iVES OENO One vine & wine

DOI: https://doi.org/10.20870/oeno-one.2023.57.3.7567

OPEN ACCESS

ORIGINAL RESEARCH ARTICLE

Effects of laccase from *Botrytis cinerea* on the oxidative degradation of anthocyanins

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Associate editor: Luca Rolle

Received: 17 May 2023

Accepted: 17 July 2023

Published: 7 September 2023

ABSTRACT

This work aimed to study the degradation kinetics of five grape anthocyanins caused by laccase from *Botrytis cinerea*. In individual solutions, the anthocyanins with three substituents in the B-ring—petunidin, delphinidin and malvidin 3-*O*-glucosides—were degraded much faster than those with two substituents. In the latter case, cyanidin 3-*O*-glucoside did not degrade as quickly, and peonidin-3-*O*-glucoside, in particular, was not degraded by laccase at all. In contrast, when an equimolar solution of the five anthocyanins was used, the differences in the degradation kinetics of all anthocyanins were lessened, probably because the less reactive anthocyanins were able to polymerise with the quinones formed by the laccase action on the more reactive anthocyanins. Finally, supplementation with (-)-epicatechin, glutathione and especially seed tannins seemed to protect the red colour from laccase.

KEYWORDS: laccase; *Botrytis cinerea*; anthocyanins; oxidative degradation; oxidasic haze; browning

INTRODUCTION

Polyphenol oxidases are multi-copper oxidative enzymes found in plants, fungi and bacteria that belong to the family called multi-copper oxidases (Ma *et al*., 2009; Strong and Claus, 2011). This family of enzymes, highly important from an oenological point of view, includes tyrosinase (EC 1.14.18.1, IUBMB, 2023), which is naturally present in grape berries (du Toit *et al*., 2006; Fronk *et al*., 2015) and laccase (EC 1.10.3.2, IUBMB), which is only present in grapes infected by epiphytic fungi, mainly *Botrytis cinerea* (Strong and Claus, 2011; Claus *et al*., 2014). Both tyrosinase and laccase can oxidise several substrates such as caftaric and cutaric acids, catechin, anthocyanins, flavanols and flavanone as substrates, but laccase acts on a far wider range of substrates than tyrosinase (Oliveira *et al*., 2011; Steel *et al.*, 2013).

Botrytis cinerea, a necrotrophic pathogenic fungus, causes grey rot. This is probably the worst plague affecting vine culture since it causes huge economic losses each year for agriculture, especially in grape and wine production (Steel *et al*., 2013). In addition to the release of laccase, which seriously affects wine colour (La Guerche *et al*., 2007; Ky *et al*., 2012; Vignault *et al*., 2019; Giménez *et al*., 2022), *Botrytis cinerea* causes several other problems, such as contamination with non-desirable microorganisms (Barata *et al*., 2008; Lleixà *et al*., 2018), problems of settling and filtration (Villettaz *et al*., 1984; Jadhav and Gupta, 2016), presence of ochratoxin A (Ponsone *et al*., 2012; Valero *et al*., 2008) and mouldy odours (Lorrain *et al*., 2012; Meistermann *et al*., 2021).

It is, therefore, clear that the infection of grape berries with *Botrytis cinerea* is undoubtedly one of the main problems in viticulture today since their presence seriously affects the quality of the final wine product. In the long list of problems that *Botrytis cinerea* causes, wine colour deterioration is probably the one that worries winemakers the most.

The main consequence of polyphenol oxidases, irrespective of whether tyrosinase and/or laccase are the enzymes responsible, is that diphenols are oxidised to quinones (Claus, 2004; Li *et al*., 2008). These quinones can polymerise through several reactions, forming brown pigments called melanins (Queiroz *et al*., 2008; Oliveira *et al*., 2011). These pigments, which are relatively insoluble depending on their degree of polymerisation (Moon *et al*., 2020), are responsible for increasing the brown colour in white wines (browning) and for the precipitation of the colouring matter in red wines (oxidasic haze) (Ribéreau-Gayon *et al*., 2006).

Red wines are particularly appreciated for the intensity and stability of their colour. For this reason, the presence of laccase from *Botrytis cinerea* in red grapes poses a serious problem since it typically makes those wines have a less intense and less stable red colour. Several studies about the effect of laccase on the browning of white grape must and wines (Gómez *et al*., 1995; La Guerche *et al*., 2007; Li *et al*., 2008; El Hosry *et al*., 2009; Oliveira *et al.,* 2011; Ky *et al*., 2012; Zimdars *et al*., 2017; Vignault *et al*., 2019; Giménez *et al*., 2022) have been reported, but only very little information exists about the effects of laccase on red wine colour (Ky *et al*., 2012; Steel *et al*., 2013; Vignault *et al*., 2019; Kelly *et al*., 2022) and to our knowledge even less about the effect of laccase on grape anthocyanins (Ky *et al*., 2012; Fang *et al.,* 2015; Detering *et al*., 2018).

