



Received for publication: April 20, 2018

Accepted: July 10, 2018

Original paper

The influence of oenological tannins on the fermentation and color stability of a Romanian red wine

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Abstract

The study aimed to assess the influence of three different commercial oenological tannins characterized by the complexity of their composition, condensed, ellagic and gallic tannins on the Fetească Neagră red wine characteristics. In order to highlight the tannin effect, the color intensity was measured spectrophotometrically at various wavelengths 420 nm, 520 nm and 620 nm and displayed increased values for all the studied variants of wine compared to the control sample.

The total polyphenols content revealed an increase once the dose of oenological tannins was raised from 20 g/hL to 40 g/hL for the wines obtained from grapes with a corresponding phytosanitary status and for those obtained from grapes with *Botrytis cinerea* in a proportion of 10%. By using oenological tannins in different doses, many advantages were outlined such as color loss prevention by accentuating the violet tones and blocking the color evolution to orange-colored shades. Moreover, the intense extractions that allow the bitter and harsh tannins to pass through the solids into the liquid fraction were also limited.

Keywords

: red wine, Fetească Neagră, oenological tannins, color stability, fermentation

To cite this article: BICHESCU C., STANCIU S. The influence of oenological tannins on the fermentation and color stability of a Romanian red wine. *Rom Biotechnol Lett.* 2019; 24(1): 57-65. DOI: 10.25083/rbl/24.1/57.65

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Introduction

The complexity of winemaking in terms of grape variety and wine composition gives each wine its uniqueness. To assess whether a wine has a high quality or not, the presence of polyphenols is imperative. These constituents give the wine a high antioxidant activity which accounts for the obvious diversity and for the ageing characteristics of red wines. Moreover, the quantity of total wine phenolics is a primary consideration. The phenolic compounds represent the secondary metabolites of plants that usually affect the organoleptic properties of wine, such as color intensity, aroma, bitterness and astringency (M. MONAGAS & al. [1], E. OBREQUE-SLIER & al. [2]), the flavonoids being of particular interest in winemaking as they are present in a wide variety. Besides the flavonoids, other types of compounds like non-flavonoid phenols are also present, hydroxycinnamic acids being the largest group found in grapes and wine. Regarding the red wine, the following phenolic compounds are present at a high concentration such as: tannins, which are responsible for the astringency and the color of red wines, anthocyanins responsible for the pigment of the grapes and also for the color of red wines, phenolic acids, flavonols and dihydroflavonols, stilbenes, which do not affect the sensory characteristics but are nevertheless important from the health perspective (J. MORENO & al. [3]).

During red wine production, the skin and seeds of the grape are in contact with the fermenting juice for a long period of time so that the pigments (primarily anthocyanins and tannins) and the polyphenols (predominantly catechins and proanthocyanidins) are extracted at a high rate. During the maturation and ageing processes, the biologically active compounds and other wine components react with each other to give the pigmented polymeric compounds that stabilize the wine colour (Z. PENG & al. [4], V.I. PETROPULOS & al. [5]).

In winemaking, the use of oenological tannins is a common practice, their use being authorized by the International Organization of Vine and Wine (OIV). These types of compounds are primarily used for the clarification of musts and wines (O. PASCUAL & al. [6]). Furthermore, oenological tannins present many other useful properties such as the antioxidant activity

(A. RICCI & al. [7]), the ability to scavenge for peroxy radicals (L.M. MAGALHÃES & al. [8]), the direct consumption of dissolved oxygen (M. NAVARRO & al. [9]), the ability to chelate Fe²⁺, the oxidative damage prevention mediated by Fenton-based reactions (C.A. PEREZ & al. [10]), antibrowning activity (D. OBRADOVIC & al. [11]), improvement of sensory properties (N. VIVAS [12]), red wines color improvement and stabilization (V. CANUTI & al. [13]), copigmentation effect (A.C. NEVES & al. [14]), new pigments formation (A.VERSARI & al. [15]), reduction odors elimination (N. VIVAS [12]) and bacteriostatic effects (O. PASCUAL & al.[6]). Obviously, to all these benefits, the interactions between the oenological tannins and proteins in order to prevent protein haze must be also added, with effects on the wine's astringency and bitterness (O. PASCUAL & al. [6]).

