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Received : 13 December 2018; Accepted : 14 February 2019; Published : 4 March 2019
DOI : 10.20870/oeno-one.2019.53.1.2361

OENO One
VINE AND WINE OPEN ACCESS JOURNAL

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ABSTRACT

Aims : The aim of this research was to determine and quantify the ability of enological tannins to reduce laccase activity and, consequently, to protect wine color against enzymatic browning and/or oxidasic haze.
Methods and results : Botrytized grape juice with laccase activity was obtained by inoculating *Botrytis cinerea* in healthy mature grapes. Laccase activity was determined in grape juice before and after supplementation with enological tannins using the syringaldazine method. White micro-fermentations were performed in the presence or not of laccase activity and supplemented or not with enological tannins in order to determine how the color was affected. Similarly, red micro-fermentations were performed using white grape juice supplemented with malvidin-3-O-glucoside. All enological tannins inhibited laccase activity and protected the wine color.
Conclusion : Supplementation with enological tannins is an interesting tool to inhibit laccase activity and protect the color of white wines from browning and the color of red wines from oxidasic haze.
Significance and impact of the study : This is the first scientific study evidencing the inhibitory effect of enological tannins on laccase activity in winemaking conditions.

KEYWORDS

enological tannins, *Botrytis cinerea*, grey mould, laccase activity, contact time, dose effect

INTRODUCTION

Enological tannins are usually classified in two families, namely hydrolysable and condensed tannins. Their use in winemaking is a common practice (Obradovic *et al.*, 2005), with doses ranging from 5 to 100 g/hL depending on the addition strategy and the desired effect. Nevertheless, up to date, they are only authorized by the International Organization of Vine and Wine (OIV) in order to facilitate the fining of musts and wines (OIV, 2015).

On the one hand, the family of hydrolysable tannins comprises gallotannins and ellagitannins. Gallotannins are polymers formed by esterification between D-glucose and gallic acid. Tannic acid is the commercial name for gallotannin extract comprising mixtures of polygalloyl quinic acid ester or polygalloyl glucoses (Pascual *et al.*, 2017). The main sources of commercial gallotannins are nut galls and tara. Ellagitannins are polymers of ellagic, gallic and/or hexahydroxydiphenic acids (Versari *et al.*, 2013). More precisely, a nonahydroxyterphenoyl unit (NHTP) is esterified in positions 2, 3 and 5 with a C-glycosidic bond, while an open-chain glucose is esterified in positions 4 and 6 with a hexahydroxydiphenoyl unit (HHDP) forming the chemical structure of ellagitannins (Quideau *et al.*, 2004). In addition to the major phenolic compounds, hydrolysable tannin preparations contain monosaccharides : oak, quebracho and hazel contain arabinose, xylose, glucose and fructose, while tannins from grapevine bunch and nut gall contain only fructose and glucose (Versari *et al.*, 2013). The main sources of commercial ellagitannins are oak, chestnut and myrobalan.

On the other hand, the family of condensed tannins, also called proanthocyanidins, mainly includes procyanidins, prodelphinidins and profisetinidins. They differ mainly in respect to the type of monomer released after acidic cleavage, the degree of polymerization (mDP), and their levels of galloylation (Versari *et al.*, 2013). Grape-skin tannins are composed of procyanidins and prodelphinidins since their acidic cleavage releases cyanidin and delphinidin. However, grape-seed tannins are only composed of procyanidins. Grape-skin tannins have a high mDP and a low level of galloylation, while grape-seed tannins have a lower mDP and a high level of galloylation (Souquet *et al.*, 1996). Quebracho tannins are profisetinidins, because their acidic cleavage gives fisetinidin, and they are characterized by a high level of ramification, while mimosa tannins are prorobinetidins releasing robinetinidin (Celzard *et al.*, 2015). The main sources of commercial condensed tannins are grapevine seeds and skins and other plant sources such as quebracho, mimosa and acacia (Versari *et al.*, 2013).

Furthermore, each group of tannins has different composition, nature, and chemical structure associated with varied properties. For this reason, enological tannins are also used for other goals than fining of must and wine. These properties include the improvement and stabilization of red wine color by means of their effect as copigments (Neves *et al.*, 2010) and their participation in the formation of new pigments (Versari *et al.*, 2013). Enological tannins are also used as antioxidant (González-Centeno *et al.*, 2012 ; Pascual *et al.*, 2017) and as antioxidasic (Versari *et al.*, 2013). Concerning the interaction with proteins, enological tannins are mainly used to help protein fining and prevent protein haze (Ribéreau-Gayon *et al.*, 2006), while avoiding gelatin over-fining (Peynaud, 1984). Finally, they are also used to improve wine structure and mouthfeel (Preys *et al.*, 2006) and to eliminate reduction odors (Vivas, 2001).