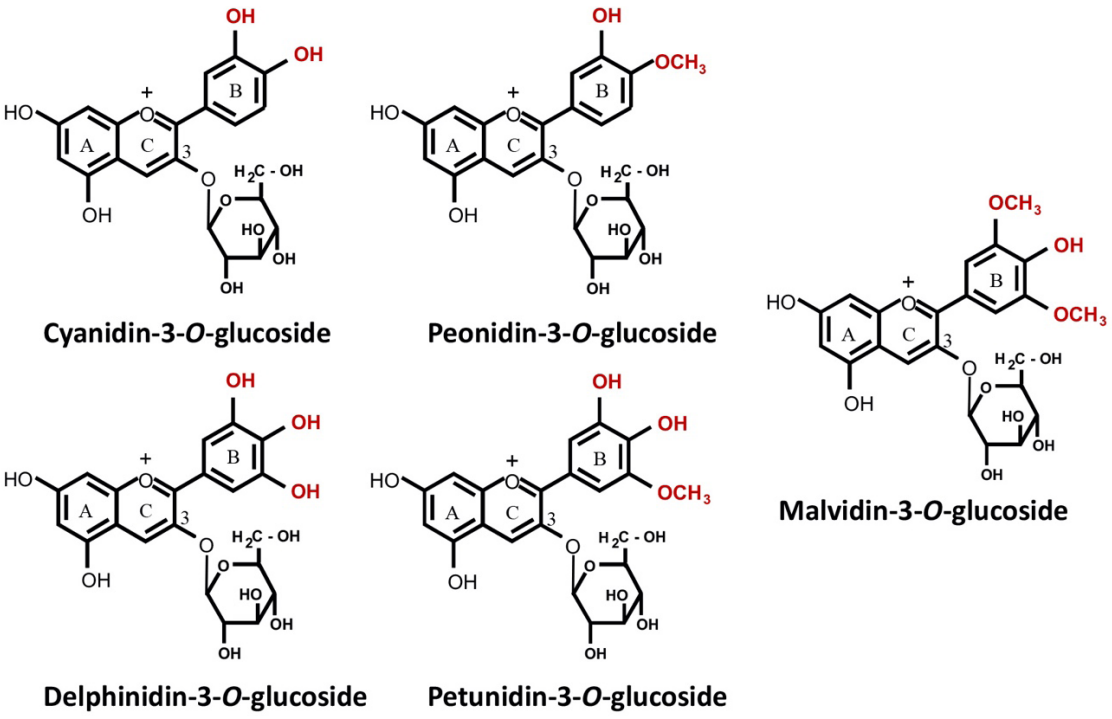

FIGURE 1. The five natural *Vitis vinifera* anthocyanins.

Moreover, the differences that exist in the B ring of the various grape anthocyanins (Figure 1) make the study of the relationship between the anthocyanin structure and the laccase degradation kinetics a matter of great interest.

Otherwise, the most common solutions that winemakers use to protect grape juice from the browning generated by polyphenol oxidases are basically to increase the dose of sulphur dioxide (Ribéreau-Gayon *et al*., 2006). However, the current trend in oenology is to try to reduce or even eliminate the use of this unfriendly additive (Lester, 1995; Costanigro *et al*., 2014; D'Amico *et al*., 2016; Massov, 2019). To this end, the use of oenological tannins (Vignault *et al*., 2019; Vignault *et al*., 2020) and glutathione (El Hosry *et al*., 2009; Giménez *et al*., 2022; Giménez *et al*., 2023) have been suggested as alternatives to protect wine colour from laccase action.

This work aimed to study the effects of laccase activity on the degradation of different anthocyanins in a synthetic media similar to grape juice and determine the possible protective effects of oenological tannins and glutathione.

MATERIALS AND METHODS

1. Chemicals and equipment

Polyvinylpolypyrrolidone (PVPP, CAS No.: 9003-39-8, purity ≥ 98 %), syringaldazine (purity ≥ 98 %), L-ascorbic acid (purity ≥ 99 %), L-glutathione reduced (purity ≥ 98 %), $FeSO_4 \cdot 7H_2O$ (purity ≥ 99 %) and (-)-epicatechin (purity ≥ 98 %) were purchased from Sigma-Aldrich (Madrid, Spain). L-(+)-tartaric acid (purity ≥ 99.5 %), sodium hydroxide (purity ≥ 98 %), methanol (purity minimum 99.9 %) and formic acid (purity ≥ 99.9 %) were high-performance liquid chromatography (HPLC) grade, sodium acetate (purity ≥ 99 %) and CuSO4 (purity ≥ 99 %) were purchased from Panreac (Barcelona, Spain). Ethanol (96 % vol.) was supplied by Fisher Scientific (Madrid, Spain). Delphinidin-3-glucoside-chloride (purity = 97.38 %), peonidin-3-glucoside-chloride (purity = 98 %), petunidin-3-glucoside-chloride (purity = 96.71 %) were purchased from Phytolab. D-glucose and D-fructose were purchased by VWR International (Leuven, Belgium). Cyanidin-3-glucoside -chloride (purity ≥ 98 %) was supplied from TargetMol (Wellesley Hills, USA). Tannins from grape seeds (purity ≥ 85 %) were from Alvinesa Natural Ingredients S.A. (Daimel, Spain). Malvidin-3-glucoside (purity ≥ 95 %) was supplied by Extrasynthese (Genay Cedex, France).

The equipment used was high-performance liquid chromatography (HPLC), an Agilent 1200 series liquid chromatograph equipped with a G1362A refractive index detector (RID), a G1315D diode array detector (DAD), a G1311A quaternary pump, a G1316A column oven and a G1329A autosampler (Agilent Technologies, Santa Clara, CA, USA); a UV-Vis Helios Alpha™ spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA), a Heraeus™ Primo™ centrifuge (Thermo Fisher Scientific Inc., Waltham, MA, USA) and an Entris II Series Analytical Weighing Balance (Sartorius, Göttingen, Germany).