Many commercial types of tannin of different plant origins and chemical compositions are available on the market (M. MALACARNE & al. [16], E. OBREQUE-SLIER & al. [17]). The oenological tannins comprise different polymers of gallic and ellagic acids, whereas condensed tannins or proanthocyanidins are composed of flavan-3-ol subunits like catechin, epicatechin, epigallocatechin and gallic acid (G. VAZALLO-VALLEUMBROCIO & al. [18]). The condensed tannins from the skins and seeds of grapes are extracted during the winemaking process, whereas the hydrolyzable tannins are transferred to the wine from oak wood barrels during aging. Different oenological products, such as chips, staves and commercial oenological tannins are widely used to add more phenolic compounds to a wine. These types of compounds are natural substances obtained from different botanical species that are rich in proanthocyanidins (from skins and seeds of grapes) and/or hydrolyzable tannins (from oak wood) (M. MALACARNE & al. [16]).

Also, the high antioxidant properties of red wines are also attributed to the high concentration of biologically active compounds. The oenological tannins act as fining agents that stabilize the wine color, improve the wine structure and contribute to a number of high beneficial biological effects (G. VAZALLO-VALLEUMBROCIO & al. [18]).

Romania, as a wine-consuming country, favors many varieties of wines, one of the most preferred types being the red wine. Among the red wines grape variety, Fetească neagră is one of the oldest Romanian varieties, whose origin and extraordinary quality are incontestable, being cultivated since the Dacian period. The wine obtained from Fetească neagră grape variety has a memorable scent of dry plums and dark fruits, a fragrant bouquet which is indisputable, being among the most popular types of wine.

The concept of modern wines, especially for the fruitful and expressive dry red wines intended for rapid commercialization, calls for the need to use complex oenological tannins in their biotechnology. In order to diversify the range of accepted oenological tannins by the current regulations, a very rigorous quality control is required, as well as numerous tests and experiments that are aimed to offer the desirability of their use in wine production and on the consumption safety of these products (lack of organic solvents, concentrations below critical limits in heavy metals, absence of various contaminants).

The most important properties of tannins are related to the ability to form complex compounds with proteins, the antiradical ability and the capacity of these compounds to consume the dissolved oxygen. In order to efficiently use exogenous oenological tannins, it is always necessary to perform laboratory experiments to determine the type of tannin required, the optimal dose and the expected sensory effects. Exogenous oenological tannins do not replace natural tannins in musts and wines, but they guarantee that their initial concentration and all the beneficial attributes resulting from the treatment will improve the chromatic, olfactory and gustatory characteristics, as well as provide the beneficial effect by protecting the human body against cardiovascular diseases.

Based on these characteristics, tannins exert interesting antioxidant properties both in the pharmacological, food and oenological fields as well as imprinting interesting taste properties reassembled under the astringency term with implications in the sensory quality of red wines. The main objective of this study was to emphasize the influence of the tannins addition in the fermentative phase on the color and phenolic composition of a red wine obtained from Fetească neagră grapes variety.

Materials and Methods

1. The grapes and the winemaking

The study highlighted the Fetească neagră grape harvest of 2016-2017, Murfatlar vineyard. The grapes were harvested at their phenolic maturity and were carefully transported to the cellar. The Fetească neagră red wine was obtained through a classical technology that started with the grapes destemming and crushing. The obtained mash was afterwards sulphited and moved to other recipients for maceration and fermentation. At the initial stage of the experiment, a quantity of 1 g/L tannins of different origin (condensed, ellagic and gallic) was added to a red wine obtained from the Feteasca neagră harvest.

2. Oenological tannins treatment

For the oenological tannins treatment, 3 different commercial products were purchased from Sodinal (Bucharest, Romania) such as: Fermotan characterized by the complexity of its composition, being a multi-component tannin product composed of condensed, ellagic and gallic tannins; Gallotan product containing gallic tannins and Protan Raisin composed only of condensed tannins. The initial phase was preceded by a repeated periodic aeration (at an interval of 15 days) using an oxygen intake of 5 mg/L at each aeration, for a period of three months after which the final stage of the experiment was completed. In order to highlight the tannin effect, the color intensity was measured spectrophotometrically at various wavelengths ($\lambda = 420$ nm, 520 nm and 620 nm) and also by determining the polymerized pigments index at the initial and final stage of the experiment for each variant in comparison to the control variant.