Although these properties of enological tannins have been widely described in the literature, the antioxidasic properties (anti-laccase) are not yet well documented. Botrytis bunch rot (BBR), caused by the necrotrophic pathogenic fungus *Botrytis cinerea*, is responsible for huge economic losses each year. The infection of the bunch with *B. cinerea* provokes serious biological and chemical changes that impact negatively on the organoleptic qualities of the wine (Ribéreau-Gayon *et al.*, 1980). When climatic conditions are wet under mild weather conditions (Ciliberti *et al.*, 2015), *B. cinerea* can infect directly grape berries from veraison onwards. A vintage contaminated by the pathogen at the rate of 5 % in severity already shows irreversible consequences on the organoleptic features of a qualitative red wine (Ky *et al.*, 2012). The infection of grape berries by *B. cinerea* is accompanied by the excretion of fungal metabolites (glycerol, gluconic acid and □-glucans) and enzymes (pectinases, proteases, tyrosinases and laccases) in the host cells. Laccases (EC 1.10.3.2) are *o*-diphenol and *p*-

diphenol : dioxygen oxidoreductases. Generally, in *B. cinerea*, two genes are found encoding laccases with molecular weights of about 60 kDa (Claus *et al.*, 2014). These multi-copper glycoproteins catalyze the oxidation of mono- and/or di-phenolic substrates in the presence of oxygen. One of the important features of laccase is that it is very stable at wine pH (More *et al.*, 2011). The oxidation of the polyphenols, and thus the alteration of the color, begins in the grape berry and continues in the grape juice. In skins from infected grape berries and in musts derived from botrytized grapes, the laccase produced by the fungus oxidizes the polyphenols and leads to the formation of quinones. These quinones will polymerize and form brown compounds, which is called oxidase case and leads to color degradation and instability (Pourcel *et al.*, 2007). This phenomenon takes place in red and white grape juices, giving brick and brown tints, respectively. Furthermore, the organoleptic qualities of wines are also impacted by changes in their equilibrium, body and mouthfeel (Claus *et al.*, 2014). Laccase activity in musts can be determined by a manual spectrophotometric method developed previously by Grassin and Dubourdieu (1986).

Until now, it is only possible to inactivate or inhibit the enzyme by thermovinification or addition of sulfur dioxide (Ribéreau-Gayon *et al.*, 2006) or to protect the grape juice against oxidation using inert gas or ascorbic acid (Li *et al.*, 2008). In our days, the consumer wants healthier and more eco-friendly products and for this reason, the wine industry is searching for alternative products for reducing or even eliminating the use of sulfur dioxide. Thus, the aim of this research was to determine the ability of enological tannins to reduce the laccase activity produced from the pathogenic infectious process by *B. cinerea* and, consequently, to protect the wine color against enzymatic browning and oxidasic haze.

MATERIALS AND METHODS

1. Chemicals and equipment

All samples and standards were handled without any exposure to light. L-(+)-tartaric acid, sodium hydroxide, sodium acetate, polyvinylpolypyrrolidone (PVPP), Tween 80 and syringaldazine were purchased from Sigma-Aldrich (Madrid, Spain). Malvidin-3-O-glucoside was purchased from Extrasynthese (Genay, France). D-(+)-glucose, peptone, agar and yeast extract were purchased from Panreac (Barcelona, Spain) and potassium metabisulfite was purchased from Acros Organics (Madrid, Spain). Ethanol (96 %) and hydrochloric acid were supplied by Fisher Scientific (Madrid, Spain). Yeast (Zymaflore® Spark) and nutrients (Nutristart®) were provided by Laffort (Floirac, France).

The equipment used was as follows : a spectrophotometer UV-Vis Helios Alpha™ (Thermo Fisher Scientific Inc., Waltham, MA, USA) ; an incubator IPP 260 (DD Biolab, Barcelona, Spain) ; a centrifuge Heraeus™ Primo™ (Thermo Fisher Scientific Inc., Waltham, MA, USA) ; and a CB Standard Balance (Cobos, Barcelona, Spain). All the materials for the micro-vinification were provided by the cellar "Mas dels Frares" of the Enology Faculty of the Rovira i Virgili University (Constanti, AOC Tarragona, Spain).

2. Commercial tannins

Five commercial tannins, representing the main botanical origins, were considered in this study. Specifically, three condensed tannins were used : one procyanidin from grape seeds, one procyanidin/prodelphinidin from grape skin and one profisetinidin from quebracho, as well as two hydrolysable tannins : one gallotannin from nut galls and one ellagitannin from oak. All these tannins were provided by Laffort (Floirac, France).

3. Fruit sampling

During the 2017 vintage, healthy grapes (*Vitis vinifera* cv. Muscat d'Alexandrie) were collected on September 18th (around 50 kg) from the experimental vineyard, planted in 1992, at the Enology Faculty of the Rovira i Virgili University in Constanti (AOC Tarragona ; 41°8'54.17" N and 1°11'53.89" E). The vineyard is at 87 m above sea level, and groundwater is located at a depth of around 4 m. The vines were trained on a vertical trellis system and arranged in rows 2.80 m apart, with 1.20 m spacing between vines. They were pruned using a double "Cordon de Royat" system, with 16 buds, 8 on each cane. Half of the grapes were kept at 4 °C to obtain healthy grape juice, whereas the other half was inoculated with *B. cinerea*.

4. Inoculation with *B. cinerea*

The *B. cinerea* single-spore isolate 213, originally isolated from grapevine leaves in 1998, was selected from the collection of UMR SAVE, Bordeaux (Martinez *et al.*, 2003). It was selected because of its virulence on grapevine leaves and berries and because it is a transposa type strain (Martinez *et al.*, 2003 ; Martinez *et al.*, 2005 ; Ky *et al.*, 2012). The pathogen was inoculated on Yeast Peptone Dextrose (YPD) Petri plates (20 g/L of peptone and glucose, 10 g/L of yeast extract and 17 g/L of agar in distilled water) and grown about 1 week at 20 °C in an incubator. Half of the grapes harvested (around 25 kg) were placed in five plastic boxes (600x400x200 mm) and inoculated by spraying a spore suspension ($1{\cdot}10^6$ conidia/mL, 1 drop of Tween 80 and 50 g/L of glucose in sterilized water) until the complete fruit surface was covered by the spore suspension. The plastic boxes containing the grapes were then incubated for around 3 weeks, at 20 °C, surrounded by two plastic boxes containing sterile water to maintain the humidity (90-100 %).