2. Synthetic grape must solution buffer

A solution of 4 g/L of L-(+)-tartaric acid, a solution containing 100 g/L of D-glucose, and 100 g/L of D-fructose adjusted to pH 3.5 with sodium hydroxide was used for all experiments.

3. Extracellular laccase production and enzymatic activity measurement

Active laccase extracts were obtained from the *Botrytis cinerea* isolate-213 strain following the methodology reported by Vignault *et al*. (2020). This laccase extract was treated with 0.16 g of PVPP/mL for 10 min and then centrifuged at 7500 rpm for 10 min. The supernatant was subsequently dialysed with a 3.5 kDa cellulose membrane for 2 days in a 0.3 M ammonium formate solution and for 2 more days in distilled water. The laccase activity of this extract was determined using an adaptation of the syringaldazine test method (Grassin and Dubourdieu, 1986). The purified laccase solution used had exactly 100 UA of laccase activity/mL.

4. Colour measurements

Degradation of the red colour (A520 nm) and increase in the yellow colour (browning) (A420 nm) of the samples were determined by spectrophotometry.

5. Anthocyanins quantification by HPLC

Delphinidin-3-*O*-monoglucoside, cyanidin-3-*O*-monoglucoside, petunidin-3-*O*-monoglucoside, peonidin-3-*O*-monoglucoside and malvidin-3-*O*-monoglucoside concentration were determined by reverse-phase HPLC analyses with an Agilent 1200 series liquid chromatograph (HPLC–diode array detection) using an Agilent Zorbax Eclipse XDB-C18, 4.6 × 250 mm 5-µm column (Agilent Technologies, Santa Clara, CA, USA), in accordance with the method described by Gil *et al*. (2012). This quantification aimed to estimate the possible losses of each anthocyanin due to enzymatic oxidation.

6. Quantification of anthocyanins by spectrophotometry

Anthocyanins were determined using the adaptation of a method reported by Ribérau-Gayon and Stonestreet, 1965. A sample of 70 µL was extracted from the original thermostated cuvette. This sample was homogenised in a plastic vial with 70 µL of pure ethanol and 280 µL of 2.8 % hydrochloric acid. Subsequently, 210 µL of this plastic vial was mixed with 83.7 µL of distilled water (plastic vial 1), while the other 210 µL was mixed with 83.7 µL of a 15 % potassium metabisulfite solution (plastic vial 2), stirred and left to react for 10 min. Once the reaction time had passed, the absorbance of the two samples (plastic vials 1 and 2) was measured at 520 nm. Anthocyanin concentration was obtained from the difference between plastic vials 1 and 2 by multiplying the factor corresponding to the molar absorptivity coefficient of malvidin (Ribéreau-Gayon and Stonestreet, 1965) and the corrections corresponding to the applied dilution factor. In our experimental conditions, the

absorbance difference multiplied by 238.82 gives the total quantity of anthocyanins (mg/L).

7. Laccase degradation kinetics of individual anthocyanins

Solutions of the five anthocyanins were prepared at a concentration of 300 µM µM in the synthetic grape must solution buffer. The reaction mixture was prepared in a 1 mL spectrophotometer cuvette (ref: 7592 20, UV cuvette micro, BRAND®, Lab Unlimited, Dublin) mixing 600 µL of a stock solution of each of the anthocyanins (500 µM), 380 µL of synthetic grape must solution buffer and 20 µL of laccase solution. This reaction mixture, therefore, had an anthocyanin concentration of 300 µM and 2 UA of laccase activity/mL.

After mixing, the cuvettes were kept at 28 °C throughout the experiment. Absorbance at 520 nm was measured at 0, 1 and 2 hours. At exactly the same frequency (0, 1 and 2 hours), aliquots of 40 µL were extracted, and the reaction was stopped by adding 5 µL of sodium azide (10 mM). These aliquots were immediately used for an HPLC anthocyanin analysis.

8. Laccase degradation kinetics of a mixture of the five grape anthocyanins

A similar procedure to that reported in Section 7 was performed using a mixture of the five anthocyanins at an individual concentration of 60 µM, representing a total anthocyanin concentration of 300 µM. In this case, samples were used for colour measurements and HPLC analysis at 0, 1, 2, 6, 10 and 24 hours, as in the previous experiment, to extend the laccase action time.

9. Study of the possible protective effect of seed tannins, (-)-epicatechin and glutathione on the laccase degradation kinetics of a mixture of the five grape anthocyanins

A similar procedure to that reported in Section 8 was performed by adding or not adding seed tannin (200 mg/L), (-)-epicatechin (200 mg/L) or glutathione (20 mg/L). In addition to measuring the red colour (A520) and anthocyanin by HPLC, the absorbance at 420 nm (A420), indicative of browning and an anthocyanin analysis by spectrophotometry were also carried out.

10. Statistical analysis

The data shown are the arithmetic means of triplicates with the standard deviation for each parameter. Two-way ANOVA Tukey comparison tests were carried out using the XLSTAT software (Addinsoft, Paris, France).

RESULTS AND DISCUSSION

1. Laccase degradation kinetics of the five different grape anthocyanins

Figure 2 shows the degradation kinetics of the five different pure grape anthocyanins by laccase action from *Botrytis cinerea*.

As can be seen in the graphs, petunidin-3-*O*-glucoside and delphinidin-3-*O*-glucoside showed the maximal degradation rate (85.75 µM/h and 80.4 µM/h, respectively), followed in descending order by malvidin-3-*O*-glucoside (57.66 µM/h) and cyanidin-3-*O*-glucoside 20.88 µM/h). Surprisingly, peonidin-3-*O*-glucoside was not degraded by the action of laccase.