Further, the experiments followed the Fermotan product treatment at doses of 20 g/hL and 40 g/hL on the Fetească neagră wine. The grape samples used in the experiment were healthy grapes with a corresponding phytosanitary status and grapes partially contaminated with *Botrytis cinerea* mold in a proportion of 10%. The evaluation of the effect of Fermotan addition was achieved by monitoring the color intensity and total polyphenols indexes as well as the sensory properties of the wines.

In order to highlight the interaction between tannins and polysaccharides, the wine obtained from the Fetească neagră variety was treated with autolysed yeast derivatives (Batonage Plus 150 KDa, 40 g/hL) and with a tannin complex consisting of condensed,

ellagic and gallic tannins (Fermotan, 20 g/hL) coupled with autolysed yeast derivatives (Batonnage Plus 150 KDa, 40 g/hL). The chromatic characteristics of the samples obtained from the three variants of wine were evaluated after 30 days.

3. Determination of the polymerized pigments index

By measuring the color intensity of the wine after adding SO₂, compared to the control sample, allowed the determination of the polymerized pigments index at the wine pH. To assess the polymerized pigments index (Equation 1), two assays were considered as follows: firstly, a volume of 45 mL of a synthetic solution with a pH of 3.2 and 0.2 mL of sodium metabisulphite 20% solution were added to 5 mL of wine, and after 5 minutes the first color intensity (Ci₁) was determined; secondly, the same volume of synthetic solution and 0.2 mL of water were added to 5 mL of wine, and the second color intensity (Ci₂) was determined. To calculate the Ci₁ and Ci₂, the absorbance of the two samples was read at 420, 520 and 620 nm, with water as blank.

$$I_{PP} = \frac{Ci_1}{Ci_2} \times 100 \quad (1)$$

4. Determination of anthocyanins ionization index

The anthocyanins ionization index (Equation 2) represents the percentage of colored anthocyanins from wine (free and combined, decolorable by SO₂). Thereby, two determinations were carried out that included the discoloration of the wine with an excess of SO₂, at the pH of the wine and at pH 1.15. The index was given by the ratio of the two discolorations expressed as percentage.

$$I_i = \frac{\Delta DO_\alpha}{\Delta DO_\gamma} \times 100 \quad (2)$$

The wine discoloration with SO₂ at the pH of the wine consisted of two parts: the first optical density (DO₁) was read at 520 nm for 10 mL of wine with 2 mL of distilled water; the second optical density was assessed after 15 minutes for 10 mL of wine with 2 mL 15% NaHSO₃. Equation 3 represents the contribution of colored anthocyanins at 520 nm.

$$\Delta DO_\alpha = (DO_1 - DO_2) \times \frac{12}{10} \quad (3)$$

The wine discoloration with SO₂ at the pH of 1.15 also included two parts: the first optical density (DO₁') was read at 520 nm for 1 mL of wine with 7 mL 0.1 N HCl and 2 mL of water; the second optical density (DO₂') was read after 15 minutes at 520 nm for 1 mL of wine with 7 mL 0.1 N HCl and 2 mL 15% NaHSO₃. Equation 4 represents the contribution of all the colored compounds (anthocyanins and polymerized pigments) at 520 nm.

$$\Delta DO_\gamma = (DO_1' - DO_2') \times \frac{100}{95} \quad (4)$$

5. Determination of total polyphenols index or D280 index

The total polyphenols index (TPI or D280 index) is a parameter that describes the content of total phenolic compounds (phenolic acids, tannins, anthocyanins, flavones etc.) from wines. It has values ranging from 3 to 15 for white wines and 20 to 100 for red wines. The principle of the method is based on the strong absorption of the ultraviolet light by the benzene nuclei, which is characteristic for the phenolic compounds and reaches a maximum absorption at the 275 - 280 nm wavelength interval. The D280 index is a spectral characteristic of all phenolic compounds (phenolic acids, tanning substances and coloring agents) present in the wine and was determined as follows: the wine was diluted 1% with distilled water, after which the absorbance was measured at 280 nm in comparison to distilled water. The result thus obtained was multiplied by the dilution factor and the value of D280 was obtained.

Results and Discussions

1. The effect of oenological tannins on the color intensity and on the polymerized pigments index

The evolution of the main parameters that were analyzed (color intensity and percentage of condensed tannins in a polymerized form) in the initial and final phase of the experiment for each of the three studied variants compared to the control variant is presented in Figures 1 and 2.