5. Obtaining of healthy and botrytized grape juice

The healthy grapes were crushed and pressed to obtain a healthy grape juice using a small pneumatic press (Venmhidrprei-040, Invia, Vilafranca del Penedès, Spain). The juice was also recovered under dry ice to keep the grape juice protected from oxidation and was slightly sulphited (60 mg of $K_2S_5O_7$/L). This healthy grape juice was settled with previous addition of 20 mg/L of pectolytic enzymes at 4 °C for 18 hours. The botrytized grapes were previously sorted visually to remove undesirable rotten berries due to other fungal development, notably by *Penicillium* spp. (blue-green color) or other fungal species (*Alternaria* spp. or *Clostridium* spp.), or acetic bacteria (red-pink color). Then, these selected botrytized grapes were crushed and pressed to obtain the botrytized grape juice using a small pneumatic press. The juice was also recovered under dry ice in order to keep it protected from oxidation, but without any addition of sulfur dioxide in order not to inhibit laccase activity. This botrytized grape juice was centrifuged at 8,500 rpm for 5 minutes. Both grape juices, healthy and botrytized, were then immediately stocked in glass bottles at - 4 °C.

6. Laccase activity measurement

Laccase activity was determined using an adaptation of the syringaldazine test method (Grassin and Dubourdieu, 1986). Five mL of the different samples were added with 0.8 g of PVPP (to remove phenolic compounds that can cause interference), stirred and centrifuged for 10 minutes at 8,500 rpm. One mL of the supernatant was introduced into a plastic spectrophotometer cuvette to which were also added : 1.4 mL of buffer solution (8.2 g/L of sodium acetate in deionized water, pH 5.5) and 0.6 mL of syringaldazine solution (60 mg/L of syringaldazine in ethanol 96 %). The solution was then homogenized by inverting the cell and the absorbance was measured at 530 nm every minute for 5 minutes (including time measurement at 0 minute). All analyses were performed in triplicate. By definition, a laccase unit (UL) corresponds to the amount of enzyme catalyzing the oxidation of a micromole of syringaldazine per minute. The following equation was used for the calculation of laccase activity by using the slope of the line obtained by a calibrating linear regression (ΔA) expressed in absorbance units/minute :

$$Laccase\ activity = 46.15\ x\ \Delta A\ \mu mol.ml^{-1} = 46.15\ x\ \Delta A\ UL$$

7. Inhibition effect of enological tannins on laccase activity

A solution of each one of the different tannins was prepared at 2 g/L in a model wine solution (12 % ethanol, 4 g/L of tartaric acid, pH 3.5). Then, the first tube, representing the control, was filled with 4 mL of botrytized grape juice supplemented with 1 mL of deionized water. The second tube, corresponding to a dose of 20 g/hL, was filled with 4 mL of botrytized grape juice and completed to 5 mL by adding 0.5 mL of tannin solution and 0.5 mL of deionized water. The third tube, corresponding to a dose of 40 g/hL, was filled with 4 mL of botrytized grape juice and completed to 5 mL by adding 1 mL of tannin solution. After 5 minutes, the tubes were supplemented with 0.8 g of PVPP, stirred, centrifuged for 10 minutes at 8,500 rpm and used for laccase activity measurement. All analyses were performed in triplicate. The following equation gives the residual laccase activity :

8. Inhibition kinetics of enological tannins on laccase activity

The estimation of the inhibition kinetics of enological tannins on laccase activity was carried out with the highest dose (40 g/hL) at 0, 1, 2, 3, 4, 5 or 10 minutes of incubation before adding the PVPP and centrifuging. These data were used to determine the time needed to reach the maximal inhibition of laccase activity. All analyses were performed in triplicate.

9. Small-scale winemaking trials

Two types of winemaking trials were performed using the grape juices obtained from the healthy and botrytized grapes. The first trial was carried out directly with the white grape juices (white winemaking), whereas the second trial was performed with the white grape juices supplemented with malvidin-3-O-glucoside (pseudo-red winemaking) in order to approach what happens in red winemaking. This strategy was selected instead of using real red winemaking because we wanted to determine the protective effect of the different enological tannins on the color of anthocyanins without the presence of the natural proanthocyanidins from seeds and skins that can also exert an inhibitory effect on laccase activity. All winemaking trials were carried out in plastic tubes of 30 mL which were used as fermentation vessels. Working solutions of the five enological tannins were prepared at 10 g/L in a model wine solution (12 % ethanol, 4 g/L of tartaric acid, pH 3.5) for supplementing the different winemaking trials. The healthy grape juice was used without any treatment. However, the botrytized juice was drastically treated with PVPP (160 g/L) and centrifuged for 10 minutes at 8,500 rpm to eliminate the interferences that its dark brown color could cause in the final wines. In the case of fermentations without laccase addition, 22 mL of healthy grape juice were added to each vessel. In the case of fermentations with laccase addition, 17 mL of healthy grape juice were supplemented with 5 mL of botrytized grape juice (corresponding to a 1.5 UL/mL of final volume). Immediately, 1 mL of deionized water was added for the controls or 0.5 mL of each tannin solution and 0.5 mL of deionized water (corresponding to a dose of 20 g/hL), or 1 mL of each tannin solution (corresponding to a dose of 40 g/hL). All the vessels were inoculated with 1 mL of a solution containing the yeast (10 g/L of Zymaflore Spark®, Laffort, Floirac, France) and 1 mL of nutrient solution (10 g/L of Nutristart®, Laffort, Floirac, France). The fermentations were carried out at room temperature (20 ± 2 °C) and were monitored by weighing the vessels. Residual sugars were determined to ensure that alcoholic fermentations were finished. With the aim of approaching red winemaking, the same experiment was done with supplementation of 50 mg/L of malvidin-3-O-glucoside (Extrasynthese, Genay, France). All these fermentations were performed in triplicate.