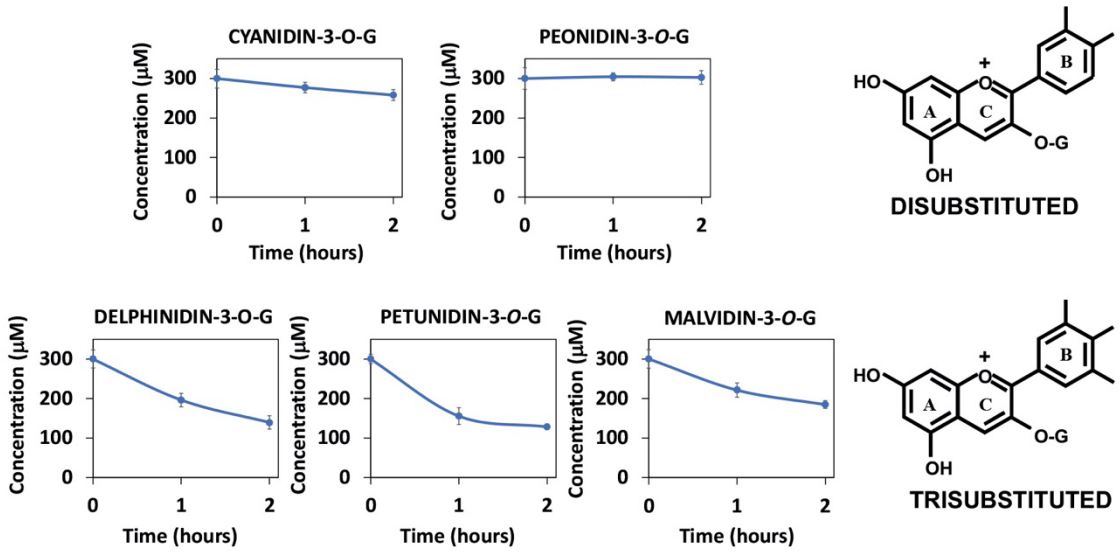

FIGURE 2. Degradation kinetics of the five different pure grape anthocyanins by the action of laccase determined by HPLC.

Results are expressed as mean ± standard deviation of three replicates.

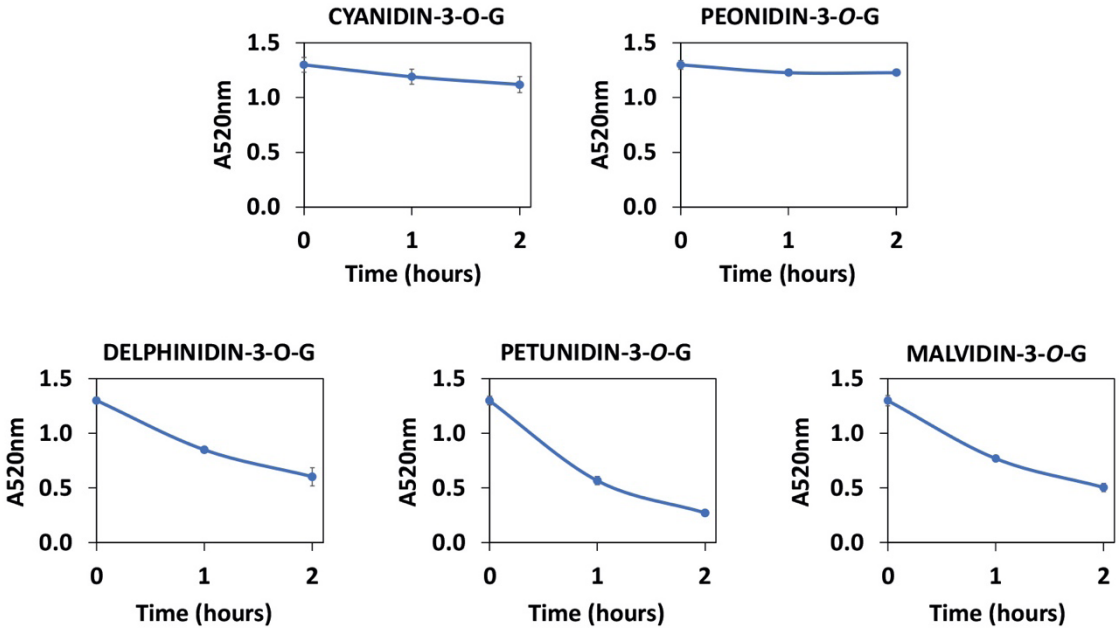

FIGURE 3. Degradation kinetics of red colour (A520 nm) of the five different grape anthocyanins by the action of laccase.

Results are expressed as mean ± standard deviation of three replicates.