The final stage of the experiment usually highlights higher values of the color intensity and higher proportions of condensed tannins in a polymerized

form, which was also the case for the three variants of wine compared to the control variant.

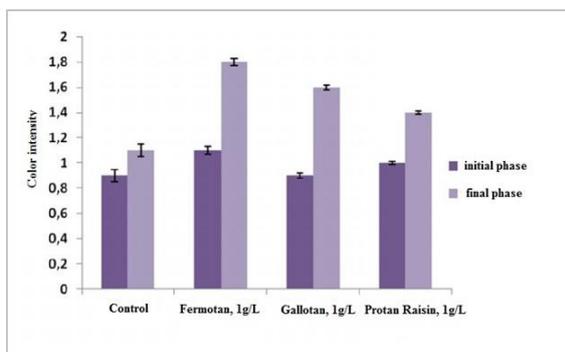


Figure 1. The color intensity of the wine samples in accordance to the type of tannin used during the alcoholic fermentation

The obtained results accentuated the synergistic action of tannin supplementation and micro-oxygenation on the color intensity increase and on the proportion of polymerized pigments. By using tannins in the fermentative step, a number of advantages were emphasized such as the ability to polymerize with anthocyanins and to form stable compounds thus avoiding the color losses as a result of the reactions between anthocyanins and other compounds. Moreover, another important property is the ability to react with oxygen hence avoiding the oxidation of polyphenols and attenuating the unwanted sensory character ("notes de réduction") by preserving varietal aromas. Also, they can prevent the early aging of wines by increasing the structural character of wines and reducing the doses of sulphurous anhydride in the pre-fermentative stage.

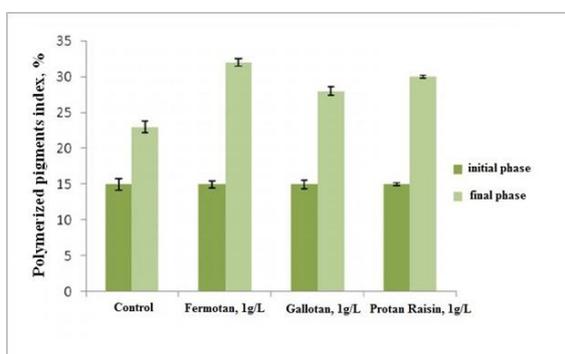


Figure 2. The polymerized pigments index of the wine samples in accordance to the type of tannin used during the alcoholic fermentation

C. GHANEM & al. [19] studied the chromatic properties and the antioxidant activity of a Cabernet Sauvignon red wine after the treatment with

commercial tannins and mannoproteins. The addition of fining agents and oenological additives decreased the color intensity and increased the hue of most of the treated wines compared to the control. M.E. ALAÑÓN & al. [20] studied the behavior of different aging chestnut treatments that involve the exogenous tannins on the polymerized pigments index of Tempranillo red wines. Regardless of the type of wine, the values of the parameter were significantly higher, ranging from 30.14 to 41.78% when compared to the control sample.

The final step of the experiment emphasized higher values of the color intensity and higher concentrations of condensed tannins in a polymerized form for the three studied variants compared to the control variant. The obtained results also drew attention on the synergistic action of tannin supplementation and micro-oxygenation that produced the color intensity and the proportion of polymerized pigments increase. In the case of the wines obtained from crops with a suitable phytosanitary status and those obtained from crops partially contaminated with *Botrytis cinerea* in a proportion of 10%, an increase of the color intensity was also reported almost at the same level as the increase induced by the Fermotan doses used for both wines (Figure 3).

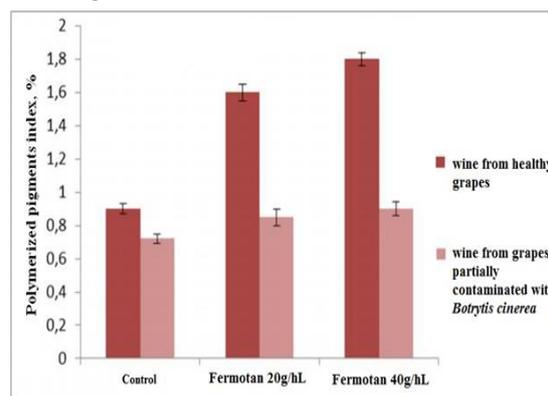


Figure 3. The color intensity evolution of the wine samples in accordance to the used Fermotan dose