10. Impact of *B. cinerea* on the color of the wines

Once the fermentations were completed, the impact of *B. cinerea* on the color of the wine was determined by measuring the absorbance at 420 nm (yellow) for white winemaking and at 520 nm (red) for "red winemaking" (Glories, 1984). In the case of "red winemaking", the total anthocyanin concentration was also analyzed (Ribéreau-Gayon and Stonestreet, 1966). Finally, the total color difference (ΔEab*) between samples was obtained using the CIELAB coordinates (Ayala *et al.*, 1997) and was calculated using the following equation:

$$\Delta \mathrm{Eab}* = \sqrt{(\mathrm{L2}* - L1*)^2 + (a2* - a1*)^2 + (b2* - b1*)^2}$$

The ΔEab* represents a measure of the difference between two colors. ΔEab* is used to know whether the difference between two samples can be detected visually by the human eye. Generally, it is considered that the difference is visible to the human eye when ΔEab* > 3 (García-Marino *et al.*, 2013).

11. Statistical analysis

All the chemical and physical data are expressed as mean values ± standard deviation. The statistical analyses were carried out using the XLSTAT 2017 statistical package. The two hypotheses of normality and homoscedasticity of the data were tested, for all parameters, by using the Shapiro-Wilk test and Levene's test, respectively. When populations were distributed normally and presented homogeneity in variance, parametric tests (ANOVA and Tukey) were used to detect significant differences at p-value < 0.05. In contrast, when populations were not distributed normally and/or presented heterogeneity in variance, non-parametric tests (Kruskal-Wallis and pairwise-Wilcox) were used. Differences were considered to be statistically significant at p-value < 0.05.

RESULTS AND DISCUSSION

Figure 1 shows the residual laccase activity in botrytized grape juice supplemented with the different enological tannins at 0, 20 and 40 g/hL. The initial laccase activity of the grape juice was 8.58 ± 1.34 units. Residual activity was expressed in all cases as a percentage of residual activity with respect to the control (% of residual activity). The supplementation with all enological tannins caused a significant decrease in the residual laccase activity compared to the control. The residual activities following supplementation with the different enological tannins ranged between 55.3 ± 4.5 % (in the case of the grape-seed tannin at 40 g/hL) and 81.5 ± 7.5 % (for ellagitannin at 20 g/hL). Regarding the doses of enological tannins used, the higher the dose, the lower the residual activity. In fact, the residual activity ranged between 75.5 ± 8.6 % and 81.5 ± 7.5 % for the dose of 20 g/hL and between 55.3 ± 4.5 % and 68.9 ± 5.4 % for the dose of 40 g/hL. The differences between doses were significant at $P = 0.05$, with the only exception of the grape-skin tannin. In contrast, ellagitannins presented the highest difference between the two doses, decreasing from 81.5 ± 7.5 % to 58.5 ± 3.1 %. All the tannins exerted a similar inhibitory effect at the low dose (20 g/hL). Specifically, the percentage of residual laccase activity was 81.5 ± 7.5 % for ellagitannin, 79.9 ± 5.0 % for quebracho tannin, 78.7 ± 1.6 % for gallotannin, 76.7 ± 5.6 % for grape-seed tannin and finally 75.5 ± 8.6 % for grape-skin tannin. However, significant differences were observed by testing the highest dose (40 g/hL). Specifically, grape-seed tannins and ellagitannins were the most effective, reaching at the highest dose 55.3 ± 4.5 % and 58.5 ± 3.1 % of residual activities, respectively. Quebracho tannins, gallotannins and grape-skin tannins were the less effective, reaching levels of inhibition of 63.9 ± 1.1 %, 66 ± 2.6 % and 68.9 ± 5.4 %, respectively. These data confirm that all enological tannins significantly inhibited laccase activity at the usual dose of use.

All the data are the mean ± SD of three replicates. The capital letters represent the significant differences between the different doses for the same tannin. The Greek letters represent the significant differences between the different tannins for the same dose.

The inhibition kinetics of laccase activity exerted by the different enological tannins at the highest dose (40 g/hL) is presented in **Figure 2**. The results clearly indicated that all the tannins reached their maximal inhibitory effect after 3 minutes, with the only exception of skin tannins that needed 1 minute more. This data indicated that all enological tannins can inhibit laccase activity in a very short time.