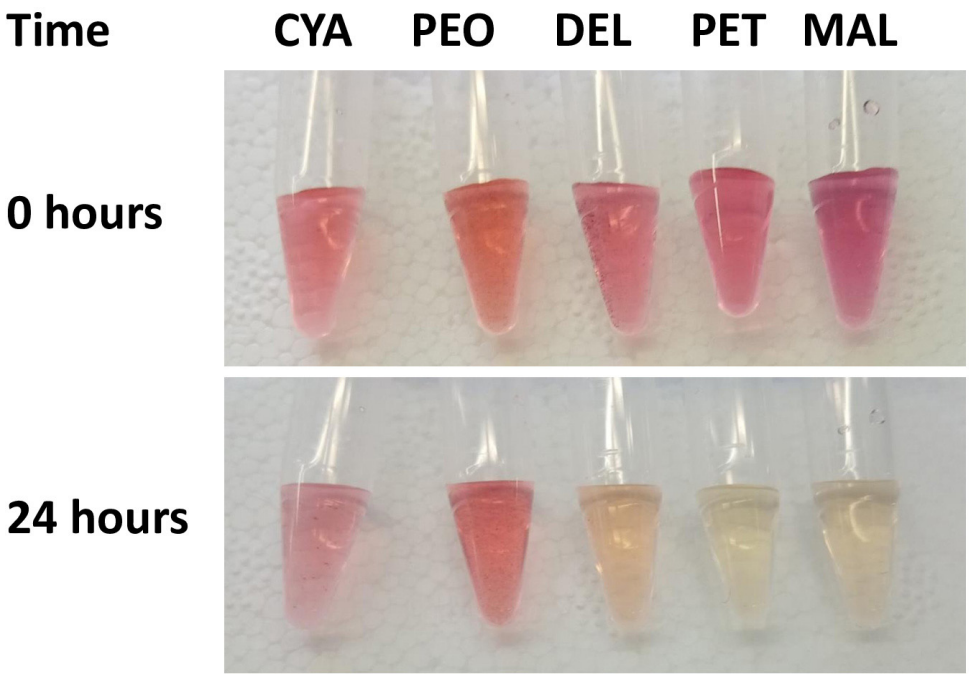

FIGURE 4. The visual aspect of the five different grape anthocyanins (300 μM) at the beginning of the experiment and after 24 hours of laccase action.

CYA: Cyanidin-3-O-G; **PEO**: Peonidin-3-O-G; **DEL**: Delphinidin-3-O-G; **PET**: Petundin-3-O-G; **MAL**: Malvidin-3-O-G.

It is worth highlighting that the three anthocyanins with three substituents in the B-ring were degraded much faster than the two anthocyanins with two substituents, as the degradation rate of cyanidin-3-*O*-glucoside was much slower than that of petunidin, delphinidin and malvidin 3-*O*-glucosides, with penodin-3-*O*-glucoside appearing to be resistant to the laccase action. It seems, therefore, that the presence of the third substituent can favour the oxidation reactivity catalysed by laccase. This activation may be related to the fact that the third substituent of the hydroxy or methoxy groups could act as an electron donor, which would induce the appearance of a delocalised negative charge in the B-ring.

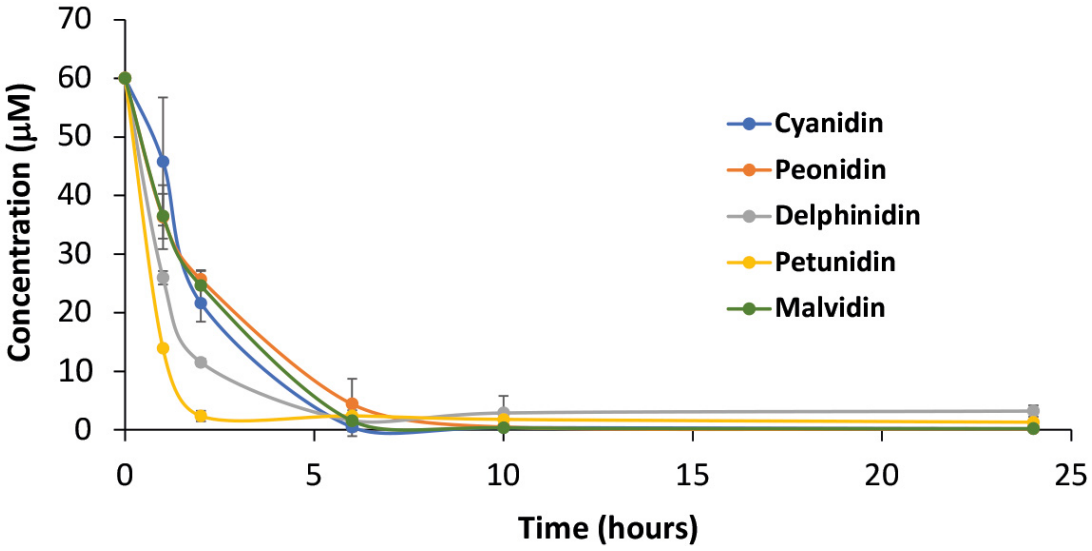

FIGURE 5. Laccase degradation kinetics of an equimolar mixture of the five grape anthocyanins. Results are expressed as mean ± standard deviation of three replicates.

Figure 3 shows the degradation of the red colour (A520 nm) in the different anthocyanin solutions by the laccase action.

In general, the red colour (A520) behaved similarly to that observed for the anthocyanin concentration, with the intensity of the red colour decreasing faster in the case of the petunidin-3-*O*-glucoside solutions and followed in descending order by delphinidin-3-*O*-glucoside, malvidin-3-*O*-glucoside and cyanidin-3-*O*-glucoside. As was the case for the concentration of anthocyanins, the colour of the peonidin-3-*O*-glucoside solution seems to be resistant to the laccase action. The variations in the colour of these solutions confirm that the presence of the third substituent makes the anthocyanin more sensitive to the degradation catalysed by laccase.

As the following images clearly illustrate, Figure 4 shows what happens to the final colour of the different anthocyanin solutions after 24 hours of laccase action. These photographs were taken at the beginning of the experiment and after 24 hours to emphasise the colour differences and make them visible to the human eye.

