance at 630 nm.

2.10. Statistical analysis

Statistical analysis was carried out by means of STATISTICA software (Statsoft Inc., Tulsa, OK, US). The equations of the calibration curves were assessed by the linear regression analysis. Differences were evaluated by the *T-test* ($p < 0.05$).

3. Results and discussion

Though the presence of gallic and ellagic tannin and low molecular weight phenols in cork was reported (Conde, Cadahía, García-Vallejo, Fernández de Simón, & González Adrados, 1997; Conde et al. 1998; Mazzoleni, Caldentey, & Silva, 1998), there are few data related to the phenolic compounds extractability and migration (Varea et al., 2001). The amount of extractable phenolic compounds can be potentially affected by a number of factors such as cork origin and production steps (Conde et al., 1997, 1998), extension of the contact surface, porosity, and duration of the extraction. Cork granules having particle size in the range 0.5–4 mm were used to extract phenolic compounds in synthetic wine, in order to have a high surface to volume ratio and to assess the amount of phenols potentially extractable from cork. The extraction of phenolic compounds from cork was carried out in a synthetic wine solution in order to simulate wine in terms of pH, acidity and alcohol content. Sodium metabisulfite and EDTA were also added for preventing phenols oxidation catalyzed by metal ions. The temperature was set to 25 °C, a value higher than that one normally occurring in cellar in order to speed up the release of phenols. The samples were shaken only when withdrawn but not during the extraction time in order to simulate the storage condition of a wine bottle.

The amount of phenolic compounds extractable from cork granules of different origin was assessed in order to set up experimental conditions suitable for the extraction of quantifiable amounts of phenolic compounds from cork. The specific surface of the granules was estimated considering them as cube shaped particles having a 0.5–4 mm side as declared by the provider. Nevertheless, the high unevenness of the particles shape could likely lead to an underestimation of the calculated surface. Based on the mean particle size of the different cork batches, the specific surfaces were calculated as 20 m^2/kg (1 mm size particles) and 6.7 m^2/kg (3 mm size particles). Thirty grams of cork granules soaked in 250 ml of synthetic wine released phenolic compounds in the range 238–374 mg/l (Table 2) after a 64 h extraction. These data showed important differences among the cork batches. The differences further increase if the phenols amount released per surface unit is considered since values in the range 11.33–46.75 μg/cm^2 were calculated considering the mean particle size of each batch. The bigger particles released higher amounts of phenolic compounds in spite of their surface to volume ratio suggesting that cork origin and production procedures (Mazzoleni et al., 1998; Peña-Neira et al., 1999), especially washing, can have different phenolic compounds depletion from cork. Our findings showed a comparable order of magnitude of the data reported by Conde et al. (1997) on cork granules (0.5–1 mm particle size), but these authors found a phenol concentration slightly higher probably due to the different extraction solvent employed (methanol 80% v/v) which allowed a more effective extraction of phenolic compounds.

Moreover, in order to evaluate the oxidative state of phenolic compounds migrated in the synthetic wine solution, assays were carried out by adding an extra amount of sulfur dioxide (50 mg/l) into the synthetic wine solution. The spectra were recorded and no difference was found in the response among the samples analyzed with and without sulfur dioxide (data not shown) suggesting that phenolic compounds were not significantly oxidized during the extraction.

On the basis of the extraction values obtained with the cork granules, cork surface in the range 200–400 cm^2 was considered suitable for monitoring the kinetic of phenolic compounds extraction from disks and stoppers, therefore, 20 cork disks (overall surface 310 cm^2) were used. Cork stoppers and disks are commercially classified in four classes defined with letters from A (best quality) to D (worst quality), according to their visual appearance (Disciplinare sulla produzione ed utilizzo del tappo di sughero in enologia, 1996). The cork surface covered with wider lenticels, crevices and fibrous tissue is of lower quality. Since the roughness of cork can increase the surface of the stoppers and different cork classes were suspected to release different amount of phenolic compounds, the kinetics of phenols release from class A and class D stoppers and disks were investigated. Both classes of the cork disks were produced following the same procedures: a washing step was carried out and no coating material was used. The phenolic compounds content was monitored for two weeks and the results are shown in Fig. 2. Both classes of cork disk showed a similar trend of phenolic compounds release though a 36% higher amount ($p = 0.049$) was dissolved from the class D disks after a 2 weeks extraction. Data dispersion is variable among the sampling times due to the inhomogeneity of cork. The phenol concentration detected following to three days of extraction showed a specific extractability values of 40.0 ± 4.8 and 92.0 ± 32.0 μg/cm^2 for the classes A and D, respectively (Fig. 2). Such values are up to 8 times higher than the values showed by the cork granules, probably because the washing step depleted higher amount of phenols from granules due to their high surface to volume ratio.