The evolution of wine total polyphenols content revealed an increase once the dose of Fermotan, was raised from 20 g/hL to 40 g/hL for both wines obtained from grapes with a corresponding phytosanitary status and from those obtained from grapes with *Botrytis cinerea* in a proportion of 10% (Figure 4). The efficacy of Fermotan was explained by the complexity of its composition, being a multi-component tannin

composed of condensed, ellagic and gallic tannins, designed to achieve a maximum efficacy in stabilizing the color of the red wines by precipitating the unstable protein.

G. VAZALLO-VALLEUMBROCIO & al. [18] displayed the characteristics of Carménère red wine that were assessed after the treatment with different commercial enological tannins. Regarding the color intensity and the hue values of the enriched wines, the third variant of wine registered the lowest color intensity values at the end of the study (90 days) in respect to the other sampling dates, while the rest of the wines presented similar values throughout the whole study. In contrast, all wines showed increased hue values at the third sampling date.

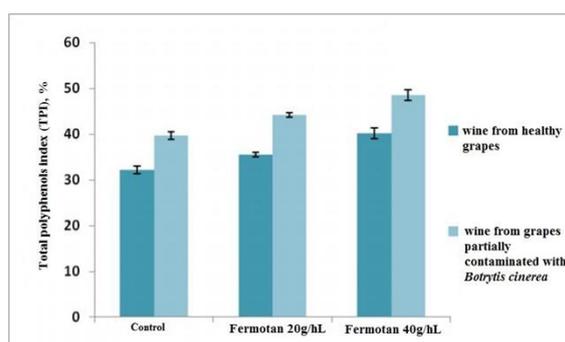


Figure 4. The total polyphenols index of the wine samples in accordance to the used Fermotan dose

M.E. ALAÑÓN & al. [20] presented the effect of 2 different oenological practices on the levels of different classes of phenolic compounds in the wines made from *Vitisvinifera* L. cv Tempranillo grapes. Several differences were observed between the phenolic composition of the wines made from the same grapes and with different oenological practices. The

values of total polyphenols index and the color intensity for the treated variants of wine presented smaller values when compared to the control sample. G. VAZALLO-VALLEUMBROCIO & al. [18] evaluated the effect produced by the addition of different commercial enological tannins on the characteristics of Carménère red wine. All the wines enriched with tannins were analyzed after 5, 45 and 90 days, and they presented very high values of total phenols compared to the control wine (WT0) which exhibited the lowest concentration.

2. The chromatic characteristics of the Fetească neagră red wines

Table 1 presents the chromatic characteristics of the wines 30 days after the completion of the alcoholic fermentation. Analyzing the data, it was observed that the yellow color component decreased in both cases compared to the control variant, while the red color values (as well as the intensity and brightness levels of the red color) underwent progressive increases compared to the blank sample.

The degree of polymerization and the degree of ionization also displayed an increase compared to the control variant. The evolution of the color intensity followed a progressive increase, while the wine tint (the ratio between the yellow and the red color, OD420nm/OD520nm) was stabilized while the violet color decreased insignificantly.

The redness degree (derived from French "indice de rosissement" - the quantification of the wine oxidability with H₂O₂) diminished considerably in the variant treated only with Batonnage 150 KDa and showed a notable but less accentuated reduction in the variant treated with both Batonnage 150 KDa and Fermotan product.

Table 1. Chromatic characteristics of different variants of wine			
Chromatic characteristics	Control	Wine variant with Batonnage Plus 150 KDa, 40 g/hL addition	Wine variant with Batonnage Plus 150 KDa, 40 g/hL and Fermotan 20g/L addition
Yellow color	30.0 ± 0.032	29.5 ± 1.060	29.0 ± 1.191
Red color	60.3 ± 2.054	61.1 ± 0.191	62.9 ± 0.097
dA, %	67.1 ± 0.140	68.5 ± 0.131	69.2 ± 0.543
Total polyphenols index (TPI)	54 ± 0.002	52 ± 0.028	53.5 ± 0.611
Color intensity	1.8 ± 0.531	2.3 ± 0.037	2.7 ± 0.468

Wine tint	0.5 ± 0.002	0.5 ± 0.106	0.5 ± 0.202
Polymerized pigments index	43 ± 1.412	44 ± 1.258	46 ± 1.907
Anthocyanins ionization index	74 ± 2.504	76 ± 1.384	81 ± 1.503
Redness degree (RS)	727 ± 5.201	532 ± 8.347	605 ± 9.011

Hence, it was demonstrated the ability of tannins to interact with polysaccharides so that the formed macromolecular aggregates decreased the astringency and improved the chemical and colloidal stability of the red wine in regards to aging abilities.