It is clear that the solutions of the three anthocyanins with three substituents, petunidin, delphinidin and malvidin 3-*O*-glucosides, have almost completely lost their red colour, whereas the cyanidin-3-*O*-glucoside still retains some. However, the colour of the peonidin-3-*O*-glucoside solution is virtually the same as it was at the beginning. This image visually confirms what was observed by HPLC and by spectrophotometry.

2. Laccase degradation kinetics of an equimolar mixture of the five grape anthocyanins

Figure 5 shows the degradation kinetics of an equimolar mixture of cyanidin, peonidin, delphinidin, petunidin and malvidin 3-*O*-glucosides.

This figure shows that all anthocyanins, even peonidin, are degraded by the laccase action. Under these conditions (a period of 2 hours) petunidin-3-*O*-glucoside degrades the fastest (28.8µM/h), followed by delphinidin-3-*O*-glucoside (24.2µM/h), while the other three anthocyanins – malvidin (19.2µM/h), cyanidin (17.7µM/h) and peonidin (17.2µM/h) 3-*O*-glucosides – are degraded more gradually. It must be highlighted that the differences observed in the degradation rate between the different anthocyanins when they are in a mixture are smaller than when they are not mixed, although petunidin and delphinidin are still degraded the most quickly. This may be due to the fact that after the primary quinones' initial formation, chemical polymerisation occurs with other phenols without the need for the laccase action (Queiroz *et al.*, 2008; Oliveira *et al.*, 2011). Consequently, the less reactive anthocyanins, such as peonidin and cyanidin 3-O-glucosides, can be used to form polymers without the laccase action. This is probably the reason why peonidin-3-*O*-glucoside cannot be degraded by laccase when it is alone, whereas it can be degraded in the presence of other, more reactive anthocyanins. This effect would also probably occur in the presence of other phenols, which could generate insoluble polymers that, when precipitated, would cause oxidasic haze.

3. Influence of the supplementation with seed tannin, (-)-epicatechin or glutathione on the degradation kinetics of an equimolar mixture of the five grape anthocyanins

Figure 6 shows the effect of the supplementation with seed tannin, (-)-epicatechin or glutathione on the degradation kinetics of an equimolar mixture of the five grape anthocyanins.

As shown in Figure 6A, in a period of 2 hours, no great differences in the degradation rate of total anthocyanins were observed between the control conditions (107 µM/h) and when the solution was supplemented with (-)-epicatechin (114.9 µM/h) and glutathione (122.5 µM/h). In contrast,

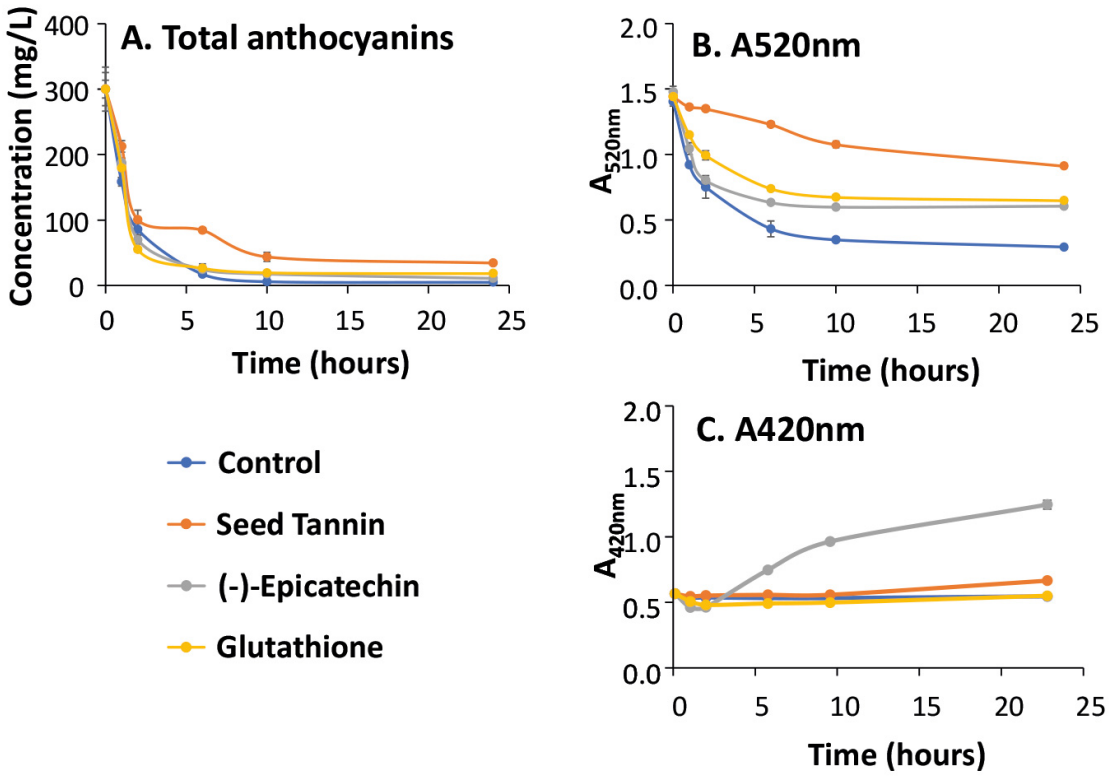

FIGURE 6. Influence of the supplementation with seed tannins, (-)-Epicatechin or glutathione on the degradation kinetics of a mixture of the five different grape anthocyanins by the action of laccase.