The formation of protein-haze was evaluated for class A and D agglomerated cork stoppers, corresponding to the class A and D cork disks. This assay was carried out by adding animal gelatin to the synthetic wine, an unstable protein commonly used as fining coadjutant (Manfredini, 1989). Protein-haze was evaluated in synthetic wine solution where 50 mg/l of animal gelatin and amounts of phenolic compounds up to 50 mg/l were added. The level of animal gelatin added for the assay was chosen in accordance to the protein amount reported in the literature about white wine (Ferreira, Piçarra-Pereira, Monteiro, Loureiro, & Teixeira, 2001; Riberau-Gayon et al., 2006; chap. 5). The absorbance measurements showed non-linear response for phenolic compounds concentration vs. turbidity (Fig. 3). Moreover a phenol to protein ratio exceeding 0.3 (mg/l phenols divided by mg/l protein) was needed to promote the animal gelatin precipitation.

The protein-haze was monitored by using agglomerated cork stoppers of either class A or D and the haze appeared after 3 days in all the bottle-neck shaped tubes. The use of the equipment in Fig. 1 allowed the simulation of the interactions potentially occurring at the contact surface of wine and cork stopper in a bottle neck. The haze was clearly visible close to the cork stopper head and it decreased as the distance from the stopper increased. The mean turbidity value was slightly lower for the agglomerated cork stoppers of class A (0.204 ± 0.123 AU) in comparison to those of class D (0.346 ± 0.194 AU) but the difference was poorly significant ($p = 0.076$). The phenolic compounds release in the gelatin-free samples were 39.1 ± 13.3 μg/cm^2 and 86.6 ± 26.0 μg/cm^2 for class A and class D agglomerated cork stoppers, respectively, but the difference was poorly significant ($p = 0.056$). However, these data were in accordance to those related to a three days extraction as above reported for the cork disks.

Table 2
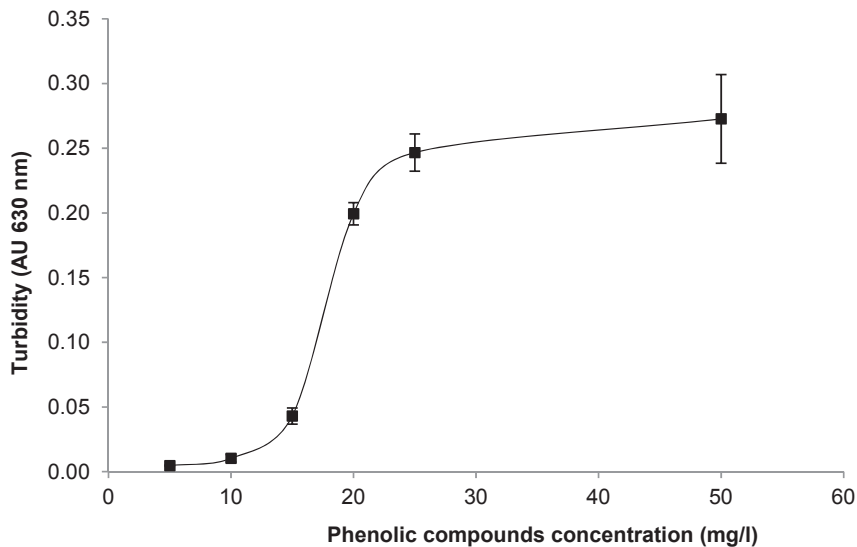Amount of phenolic compounds released from cork granules after a 64 h extraction.

Sample code	Total surface (cm^2)[a]	Phenols	
		(mg/l)[b]	(μg/cm^2)
1	6000	310 ± 7	12.91
2	6000	272 ± 5	11.33
3	2000	319 ± 2	39.88
4	2000	374 ± 9	46.75
5	2400	238 ± 6	24.79