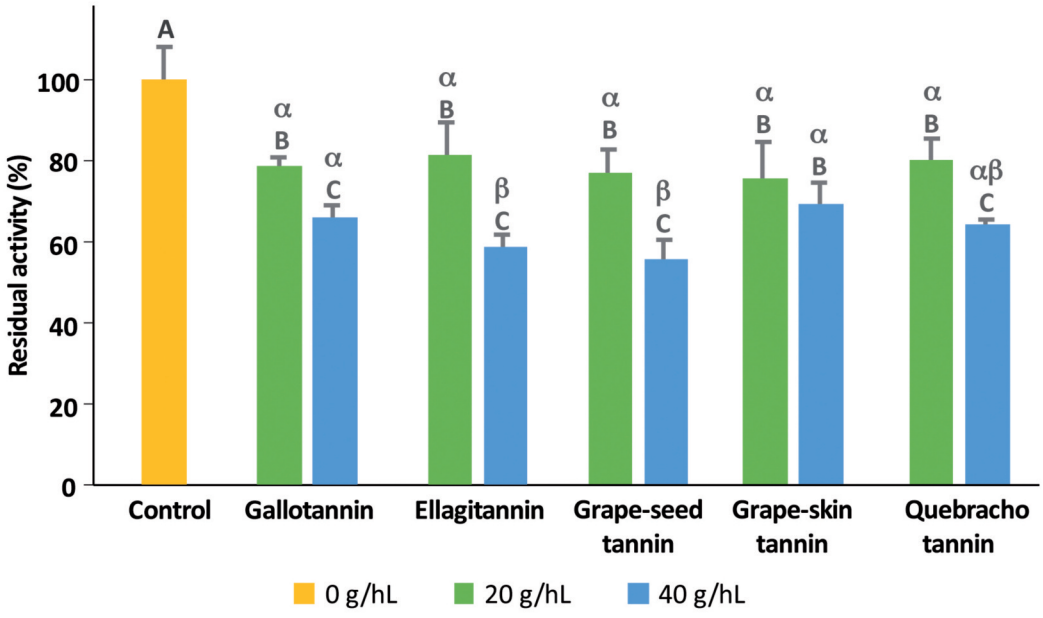

FIGURE 1. Inhibition effect of enological tannins on laccase activity.

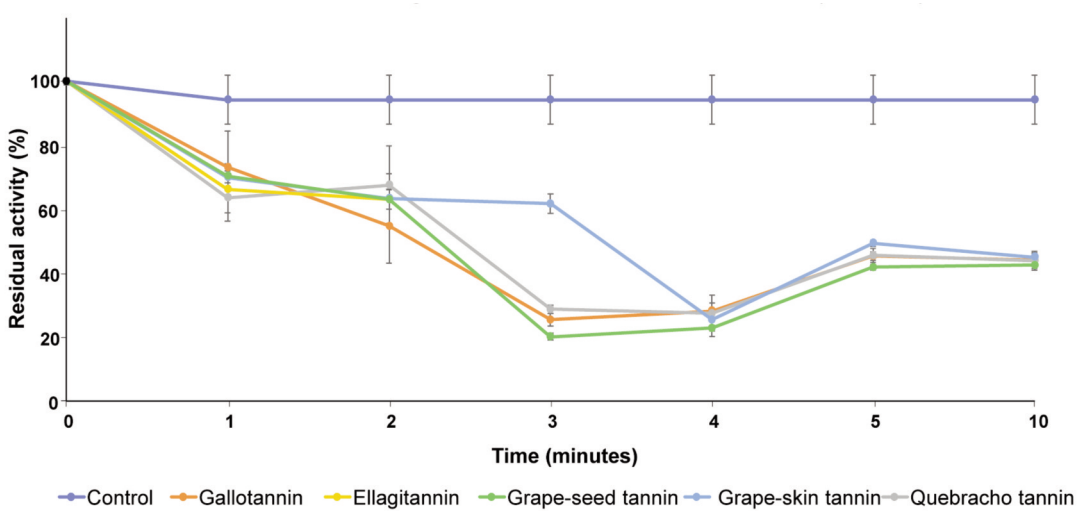

FIGURE 2. Inhibition kinetics of enological tannins on laccase activity.

All the data are the mean ± SD of three replicates.

The intensity changes of yellow color (A_{420}) in the white micro-fermentations are shown in **Figure 3**. As expected, the A_{420} of the control supplemented with laccase was significantly higher than in the control without laccase. This data confirmed that laccase, as it is well known, caused browning (Ribéreau-Gayon *et al.*, 1980). No significant differences were found in A_{420} when enological tannins were added in wines obtained without addition of laccase, despite a certain decreasing tendency. This trend suggests that the supplementation with enological tannins may play a certain role in protecting the wine against oxidation. In fact, it has been reported that enological tannins consume oxygen (Pascual *et al.*, 2017; Vignault *et al.*, 2018). The decrease in A_{420} following addition of enological tannins was quite more important when laccase was present, approaching the values of the wines obtained without laccase. This decrease reached its maximal effect at 20 g/hL, being similar at 40 g/hL for all the enological tannins with the exception of ellagitannins, for which the effect was, surprisingly, significantly lower at the high dose. This effect was specially marked in the case of ellagitannins, the enological tannins that have the highest inhibitory effect on laccase activity as previously shown. This data confirmed that supplementation with enological tannins really protects the color of white wine against the browning caused by the presence of *B. cinerea*.

All the data are the mean ± SD of three replicates. The capital letters indicate the existence of significant differences between with and without laccase for the same samples and the same doses.