Results are expressed as mean ± standard deviation of three replicates.

the supplementation with seed tannin seems to slow down the degradation rate of total anthocyanins to some extent (99.8 µM/h). This apparent reduction in the degradation rate can be attributed to the proven laccase inhibitory effect of oenological tannins (Vignault *et al*., 2019; Vignault *et al*., 2020).

Figure 6B shows the changes in the red colour (A520 nm) of the various anthocyanin solutions, which were either supplemented or not with the different substances. In this case, the degradation of the red colour was clearly slower when the solution was supplemented with (-)-epicatechin and glutathione, especially with seed tannins. These data indicate that the concentration of total anthocyanins determined by HPLC does not coincide with the changes in the red colour since the differences in colour, in this case, are much more evident.

The explanation for why there is more colour in the case of the samples supplemented with seed tannins and (-)-epicatechin than that which would justify the remaining concentration of anthocyanins may be associated with copigmentation and with the formation of new pigments that retain the red colour but are not detected by HPLC. In the case of glutathione, the results are more difficult to interpret. However, it has been shown that glutathione can react with the orthodiquinones formed by the action of laccase and that these reconstitute the original orthodiphenols, thereby avoiding the formation of brown pigments (Cheynier *et al*., 1989; Cheynier *et al*., 1995; Robards *et al*., 1999). Consequently, it might be possible for glutathione to react with the initial anthocyanin degradation products of laccase action to reconstitute adducts between anthocyanins and glutathione. While HPLC cannot detect these, they do contribute to the red colour.

Figure 6C shows the changes in yellow colour (A420 nm), indicative of browning, of the various anthocyanin solutions which were supplemented or not with the different substances. No great differences were observed between the control samples and those supplemented with seed tannins or glutathione. In contrast, the sample supplemented with (-)-epicatechin showed a high increase in absorbance at 420 nm. (-)-Epicatechin has been identified as a very good substrate for laccase (Gimenez *et al*., 2022). Consequently, this data seems to indicate that laccase, in addition to catalysing the degradation of anthocyanins, also oxidises epicatechin, which leads to the well-known browning process (Rigaud *et al*., 1991; Singleton and Cilliers, 1995).

Figure 7 illustrates the changes after 24 hours in the colour of the various anthocyanin solutions supplemented or not

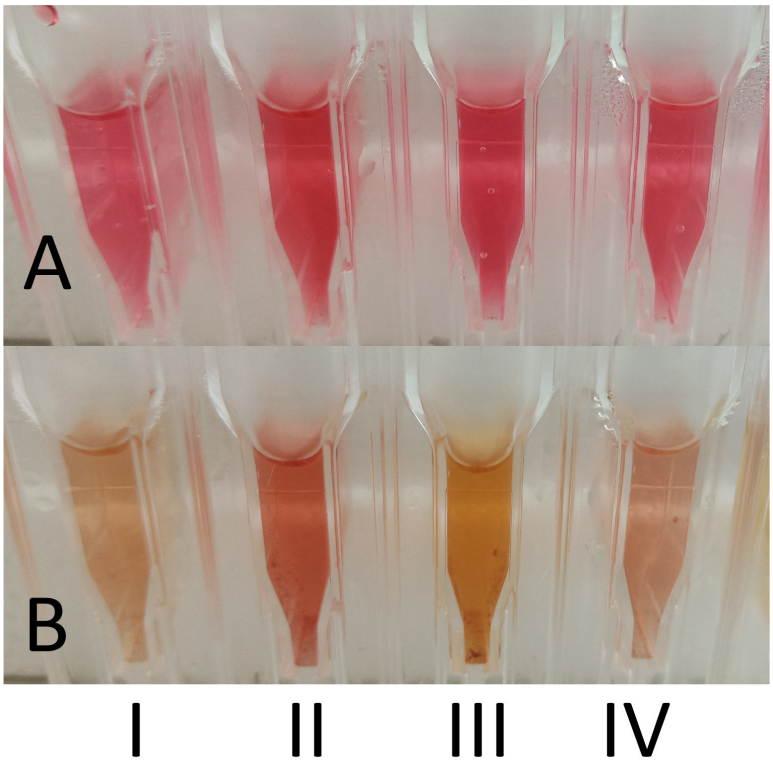

FIGURE 7. The visual aspect of anthocyanin samples after 24 hours in the absence or presence of 2 UA of laccase activity/mL.

A: Without Laccase; B: With laccase; I: Control; II: seed tannins, III: (-)-epicatechin; IV: glutathione.