[a] Surface was calculated for 30 g of cork granules.
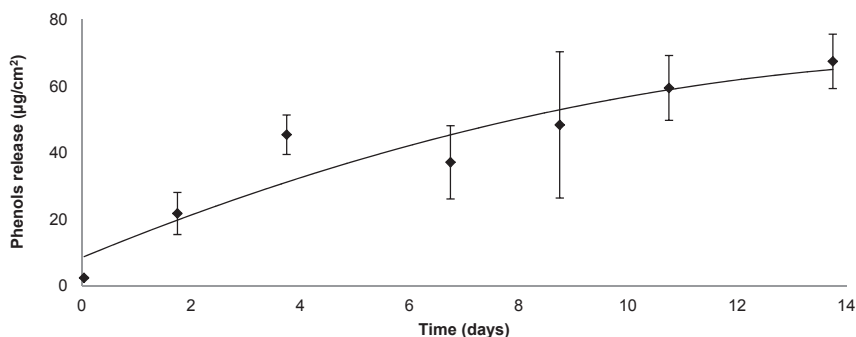[b] Mean value ± range (n = 2).
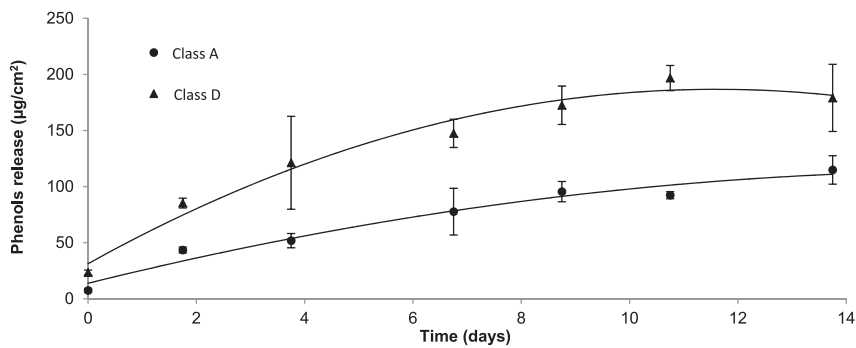
Fig. 2. Release of phenolic compounds from class A and D cork disks soaked in 150 ml of synthetic wine solution (overall surface: 310 cm^2). Mean values (n = 3) and standard deviation (vertical bars) are reported.

Fig. 3. Trend of protein-haze in synthetic wine solution added with animal gelatin (50 mg/l) and increasing amounts of phenolic compounds (5–50 mg/l).

The release of phenolic compounds from cork was also investigated for natural cork stoppers coated with paraffin. In order to carry out the phenols extraction by a cork surface comparable to the disks, 7 stoppers were employed whose total surface was 326 cm^2. The phenolic compounds extraction trend was similar to that one of the cork disks (Fig. 4) but the absolute amount released was significantly lower since less than 70 μg/cm^2 was detected in the synthetic wine solution after a two weeks extraction. This finding was expected since the natural cork stoppers were washed and then coated with paraffin. Peña-Neira et al. (1999) reported an increase of phenols concentration after cork lubrication,

but such a difference could be due to the higher alcohol concentration into the extracting solvent (methanol 80% v/v) causing the change of the chemical–physical properties of the lubricating products as well as to the extracting solvent. Our data showed noticeable differences among the replicates (Fig. 4) and this is not surprising since the natural variability of the cork is further increased by the variability of the coating on the stoppers surface. The phenolic compounds concentration in natural cork stoppers was previously reported by Varea et al. (2001) and the extraction was investigated in a model wine. Their data have comparable order of magnitude with ours though they were slightly higher probably

Fig. 4. Release of phenolic compounds from natural cork stoppers soaked in 150 ml of synthetic wine solution (overall surface: 326 cm^2). Mean values (n = 3) and standard deviation (vertical bars) are reported.

because the phenolic compounds extraction was carried out under shaking condition.

The formation of protein-haze was evaluated for the natural cork stoppers in presence of lysozyme which can be added to bottled white wine for preventing the growth of lactic bacteria (Blättel et al., 2009; Gerbaux et al., 1999). Moreover, lysozyme showed characteristics of heat stability comparable to chitinase and TLP proteins (Bartowsky et al., 2004). Agglomerated and natural cork stoppers are expected to behave in the same way once they are in the bottle neck since only the stopper head is exposed to the extractive solution whereas the round surface is poorly accessible to the liquid phase. The formation of protein-haze was firstly monitored for 3 lysozyme concentrations (50 mg/l, 75 mg/l and 100 mg/l) in the synthetic wine solution added with increasing concentration of phenolic compounds up to 50 mg/l. A linear response for phenolc concentration vs. turbidity was found as shown in Fig. 5. The slopes were comparable among the 3 lysozyme concentrations tested and the correlation indexes were higher than 0.97. The turbidity values slightly increased as lysozyme concentration increased from 50 mg/l to 75 mg/l, but no significant change was observed as concentration further increased to 100 mg/l. This finding suggests that phenols concentration could limit the protein precipitation. However, 50 mg/l of lysozyme was chosen to evaluate the haze formation due to the phenolic compounds release from cork.