Other researchers evaluated and analyzed the effect of commercial tannins and mannoproteins on the pigment, color and tannins composition of a Cabernet Sauvignon red wine. The Total Polyphenols Index was largely affected by the used treatments. The decrease of TPI was explained by the removal of some polyphenolic compounds by the used treatments. The addition of tannins especially at a high concentration led to a significant increase of the TPI compared to the control wine. All the treated wines displayed a decrease in the content of total polyphenol except the wines in which the exogenous tannins were added, although the increase was not significant except that for the maximum concentration. The obtained results were due to the effect of tannins on the anthocyanins contents of wines. The tannins addition increased the total polyphenols index by 9% at higher concentrations and the total tannins by 8% while it decreased the total anthocyanins content (C. GHANEM & al. [19]). W. TCHABO & al. [21] assessed the chromatic indicators in correlation to the phytochemical profile of a sulfur dioxide-free mulberry red wine.

The authors studied four different methods of color measurement of wine proposed by Boulton, Giusti, Glories and the Commission International de l'Eclairage (CIE). The change of the chromatic properties and phenolic composition of the non-thermal aged mulberry wine were examined by different statistical tests. W. TCHABO & al. [21] studied many chromatic properties including the lightness, redness, yellowness, chroma, hue, angle, polymeric anthocyanin (PA) and saturation and also the total phenolic index (TPI), total flavonol index (TFI) and total anthocyanin index (TAI). The results revealed for all the parameters a positive effect of the

non-thermal treatments on the phytochemical families and color stability of studied wines.

M.E. ALAÑÓN & al. [20] studied the behavior of different aging chestnut treatments that involved the exogenous tannins (chips, barrels, and aging time) on the phenolic profile and color characteristics of Tempranillo red wines. Practically, the formation of more polymerized pigments decreased the color saturation as a result of a more complex mixture between the pigments that provide the actual red color of the wine. The reduction of the anthocyanins during the aging process also caused a significant decrease of the copigmentation percentage that contributed to the total wine color and an increase of the increase of the lightness. In contrast, the yellow component of color and the hue angle did not significantly change after the aging period for the treated wines.

The wines red color presented observable purplish nuances similar to the original young wine. Furthermore, the authors identified significant differences between the control and the aged wines in terms of lightness and saturation. The color intensity of the aged wines was significantly lower than that of the control sample. This fact could be attributable to the decrease on the anthocyanin content, since they are involved not only in the reduction of copigmentation effect but also in the formation of insoluble polymeric pigments which represent the coloring matter of aged wines. On the other hand, the tint of the wine was evaluated based on the yellow color of wines. The highest values of tint observed were found in the wines aged for 6 months. The obtained values were also related to the high content of polymeric pigments that produced a color stabilization (M.E. ALAÑÓN & al. [20]).

Conclusions

The obtained results highlighted the synergistic action of the tannin supplementation and the microoxygenation on the color intensity increase and on the proportion of the polymerized pigments for the three studied variants compared to the control variant.

The synergy between the three categories of tannins developed a triple protective action on anthocyanins, namely the stabilizing action provided by the contribution of condensed tannins, the stimulation of acetaldehyde production which is indispensable for the formation of stable colored complexes and free radicals provided by the presence of ellagic tannins and the protective action against oxidation caused by the gallic tannin. Tannins possess the property to interact with polysaccharides in such a manner so that they can form macromolecular aggregates with a beneficial influence on the astringency, the chemical and colloidal stability and the ageing of the red wine.

Conflict of interest disclosure

There are no known conflicts of interest in the publication of this article, and there was no financial support that could have influenced the outcomes. The manuscript was read and approved by all authors.

Compliance with ethical standards

Any aspect of the work covered in this manuscript has been conducted with the ethical approval of all relevant bodies and that such approvals are acknowledged within the manuscript.

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