Figure 4 shows the intensity of red color (A_{520}) of the micro-fermentations supplemented with malvidin-3-O-glucoside. As expected, the intensity of the red color of the control wine with laccase was significantly lower than in the control wine without laccase. In fact, red color intensity was decreased almost by half, confirming that the presence of this enzyme seriously affects the color of red wines. This decrease in the A_{520} of the wines supplemented with laccase was mitigated by the presence of all the enological tannins and this effect was in general greater at the high dose (40 g/hL). Condensed tannins protected better the red color than hydrolysable tannins because the differences in A_{520} with the wines obtained without laccase were lower and even sometimes not significant. This data demonstrated that supplementation with enological tannins really protects the color of red wine against the presence of laccase, preventing the oxidasic haze in red wines caused by the presence of *B. cinerea*. In that case, the behavior is exactly the opposite than in the previous figure.

All the data are the mean ± SD of three replicates. The capital letters indicate the existence of significant differences between with and without laccase for the same samples and the same doses.

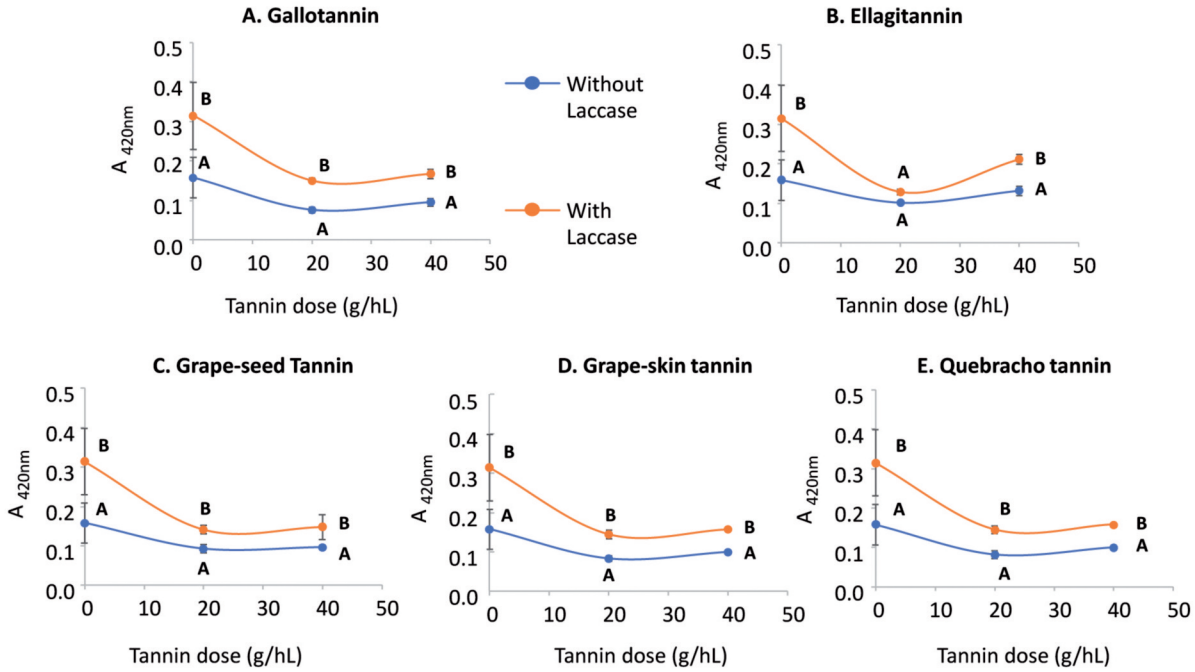

FIGURE 3. Influence of the supplementation with enological tannins on the changes in the yellow component of the color (absorbance at 420 nm) of wines made with healthy white grape juice with addition or not of 1.5 units of laccase activity.

The concentration of anthocyanins in the different wines elaborated with supplementation of malvidin-3-O-glucoside is presented in **Figure 5A**. The total concentration of anthocyanins in the control wine without addition of laccase was around 15 mg/L. This concentration was quite lower than that added to the grape juice (50 mg/L) probably due to oxidation, absorption by the yeast surface and/or formation of new pigments. In any case, the total concentration of anthocyanins in the control wine supplemented with laccase was significantly lower (at $P = 0.05$) than in the control wine without laccase. Laccase really degraded these pigments since anthocyanin concentration decreased to less than one third due to presence of this enzyme. The inhibitory effect of enological tannins on laccase activity was noticeable since the concentration of anthocyanins was significantly higher (at $P = 0.05$) when enological tannins were added in the presence of laccase.

Figure 5B further shows the protective effect of enological tannins in terms of percentage of anthocyanin loss originated by the presence of laccase according to the tannin dose. The results clearly confirmed that the presence of all type of enological tannins significantly protected anthocyanins against the laccase effect and that this protection tended to be greater when the dose of tannins was higher.

All the data are the mean ± SD of three replicates. The capital letters indicate the existence of significant differences between with and without laccase for the same samples and the same doses. The lower-case letters indicate the existence of significant differences between the samples.

The total color differences (ΔEab*) between each one of the wines supplemented with laccase and the control wine without laccase are shown in **Figure 6**. This value is a good indicator to assess whether the color of the wines was affected by the presence of laccase and degraded enough to be distinguished by the human eye. The human eye can theoretically distinguish two samples when ΔEab* ≥ 1 (Pérez-Magariño and González-Sanjosé, 2003). However, it is also generally accepted that tasters can only distinguish the color of two wines through the glass when ΔEab* ≥ 3 units (García-Marino *et al.*, 2013).