Even in this case, the haze appeared close to the stopper head surface after 2 days in all the bottle-neck shaped tubes corresponding to a phenolic compounds release of 9.1 ± 4.1 µg/cm^2 and to a turbidity value of 0.125 ± 0.041 AU. The differences among the replicates could be related to the cork variability, as it was already observed for the agglomerated cork stoppers. The phenolic compounds released in the bottle-neck shaped tubes was less than half in comparison to the phenols level measured for the extraction kinetic with the natural cork stoppers after two days of extraction (22.0 ± 6.4 µg/cm^2), though this difference was poorly significant ($p = 0.056$). The detection of this level of phenolic compounds in such a short time was surprising and it could potentially lead to a protein-haze into the bottle neck when the bottle is maintained in a stationary and lying down position as it usually occurs during the in-bottle storage of wine. Nevertheless, the high overall volume of a wine bottle should be taken into account since it could allow a lower overall concentration of phenolic compounds migrated from the cork stopper head into the wine.

The protein-haze formation was evaluated in white wine stored in the bottle-neck shaped tubes capped with either natural cork or plastic stoppers. The plastic stoppers were employed as a control since they are made with an inert material. The white wine showed low but significant protein instability (from 0.000 ± 0.000 AU to 0.003 ± 0.001 AU after heating) which increased when the cork phenol extract was added (Δ AU $= 0.025 \pm 0.004$). The appearance of protein-haze was observed after 3 weeks storage at room temperature and a precipitate was clearly detected in 5 bottle-neck shaped tubes capped with natural cork stoppers and in 7 corked with plastic stoppers. The absorbance value was 0.160 ± 0.046 AU for the white wine left into the bottle-neck shaped tubes corked with natural cork stoppers in correspondence to a phenols concentration of 19.9 ± 4.0 mg/l. The white wine in the bottle-neck shaped tubes corked with plastic stoppers showed an absorbance value of 0.147 ± 0.033 AU, slightly lower than that found for the natural cork stoppers but not significantly different ($p = 0.14$). Therefore, the haziness occurred independently to the release of phenolic compounds from the cork stopper. This finding was confirmed as no protein-haze and change of turbidity were observed in the white wine sample spiked with 50 mg/l of phenolic compounds. As a consequence, wine protein is not affected by cork phenols. As a further evidence, no significant difference was found in the protein content determined in wine samples drawn from the bottle-neck shaped tubes corked with cork (254.6 mg proteins/l) and plastic (267.0 mg proteins/l) stoppers.

4. Conclusions

Release of phenolic compounds from the cork stoppers can be responsible for the protein-haze in white wine treated with gelatin or lysozyme if suitable protein stabilization is not carried out. Major risks could arise for bottled white wine added with lysozyme as preservative. The protein-haze increased as the release of cork phenolics concentration increased, mostly if an high ratio of tannin vs. protein concentration is achieved. Such ratio can be achieved in the wine close to the cork stopper in the bottle neck when the bottle is stored in laying down position and motionless which limit the phenols diffusion in the wine. The risk of protein-haze can increase when low quality and/or uncoated cork stoppers are used since both factors allow a higher release of phenolic compounds from cork. Our data also suggest the role of cork production procedures on phenols release into wine. No effect of cork phenolic compounds on wine protein stability was evidenced.
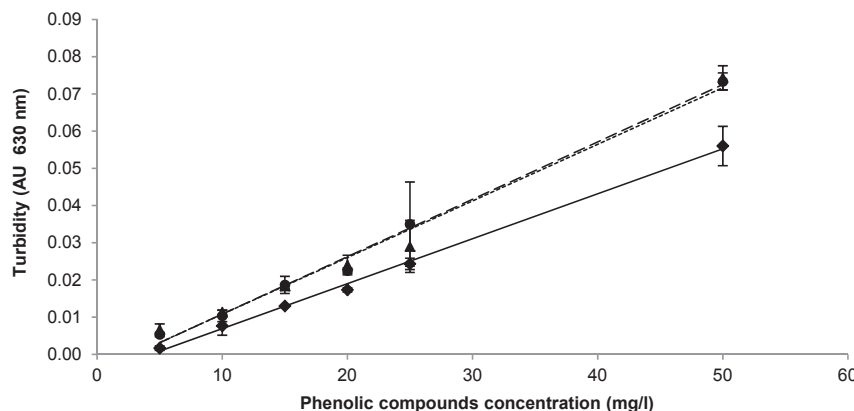
Fig. 5. Trend of protein-haze in synthetic wine solution added with lysozyme (♦: 50 mg/l; ●: 75 mg/l; ▲: 100 mg/l) and increasing amounts of phenolic compounds (5–50 mg/l).

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