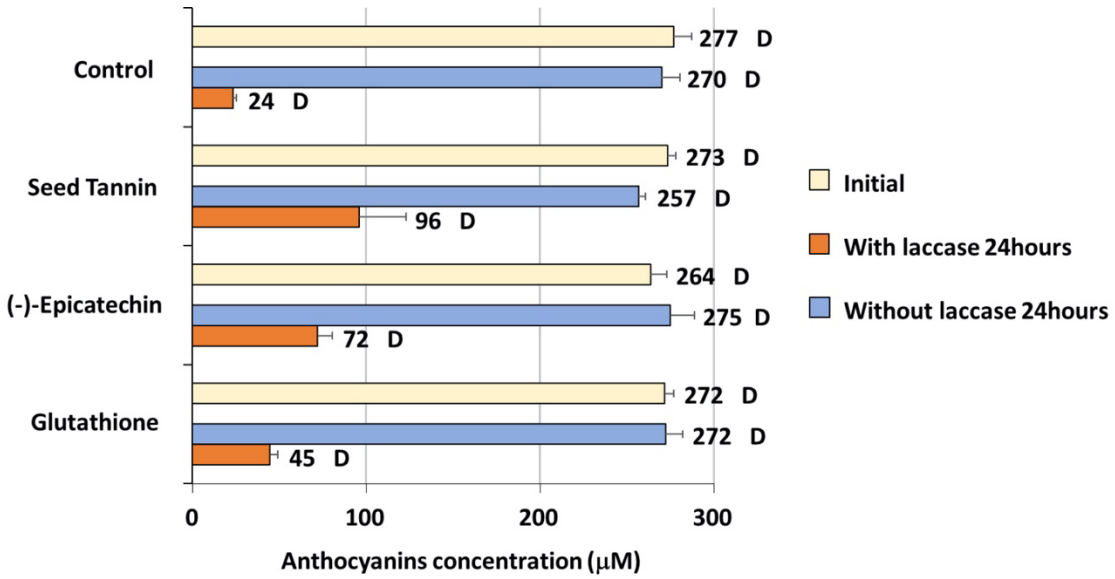

FIGURE 8. Influence of the supplementation with seed tannins, (-)-epicatechin or glutathione on initial and final anthocyanin concentration analysed by spectrophotometry.

All data are expressed as the arithmetic mean of 3 replicates ± standard deviation. Different letters indicate statistically significant differences ($p < 0.05$) between the samples.

with the different substances in the presence or not of 2 UA laccase/mL.

This picture visually demonstrates what has been previously explained and confirms that glutathione, and especially seed tannins, exert a protective effect on the red colour against laccase action. The (-)-epicatechin also protects the red colour slightly, but the browning induced by its presence predominates.

To verify if new pigments were formed in the presence of seed tannins, (-)-epicatechin or glutathione, spectrophotometric analysis of anthocyanins in the different samples was performed. Figure 8 shows the results.

The results indicate that in the absence of laccase, there are no significant differences between the initial concentration of total anthocyanins and that obtained after 24 hours.

As expected, the presence of laccase caused a drastic reduction in the total anthocyanin concentration in all the experimental groups. However, the total values of anthocyanins remaining after 24 hours of laccase reaction determined by spectrophotometry show that the supplementation with 200 mg of seed tannin/L caused a 30 % inhibition of anthocyanidin degradation. This inhibitory effect was 24 % when the solution was supplemented with 200 mg/L of (-)-epicatechin and 10 % when it was supplemented with 20 mg/L of glutathione. It should be stressed that 20 mg/L is the maximum legal dose authorised by the OIV for this antioxidant (OIV, 2021). These differences, which were not so clearly evident when anthocyanins were analysed by HPLC, seem to indicate that new red pigments were formed in the presence of these substances.

CONCLUSIONS

In this study, we analysed the degradation kinetics of anthocyanins by laccase from the *Botrytis cinerea* grape in three scenarios. The first scenario was a synthetic grape must using each one of the five grape anthocyanins separately, while the second was in a similar matrix but containing an equimolar of the five grape anthocyanins to get closer to the real grape must conditions. Finally, the third scenario was a replication of the second scenario but with the supplementation of three possible protectors: seed tannin, (-)-epicatechin and glutathione.

Our results show that the three anthocyanins with three substituents on the B-ring are more sensitive to the laccase action than those with only two when they are alone in the matrix. Even so, peonidin seems to be non-reactive to the laccase action in these conditions. These data could perhaps indicate that the varieties rich in peonidin-3-*O*-glucoside, such as Nebbiolo (Rolle *et al*., 2012), are more resistant to the colour degradation caused by laccase, although other authors (Darnal *et al*., 2023) have found that the presence of a high concentration of peonidin can be a cause of colour instability in wines. However, when all the anthocyanins were present in the matrix, peonidin was also degraded, probably because peonidin-3-*O*-glucoside can polymerise with the quinones formed by the oxidation of other anthocyanins or phenols (Queiroz *et al*., 2008; Oliveira *et al*., 2011). Consequently, the advantage of using varieties rich in peonidin-3-*O*-glucoside would not be as significant under real grape must conditions where many other phenols are present.

Our results also confirm that supplementation with seed tannin is effective in preventing oxidasic haze, even at high levels of laccase activity, since the red colour is clearly protected and the total anthocyanin concentration, as determined by spectrophotometry, is significantly higher than in the control conditions. These data suggest the formation of new red pigments is not detectable by HPLC.

On the other hand, the supplementation with (-)-epicatechin also seems to protect the red colour and anthocyanin concentration, as determined by spectrophotometry, but to a lesser extent than seed tannins. It appears, therefore, that its presence also favours the formation of new red pigments; however, despite being a very good substrate for laccase, it also causes very intense browning, a point which must be taken into consideration.

Finally, supplementation with glutathione also protected the red colour and anthocyanins determined by spectrophotometry, but in this case, the mechanism is expected to be different to that of seed tannins and (-)-epicatechin since it is not a flavanol that can react with anthocyanidins to form new red pigments. Further studies are needed to understand better the degradation of anthocyanins by laccase and the protective mechanisms exerted by seed tannins on wine colour.

ACKNOWLEDGEMENTS

This research was funded by CICYT (Efecto de las lacasas sobre la sensorialidad, calidad y salubridad de los vinos. Evaluación de la influencia de lacasas sobre el color, la astringencia y la calidad del vino—project RTI2018-095658-B-C33).

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