Specifically, **Figure 6A** shows the total color differences obtained with white wines. The comparison between the two control white wines

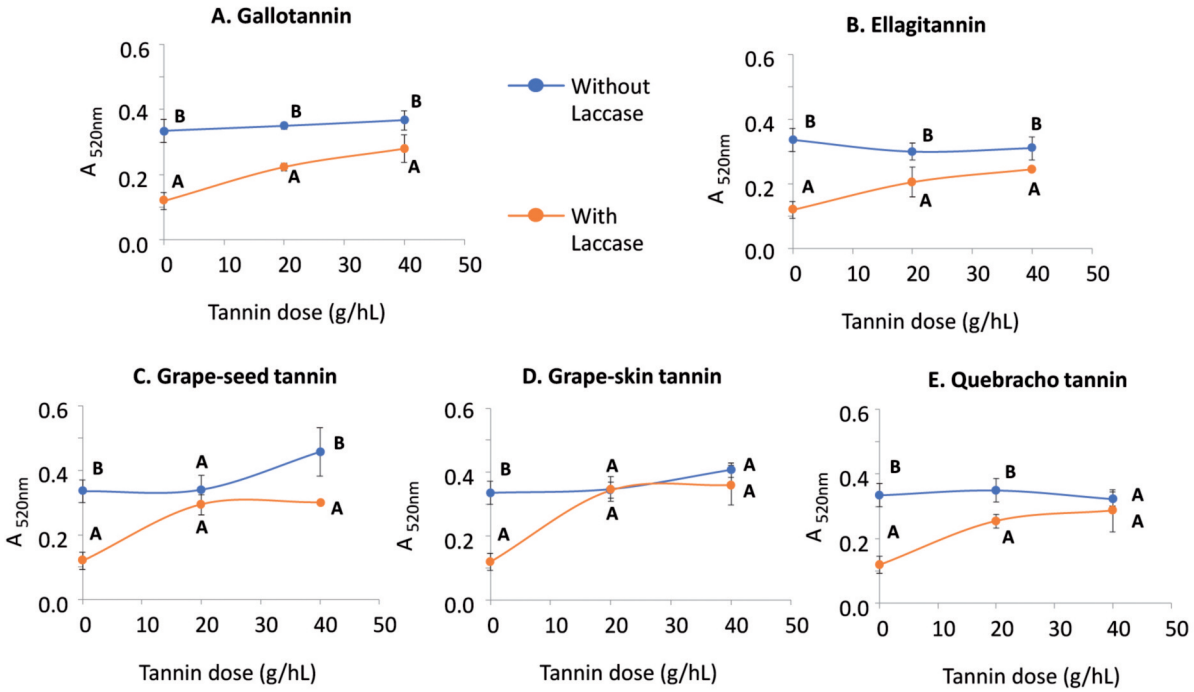

FIGURE 4. Influence of the supplementation with enological tannins on the changes in the red component of the color (absorbance at 520 nm) of wines made with healthy white grape juice supplemented with 50 mg/L of malvidin-3-O-glucoside with addition or not of 1.5 units of laccase activity.

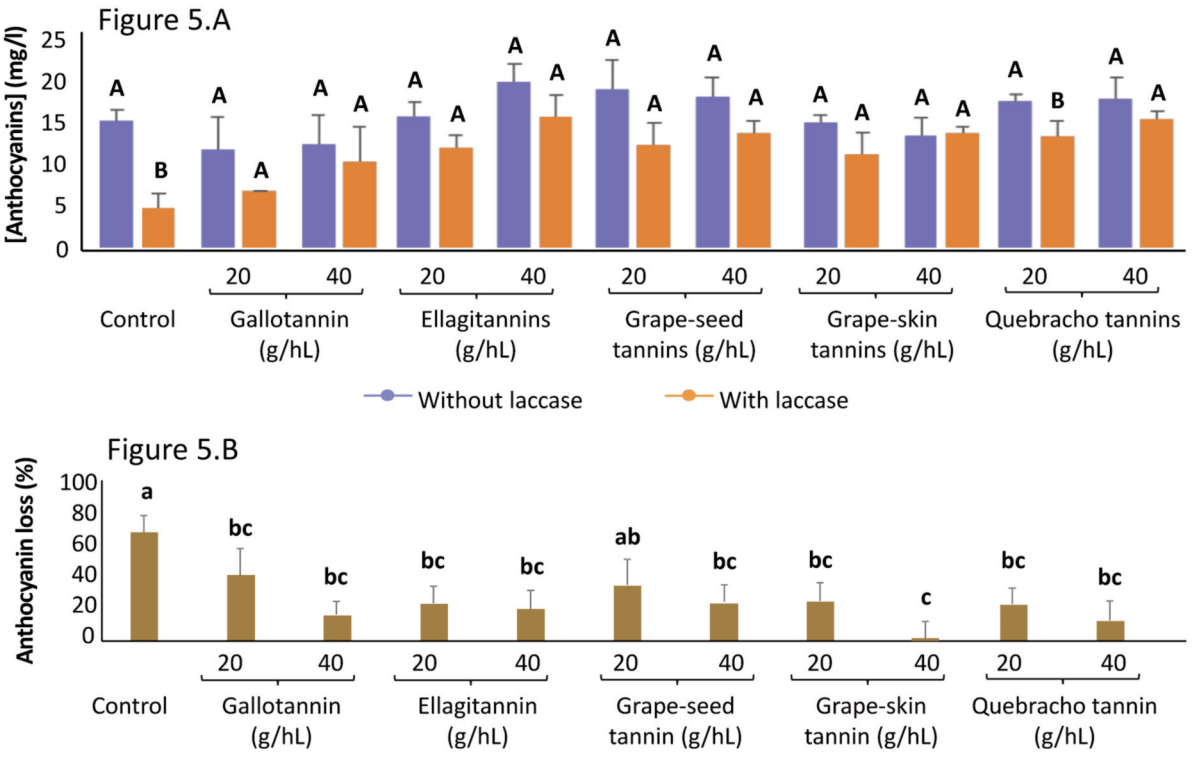

FIGURE 5. Influence of the supplementation with enological tannins on the malvidin-3-O-glucoside concentration (A) and loss (B) of wines made with healthy white grape juice supplemented with 50 mg/L of malvidin-3-O-glucoside with addition or not of 1.5 units.

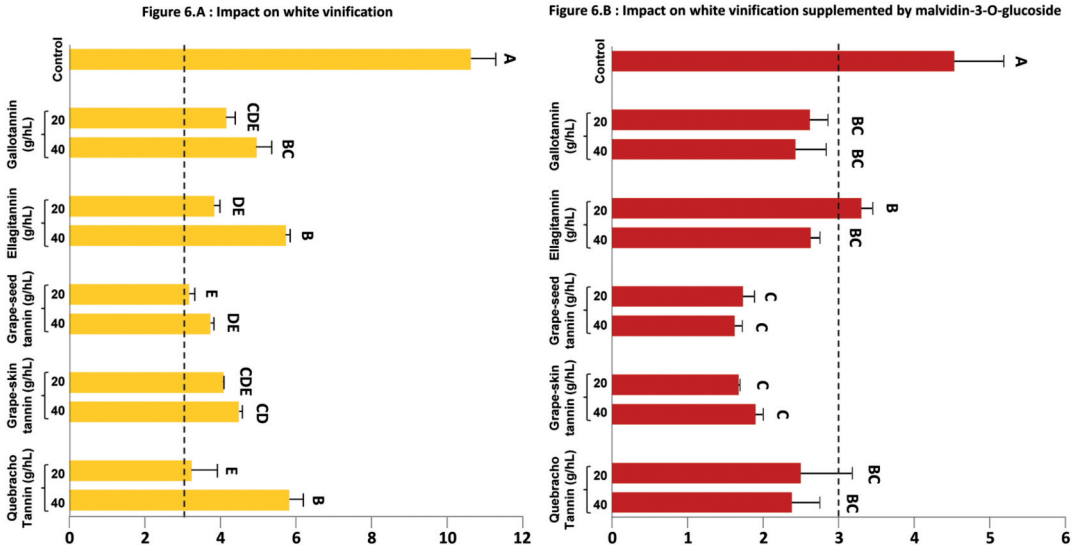

FIGURE 6. Impact of enological tannins added in botrytized wines on color visible to the human eye.

(with and without laccase) showed a ΔEab* reaching around 10 units. Since this value is much higher than 3 units, this result indicated that the presence of laccase drastically affected the quality of the wine color. The total color difference between the wines affected by the presence of laccase and the control wine without laccase was also, in all cases, greater than 3 units. However, it must be highlighted that these ΔEab* values were, in all cases, much lower than those observed between the two control wines : with and without laccase. Even in the case of the lower dose of seed tannins and quebracho tannins, the corresponding ΔEab* values were very close to 3 units.

Finally, the results for the total color differences in the white wines supplemented with malvidin-3-O-glucoside are presented in **Figure 6B**. The general trend was very similar to that observed for the white wines (Fig. 6A). The ΔEab* comparing the two control red wines, with and without laccase, was around 5 units. This value being greater than 3 units, the presence of laccase deteriorated sufficiently the color of the red wine to be distinguished by the human eye. Moreover, the supplementation with every enological tannin significantly decreased the ΔEab* compared to the control wine without laccase. The corresponding ΔEab* values in most of the cases were lower than 3 units. These results confirmed again that enological tannins protected the color of the red wines, since in most of the supplemented wines the human eye cannot distinguish between the healthy wine and the wines affected by the presence of laccase.

All the data are the mean ± SD of three replicates. The capital letters indicate the existence of significant differences between the different samples.

CONCLUSIONS

It can be concluded that enological tannins really inhibit laccase activity. Only small differences in the effectiveness of the different types of tannins were observed. In terms of winemaking process, the duration of contact needed to reach the maximal inhibition of laccase can be considered as very short (around 4 minutes). The supplementation with all enological tannins really mitigates the negative effect due to the presence of laccase by affecting the color of white and red wines. This effect seems to be more effective in the case of the protection of red color in red wines. Indeed, the presence of enological tannins allows protecting anthocyanins from oxidation. It can be concluded, therefore, that supplementation with enological tannins is an interesting tool to inhibit laccase activity and protect the color of white wines from browning and the color of red wines from oxidasic haze.

ACKNOWLEDGMENTS

The authors are grateful to Laffort industries (Floirac, France) for providing the enological

tannins, nutrients and yeast and for financial support.

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